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Ultrasound-induced biophysical effects in controlled drug delivery

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Ultrasound is widely used in biomedical engineering and has applications in conventional diagnosis and drug delivery. Recent advances in ultrasound-induced drug delivery have been summarized previously in several reviews that have primarily focused on the fabrication of drug delivery carriers. This review discusses the mechanisms underlying ultrasound-induced drug delivery and factors affecting delivery efficiency, including the characteristics of drug delivery carriers and ultrasound parameters. Firstly, biophysical effects induced by ultrasound, namely thermal effects, cavitation effects, and acoustic radiation forces, are illustrated. Secondly, the use of these biophysical effects to enhance drug delivery by affecting drug carriers and corresponding tissues is clarified in detail. Thirdly, recent advances in ultrasound-triggered drug delivery are detailed. Safety issues and optimization strategies to improve therapeutic outcomes and reduce side effects are summarized. Finally, current progress and future directions are discussed.

ultrasound, biophysical effects, drug delivery systems, ultrasound parameters, ultrasound-triggered drug delivery

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Introduction

Spatiotemporally controllable drug delivery remains a major goal in medicine as it would allow therapeutic drugs to be delivered to target sites conferring maximum drug efficacy with minimal side effects on normal tissues. In the early 20th century, Ehrlich posited the development of a drug carrier able to respond to external or internal stimuli. Since then, many methods of spatiotemporally controllable drug delivery utilizing a range of stimuli have been reported (Geers et al., 2012).

Internal and external stimuli can be used for controllable drug delivery. In general, tumoral tissues have specific properties that differ from normal tissues, including lower pH, higher enzyme concentrations, and higher redox gradients (Mura et al., 2013), which can act as endogenous stimuli. In recent years, a range of smart delivery carriers have been developed that can respond to various endogenous stimuli to achieve controllable drug delivery. However, precise spatiotemporal control in response to endogenous stimuli remains technically challenging due to the heterogenicity and complexity of tumors. Indeed, significant variation in tumoral pH levels has been reported between individuals. Consequently, few endogenous stimuli-triggered drug delivery systems have entered clinical practice, with the majority still in preclinical trials. On the other side, there is increasing interest in exogenous stimuli-triggered drug delivery. Exogenous stimuli, such as light, magnetic fields, and ultrasound, have the advantages of precise temporal and spatial controllability and non-invasiveness but are limited by penetration depth and biosafety concerns. Accordingly, exogenous stimuli-triggered drug delivery systems may have greater potential for clinical translation than systems utilizing endogenous stimuli.

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Ultrasound has wide applications in medical diagnostics (Wang et al., 2020). Ultrasonic waves cause mechanical disturbances via wave propagation of kinetic energy. Propagation of ultrasonic waves through tissues produces biophysical effects, predominantly through transfer of kinetic and thermal energy, which can be utilized for controllable drug delivery. The use of biophysical effects induced by ultrasound for drug delivery was first reported in 1954 despite the development of ultrasound in 1928. The first clinical trial of a system utilizing ultrasound as an external stimulus for drug delivery was conducted in 2013 (Kotopoulis et al., 2013). Since then, many clinical studies utilizing the biophysical effects of ultrasound have conducted demonstrating significant potential as a novel treatment technique (Figure 1). The advantages of ultrasound in drug delivery compared with other exogenous stimuli, such as light, electric fields, and magnetic fields, are summarized in Table 1. Ultrasound can directly induce mechanical and cavitation effects. When combined with microbubbles, these effects can be magnified to enhance drug delivery. Furthermore, ultrasound has better tissue penetration and possesses equivalent spatiotemporal control capability compared with other methods, thereby overcoming the limitation of poor deep tissue penetration with the use of other external stimuli. In addition, various microbubbles have been approved by Food and Drug Administration for ultrasound imaging and represent promising ultrasound-responsive delivery carriers allowing improvements in the biosafety of drug carriers (Poon and Borys, 2011; Wood et al., 2012; Zagar et al., 2014). Low cost, noninvasiveness, and absence of ionizing radiation make ultrasound a promising candidate for clinic translation.

Ultrasound-triggered drug delivery currently has applications in many clinical fields, including neurology, cardiology, and oncology (Kopechek et al., 2015; Kwekkeboom et al., 2016; Lin et al., 2020; Mozafari et al., 2016; Rich et al., 2020; Yang et al., 2019; Zhang et al., 2017). This review describes the utility of the biophysical effects of ultrasound in stimulus-triggered drug delivery and provides an overview of the recent developments, applications, and safety considerations of this technique. Finally, current progress and future directions are discussed.

