Micro/Nanobubbles Driven Multimodal Imaging and Theragnostics of Cancer

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Abstract: Ultrasound imaging has attracted great interest of researchers due to their application in cancer diagnosis and treatment. Ultrasound contrast agents, microbubbles and nanobubbles are widely explored as a multifunctional platform, not only carrying other contrast agents for multimodal imaging to complement the disadvantages of each imaging modality, but also carrying drug/gene for cancer theragnostic. In this article, the characteristics and differences of microbubbles and nanobubbles are briefly introduced and reviewed. Besides, the microbubbles and nanobubbles driven multimodal imaging and theragnostic of cancer are summarized.

Key words: Microbubbles; Nanobubbles; Multimodal imaging; Theragnostic

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Ultrasound (US) imaging is one of the most common non-invasive imaging methods in cancer diagnosis. Ultrasound imaging has the advantages of high safety, non-invasive nature, real-time imaging, deep penetration into tissue and low cost. In order to improve the accuracy and confidence of disease diagnosis, ultrasound contrast agents with high echogenicity are introduced to increase ultrasound signals and provide high-quality images. Gramiak et al. found a cloud of echoes in the heart chambers after intracardiac injection of indocyanine green, and first proposed the concept of ultrasound contrast agent in 1968 [1]. In 1972, Ziskin et al. found that the contrast effect could be produced by rapidly injection of any fluid, because of the production of bubble cavitation [2]. Since then, the study of bubbles as ultrasound contrast agents was developed rapidly [3, 4]. Microbubbles (MBs) are widely used as ultrasound contrast agents, usually composed of gaseous cores and outer shells of biocompatible materials, ranging from 1 to 10 μm in diameter. Recently, nanobubbles (NBs) have been developed as novel ultrasound contrast agents [5,6].

Although ultrasound imaging combined with

contrast agents can provide higher resolution of tissues than that of ultrasound imaging only, there are still some limitations. Combination of ultrasound imaging with other imaging modalities can improve diagnostic accuracy and reliability, due to the unique advantages and limitations of each imaging modality. Microbubbles and nanobubbles are widely explored as a platform to carry other contrast agents for multimodal imaging [7-9].

Moreover, US plays a significant role in non-invasive therapy, such as high-intensity focused ultrasound (HIFU) [10,11] and US-mediated drug delivery [12-14]. Focused ultrasound can generate thermal and non-thermal effect in target tissues, especially ultrasound-induced cavitation effects, which improve the drug delivery efficiency through increasing vascular permeability. The existence of microbubbles increases the delivery efficiency through enhancing ultrasound energy deposition and serving as cavitation nuclei [13]. Through binding drugs or gene to the shells or specific ligands, microbubbles and nanobubbles can serve as a multifunctional platform to deliver drugs or genes, increase local drug concentration, control drug release, and achieve simultaneously imaging and treatment of cancer.

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In this article, the characteristics and differences of microbubbles and nanobubbles are briefly introduced and reviewed. In addition, the microbubbles and nanobubbles driven multimodal imaging and theragnostic are summarized.

Microbubbles and Nanobubbles

Microbubbles are commonly used as ultrasound contrast agents in the clinic due to their excellent echogenicity. Several ultrasound contrast agents, such as Sonovue and Definity have been approved by the food and drug administration (FDA) for clinical applications. They can provide high quality ultrasound images of many organs and tissues, including heart, liver, spleen, blood vessels and lymph nodes. They also play a

valuable role in tumor diagnosis and evaluation.

The physiochemistry of MBs are determined by the materials of gaseous cores and outer shells [15]. Air was used in first generation MBs, but abandoned because of poor stability. Then insoluble and low diffused gases, such as perfluorocarbons, are introduced to increases stability and the circulation time of microbubbles [16,17]. Biocompatible materials, including lipids, proteins [18], and polymers [19,20] are primary choices for shell materials of MBs. The MBs can be functionalized by several methods [21–23], including attaching drugs to the surface of MBs, encasing hydrophobic drugs inside the microbubbles or embedding lipid-stabilized drugs within the microbubble membrane, as illustrated in Figure 1.

Figure 1 Schematic of different potential loading techniques for lipid microbubbles. All techniques are applicable to protein and polymer microbubbles [Reprinted with permission from reference 22].

Because the gaps between tumor vessels endothelium cells are smaller than 1 μm [24], MBs cannot penetrate the tumor vessels and enter the tumor microenvironment. Nano-sized ultrasound contrast agents are small enough to penetrate tumor vessels pores and interact with tumor cells directly, thus showing great potential in the field of molecular ultrasound imaging and drug/gene delivery [25–27]. Nanobubbles have smaller particle size, more stable performance, and longer circulation time than MBs, which leads to more accumulation in the tumor

area [28]. Researcher have confirmed that NBs can passively accumulate in the tumor area via the enhanced permeability and retention (EPR) effect [5]. Note that the echogenicity of NBs is decreased because of their small particle size, but it can be improved through shell modification and bubble aggregation [29].

Although MBs cannot penetrate tumor vessels and enter tumor tissues, ultrasound induced cavitation can enhance permeability of blood vessel wall and cell membrane, making it possible for MBs to deliver drug

or genes into tumor microcirculation. The cavitation effect depends on various factors, including ultrasound frequency, intensity, microbubble shell composition and particle size [29]. Furthermore, under high acoustic pressures, microbubbles can violently contract and expand, even burst into smaller particles or fragments. Then carried drug/gene or obtained nanoparticles can be released and accumulated in tumor area via EPR effect. This approach is a safe and efficient active targeting method, which called ultrasound-targeted MB destruction (UTMD). The delivery induced by UTMD is limited to the area of ultrasound irradiation, thus it decreases the undesired damage to surrounding tissues and reduces systemic side effects. UTMD method is widely used in treatment of various types of cancer [30], cardiovascular diseases [31] and neurological diseases [32].

Micro/Nanobubbles Mediated Multimodal Imaging

Each imaging modality has its own advantages and limitations, thus multimodal imaging, which combines different imaging modalities, are developed to improve diagnostic accuracy and reliability. Microbubbles and nanobubbles are widely explored as a platform for multimodal imaging due to facile modification.

Fluorescence imaging has high sensitivity and possesses multicolor imaging capacity, while it suffers from low tissue penetration and poor spatial resolution. Considering these limitations, US/fluorescence bimodal imaging attracts great interest of researchers. With the introduction of fluorescence dyes, including indocyanine green (ICG) [33], cyanine dyes [34-37], quantum dot (QDs) [38,39] and porphyrin [40], microbubbles and nanobubbles can act as bimodal contrast agents for tumor visualization. Huynh et al. prepared porphyrin microbubbles (pMBs) as tri-modality contrast agents for ultrasound, fluorescence, and photoacoustic imaging [40]. Bacteriochlorophyll-lipid (BChl-lipid), a kind of porphyrin-lipid, was used as microbubble shell materials to provide fluorescence and photoacoustic properties. Upon exposure to low-frequency ultrasound, pMBs were successfully converted into porphyrin nanoparticles (pNPs), which generated only fluorescence and photoacoustic contrast signals. In vivo experiments also revealed the successful conversion of pMBs to pNPs and accumulation of pNPs at the tumor site without relying on the EPR effect. This conversion of MBs to NPs has great potential in imaging and therapeutic applications.

Targeted fluorescence nanobubbles plays a significant role in cancer diagnosis because of their small size and targeting character. Li et al. used biodegradable photoluminescent polymers (BPLPs) as a shell around liquid tetradecafluorohexane (C_6F_{14}) to form nanobubbles, followed by conjugation with PNBL-

NPY, a neuropeptide Y Y1 receptors (Y1R) ligand, for targeted breast tumor imaging [41]. In vitro and in vivo experiments demonstrated that the obtained Y1R-mediated fluorescent nanobubbles had good ultrasound contrast enhancement and targeting ability to Y1 receptors-overexpressing breast cancer. Near-infrared (NIR) heptamethine cyanine dyes with fluorescence property and tumor-targeting characteristic, such as IR-780 [42,43] and IR-783 [44], are loaded into the shell of nanobubbles for tumor-specific ultrasound/fluorescence dual-modal imaging. For example, Shen et al. synthesized a novel targeted bimodal nanobubbles, FA-NBs-IR780, with a uniform nano-size (591 ± 52 nm) [43]. Because folic acid (FA) and IR-780 iodide provided dual-targeting capacity, FA-NBs-IR780 could be efficiently accumulated at the tumor site for ultrasound/fluorescence bimodal imaging. In addition, IR-780 iodide encapsulated in FA-NBs-IR780 can act as a photothermal agent to induce thermal ablation of tumors upon NIR irradiation with minimal damage to surrounding tissues. The nanobubbles combined with dual-targeting strategy have promising potentials for accurate diagnosis and treatment of cancer.

MRI provides worthwhile structural details of soft tissues with high spatial resolution, while its applications are limited by low sensitivity, long scanning time and high costs. MRI/ultrasound bimodal contrast agent has emerged as a promising method for cancer diagnosis. Superparamagnetic iron oxide (SPIO) nanoparticles are excellent T2-MRI contrast agents and can be physically embedded into the shell [45-47] or chemically conjugated on the external surface of MBs [48-50]. The echogenicity of the host MBs does not significantly decrease with the introduction of SPIO nanoparticles [49]. However, studies have demonstrated that the shell of MBs stiffen when SPIO entrapped into the shell. Thus, compared to MBs with SPIO embedded into the shell, MBs with external loading of SPIO have better performance for ultrasound imaging [51]. Chen et al. constructed novel microbubbles combined with manganese (III)-based MRI contrast agent for bimodal tumor imaging [52]. Mn-chelated porphyrin lipid (MnP) were fabricated to form the shell of microbubbles, followed by encapsulating a perfluoropropane gas core. The Mn-chelated porphyrin microbubbles (MnP-MBs) possessed good contrast enhancement ability for both US and MR imaging with a very low dose. Upon low-intensity US exposure, MnP-MBs were successfully converted into NPs and exhibited high accumulation at the tumor site for efficient MRI contrast enhancement (Fig. 2).

The combination of three-dimensional CT and real-time ultrasound provides accurate images to identify the size and location of tumors, as well as to monitor

and guide cancer therapy. US/CT bimodal contrast agents were successfully fabricated by introduction of gold nanoparticles (GNP) into microbubbles [53] and nanobubbles [54]. Ke et al. fabricated gold nanoshelled perfluorooctylbromide (PFOB) nanocapsules with PEGylation (PGsP NCs) for ultrasound/CT dual-modal contrast imaging [54]. The gold nanoshell on the surface provided CT signals, and encapsulated PFOB of PGsP NCs provided ultrasound contrast signals. Moreover, gold nanoshell could induce thermal ablation of tumors upon laser irradiation due to the excellent photothermal property of gold nanoparticles. The use of multifunctional nanobubbles offers accurate diagnostic information to improve imaging-guided cancer

treatment. Other CT contrast agents, such as iodinate compounds [55] and bismuth sulfide (Bi_2S_3) [56], are also used to construct dual-modal contrast agents for more accurate and comprehensive diagnosis of tumor. Zhou et al. constructed folate-targeted perfluorohexane nanoparticles carrying bismuth sulfide (FLBS-PFH-NPs) for tumor ultrasound/CT imaging and targeted HIFU therapy [56]. Upon ultrasonic irradiation, the capsulated PFH underwent a liquid-gas phase-transition, then the formation of MBs enhanced the efficacy of HIFU therapy. The as-designed multifunctional contrast agents depending on active targeting significantly improved the diagnosis and treatment effect of cancer.

Figure 2 (A-B) In vivo US imaging of MnP-MBs. MnP-MBs were intravenously administered into U87 glioblastoma tumor-bearing nude mice. The duration of US contrast enhancement in the tumor was monitored by a clinical US probe and the quantitative data are shown in (B); (C-D) In vivo MRI imaging of MnP-MBs. In vivo MRI monitoring of MnP-MBs with US exposure (C, upper) and without US exposure (C, bottom) and their quantitative results (D) within 24 h. [Reprinted with permission from reference 52]

Micro/Nanobubbles Driven Theragnostics of Cancer

With the guidance of ultrasound, microbubbles enable not only ultrasound contrast imaging for diagnosis, but also increasing vascular permeability and promoting drug/gene penetration into tumors. It is feasible to deliver drugs or genes through co-injection of microbubbles with drugs followed by MBs induced sonoporation [57-59]. For example, the combination of

gemcitabine, SonoVue® and ultrasound has been applied for the treatment of pancreatic ductal adenocarcinoma [57]. The results of Phase I clinical trial proved that the combined therapeutic regimen enhanced the treatment effect, improved life quality of patients and prolonged their survival without additional side effects. However, incorporating drugs into MBs is a better choice, which ensures the same bio-distribution and pharmacodynamic behaviors of drugs and MBs.

Figure 3 (A) Schematic illustration of engineering and US-induced micro-to-nano conversion of Janus camptothecin-fluoruridine MBs (CF MBs) for in situ combined cancer chemotherapy; (B) In vivo US imaging. CF MBs were intravenously administered into 4T1 tumor-bearing mice, and the tumor was imaged with a clinical US probe (3~12 MHz); (C) Time-lapse NIR fluorescence imaging of mice; (D) Quantitation of tumor site after tail vein injection. (E-F) In vivo antitumor effect of CF MBs combined with US on 4T1 tumor-bearing nude mice; (E) Images of tumor-bearing mice after different treatments; (F) Tumor volume change over time after treatment with CPT&FUDR@liposome, CF NPs, CF MBs and CF MBs + US, * $P < 0.05$. [Reprinted with permission from reference 71]

Multifunctional Micro/Nanobubbles Driven Chemotherapy

Chemotherapy plays a significant role in cancer treatment, but it faces with many problems, including low bioavailability, side effects and drug-resistance. Drug-loaded MBs combined with ultrasound provide an effective strategy to increase local drug concentration, control drug release, and reduce side effects and drug-resistance. Common chemotherapy drugs, such as doxorubicin [60-63], docetaxel [64,65], and paclitaxel [66,67] have been loaded into MBs for application of cancer theragnostics. Ting et al. prepared MBs loaded with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) for brain tumor treatment [68]. The blood-brain barrier (BBB) protects the brain from potentially harmful compounds in the blood, but it restricts the chemotherapy drugs entering brain tumors. MBs-mediated focused ultrasound (FUS) provides a feasible strategy to induce local BBB opening and improve drug delivery into brain. The combination of FUS (1MHz) with BCNU-MBs successfully triggered BBB opening, meanwhile BCNU was released from the BCNU-MBs and accumulated at the target site, thereby significantly inhibiting glioma growth and prolonging survival of tumor-bearing rats. Furthermore, multifunctional MBs loaded with DOX and SPIO nanoparticles were designed by the same group

[60,69]. The present of SPIO particles not only acted as T2-MRI contrast agents, but also allowed magnetic targeting (MT) to enhance drug delivery to brain tumors. The designed multifunctional MBs loaded with DOX and conjugated with SPIO nanoparticles (DOX-SPIO-MBs) exhibited good contrast enhancement in both US and MR imaging [69]. The DOX-SPIO-MBs simultaneously induced BBB opening and drug delivery with FUS exposure, then magnetic targeting (MT) further enhanced drug accumulation in brain tumors. They also proposed a novel theragnostic complex of SPIO-DOX-conjugated MB (SD-MBs) for drug delivery via ultrasound/magnetic dual-targeting [60]. The behaviors of DOX could be real-time monitored by MRI signals of SD complex. Such dual-targeting therapeutic strategy has promising potentials to achieve advanced tumor treatment.

Through further conjugation with targeting ligands, the chemotherapy MBs and NBs can be used as potential tumor-targeted theragnostic agents for future applications. Zhang et al. fabricated iRGD-modified paclitaxel-loaded liposomes (iRGD-PTX-PL), then successfully conjugated it to the surface of MBs through biotin-avidin linkage [67]. The obtained liposome-microbubble complexes (iRGD-PTX-LMC) had high affinity for breast tumors and great ultrasonic responsiveness for local drug release upon ultrasound irradiation. Compared with iRGD-PTX-PL and nontargeted PTX-LMC, the combination of

iRGD-PTX-LMC and ultrasound could better improve local drug delivery and enhance therapeutic effect. Gao et al. designed folic acid (FA)-conjugated lipid nanobubbles loading artesunate (Arte; FA-ALNBs) for ultrasound-guided targeted chemotherapy [70]. The FA-ALNBs had a uniform particle size distribution (781.2 ± 5.3 nm), good stability in physiological environment and excellent capability to enhance US signals. Upon FUS irradiation, the entrapped Arte was released as a result of US-mediated FA-ALNBs destruction. The FA receptor-mediated endocytosis process enhanced the cellular uptake of FA-ALNBs. Both in vitro and in vivo experiments demonstrated that FA-ALNBs combined with US exhibited great anti-tumor effect without obvious systemic toxicity.

Recent studies have revealed that one of major limitations of MB-driven chemotherapy is their low drug loading content. To enhance the chemotherapeutic efficacy with minimal side effects, Liang et al. synthesized an amphiphilic Janus camptothecin-floxuridine (CPT-FUDR, CF) conjugate as shell materials to construct CF MBs with high drug loading contents ($\sim 56.7\%$) [71]. CF MBs showed excellent US contrast capability. Upon UTMD technique with the guidance of ultrasound imaging, CF MBs were converted into nanoparticles in situ, resulting in ~ 14 times higher drug accumulation in subcutaneous breast tumor and reduced undesired drug accumulation in organs. Furthermore, the ester bond of CF was hydrolyzed in the tumor microenvironment, and CPT and FUDR were released at an exact 1:1 ratio to achieved synergistic chemotherapy for tumors. The tumor growth inhibition ratio of CF MBs combined with US was 72.4%, higher than that of CF NPs (54.1%) and liposomes loaded with CPT and FUDR (21.6%), which demonstrated that CF MBs combined with UTMD is an effective strategy for cancer theragnostics (Fig. 3). Based on CF MBs, Chen et al. successfully constructed porphyrin/camptothecin-floxuridine triad MBs (PCF-MBs) with high drug loading contents for ultrasound/fluorescence bimodal imaging and chemo-photodynamic combination therapy [72]. PCF-MBs induced PDT could reduce the expression of ATP-binding cassette subfamily G member 2 (ABCG2), thus overcoming multidrug resistance (MDR) in colorectal cancer. This combination therapeutic strategy based on PCF-MBs demonstrated a 90% tumor inhibition ratio in murine colon cancer models without recurrence.

Multifunctional micro/nanobubbles driven gene therapy

Gene delivery using ultrasound and microbubbles is another attractive approach for tumor treatment. The cationic microbubbles are suitable to deliver gene via charge-coupling with genetic material such as DNA [73,

74], microRNA (miRNA) [75] and small interfering RNA (siRNA) [76]. UTMD effectively improves gene transfection efficiency with the advantages of safety, practicality, and target. The use of miRNA-133a-microbubbles with UTMD for breast cancer therapy was reported by Ji et al. [75]. When miR-133a-microbubble injected into animal model of breast cancer, miR-133a presented prolonged circulation time, and showed better delivery efficiency upon low-frequency ultrasound. UTMD of miR-133a was a promising approach to suppressing the tumor growth and improving survival of xenografted mouse model. Zhou et al. developed targeted cationic microbubbles conjugated with CD105 antibody (CMB105), following by loading endostatin gene to achieve targeted anti-angiogenesis gene therapy and ultrasound imaging [73]. The CD105 antibody conjugated on the surface of CMB105 would not affect the binding ability of plasmid DNA. Compared with untargeted cationic microbubbles (CMB) and neutral microbubbles (NMB), CMB105 combined with UTMD presented better gene transfection efficiency and treatment effect in vivo.

Nanobubbles are potential multifunctional carriers for gene delivery [77]. Nanobubbles could pass through tumor blood vessels and passively accumulate in tumor microcirculation due to their nanoscale size. Cai et al. successfully synthesized NBs carrying siRNA (NBs-siRNA) by a biotin-streptavidin system [76]. The NBs-siRNA had high ultrasound contrast-enhancement capability and can be disrupted to induce sonoporation when exposure to ultrasound. The NBs-siRNA combined with ultrasound targeted destruction (UTD) possessed better transfection efficiency of siRNA than siRNA-NBs without UTD. In vivo experiment demonstrated siRNA-NBs combined with UTD could successfully deliver siRNA into tumor tissues to inhibit glioma tumor growth and prolong survival of mice.

Multifunctional micro/nanobubbles driven synergistic therapy

There are also theragnostic MBs and NBs with sophisticated designs that combine photothermal therapy [78-80], photodynamic therapy [81], and synergistic therapy [72,82,83]. For example, NIR fluorescence dye 1,1-dioctadecyl-3, 3, 3, 3-tetramethylindotricarbocyanine iodide (DiR) can act as a photothermal agent for tumor photoablation, while porphyrin and chlorin e6 (Ce6) can operate as a photosensitizer for photodynamic therapy. Xu et al. prepared multifunctional theragnostic microbubbles by self-assembly from porphyrin grafted lipid (PGL) and DiR with high loading contents (PGL: 5.8%; DiR: 10.38%) [84]. The PGL-DiR MBs showed excellent ultrasound/fluorescence dual-modal contrast

capability, and could be used for combined photothermal therapy and photodynamic therapy with the assistance of UTMD. Upon US exposure to tumor, the PGL-DiR MBs were converted into nanoparticles, resulting in improved accumulation and cellular internalization of two phototherapeutic agents. With the laser irradiation for PDT and PTT, the tumor growth of xenografted mouse model treated with PGL-DiR MBs were completely inhibited with no recurrence, while the tumor growth inhibition ratio of PGL-DiR NPs was only 72.6% (Fig. 4). The combination of phototherapy with PGL-DiR MBs provided an effective strategy for imaging-guided

tumor therapy. However, previous studies suggest that PDT may induce undesired overexpression of angiogenic factors in tumor [85]. To overcome this side effect, Jang et al. developed the complex of DOX-encapsulating anti-angiogenic siRNA nanoparticle and Ce6-encapsulating microbubble (DOX-siVEGF-NPs/Ce6-MBs) for combination of chemotherapy, photodynamic and gene therapy [83]. With local ultrasound irradiation, the DOX-siVEGF-NPs/Ce6-MBs was disrupted and released three drugs at the tumor microenvironment, resulted in effective inhibition of angiogenesis and enhanced therapeutic outcomes.

Figure 4 (A) The structure and conversion of the PGL-DiR MBs into nanoparticles when exposed to sufficient ultrasound, and ultrasound-mediated tumor-specific combined photothermal and photodynamic therapy; (B) PGL-DiR MBs were intravenously administrated into the 4T1 tumor-bearing mice and the tumor was imaged with a clinical ultrasound probe (3–12 MHz). At 38 s, the tumor site was irradiated by the low-frequency ultrasound with a higher mechanical index to destroy the MBs; (C) Time-lapse NIR fluorescence imaging of mice intravenously administrated with PGL-DiR NPs, and PGL-DiR MBs combined with ultrasound exposure. The dashed circles indicate subcutaneous tumor regions; (D) Cell viabilities of 4T1 cells treated by PGL NPs+US, DiR NPs+US, PGL-DiR NPs+US, PGL-DiR MBs, and PGL-DiR MBs+US. All the above groups were irradiated by 760 nm laser (1 W cm^{-2} , 10 min) and 650 nm laser (200 mW cm^{-2} , 3 min). The molar concentrations of PGL and DiR were same; (E) Tumor growth curves of different groups of mice received various treatments indicated. The data were presented as the mean $\pm$ SD ($n = 5$), $^*P < 0.05$, $^{**}P < 0.01$. [Reprinted with permission from reference 84]

Conclusion

Ultrasound imaging is an important diagnostic tool in the clinical settings due to the advantages of high safety, non-invasive nature, real-time imaging, deep penetration

into tissue and cost effectiveness. Microbubbles and nanobubbles are widely used as ultrasound contrast agents for tumor diagnostics. Several microbubbles have been approved by FDA for diagnostic applications.

Multimodal imaging can be achieved through loading certain contrast agents in microbubbles and nanobubbles. Similarly, microbubbles and nanobubbles play a significant role to deliver drug/gene for tumor treatment.

However, multifunctional MBs and NBs still suffer from limitations that confine the clinical application to some extent. One of major limitations is low delivery efficacy due to instability and short circulation time of MBs and NBs. We need to consider the choice of membrane material, size distributions and ultrasonic parameters to overcome this for effective delivery into tumors. UTMD technique is a useful targeting approach to improve drug delivery and local release. To get better therapeutic effect, it is necessary to improve drug loading of MBs and NBs. Researchers have developed novel shell materials composed of drugs. Conjugating drug liposomes to the surface of microbubbles also increase drug loading for chemotherapy and other related applications. In addition, the bioeffects associated with multifunctional MBs and NBs, including toxicity, biodistribution and pharmacokinetics should be investigated as clinical translation proceeds. Believing that with further research on multifunctional MBs and NBs, it has a wide application prospect in the clinical practice.

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Conflict of Interest

The authors have declared no conflicts of interest for this article.

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