Ultrasound-induced biophysical effects in stimulus-triggered drug delivery

Thermal effects

Propagation of ultrasound waves through tissues leads to energy dissipation due to absorption and scattering by tissues. Tissue temperature can increase as a result of kinetic energy absorption through a process known as ultrasound-

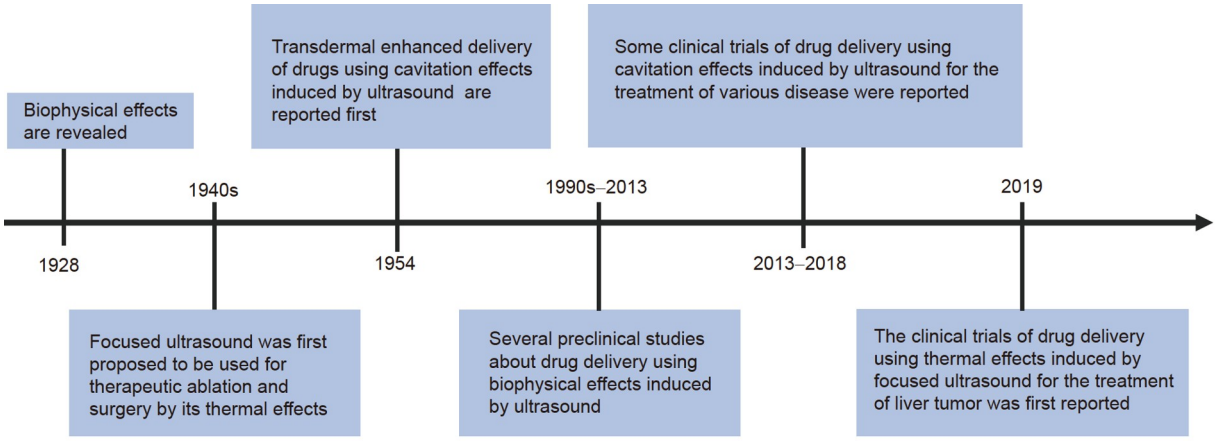

Figure 1 (Color online) History of the application of ultrasound-induced biophysical effects in controlled drug delivery.

Table 1 Advantages and disadvantages of different external stimuli

External stimuli	Advantages	Disadvantages	References
Light	Spatiotemporal control, low cost	Limited penetration (depth of penetration: 0.3–0.8 cm)	(Zhao et al., 2019)
Electric field	Spatiotemporal control	Surgical implantation required, low penetration, sensitive to surrounding medium	(Zhao et al., 2016)
Magnetic field	Spatiotemporal control, noninvasiveness, high penetration	Cytotoxicity due to accumulation of magnetic particles, high cost	(Ge et al., 2013)
Ultrasound	Spatiotemporal control, high penetration (depth of penetration: 0.1–10 cm), low cost	Technically challenging targeting of moving organs, high reflection of air and bone	(Boissenot et al., 2016)

induced thermal effects (Boissenot et al., 2016). However, temperatures greater than 43°C can result in enzyme denaturation leading to disrupted cellular structure and function. The degree of tissue damage depends on temperature change and duration of exposure (Dewey et al., 2009). Studies have reported detrimental effects of ultrasound, including protein denaturation, cell necrosis, and ablation *in vitro* at temperatures greater than 43°C or with sustained temperature increases over long periods. Thus, thermal effects are typically divided into mild hyperthermia (37°C–43°C) and strong hyperthermia or thermal ablation (>43°C) according to cellular response (Boissenot et al., 2016).

Mechanism of drug delivery induced by thermal effects

Mild hyperthermia is typically used to trigger drug delivery as strong hyperthermia or thermal ablation can induce irreversible coagulative necrosis of tumor tissues and destroy adjacent vasculature, thereby impeding drug delivery (Boissenot et al., 2016). In addition, hyperthermia may damage adjacent normal tissue resulting in severe complications such as full-thickness burns (Ge et al., 2014; Jung et al., 2011). Conversely, mild hyperthermia can increase blood flow and enhance vascular permeability by increasing microvessel pore size, thereby enhancing drug delivery (Grüll and Langereis, 2012; Jain et al., 2018; Lefor et al., 1985; Song, 1984; Song et al., 1980) (Figure 2A). Furthermore, mild hyperthermia can be used to alter the structure of thermo-responsive drug delivery systems to increase permeability and initiate drug release when temperatures rise above the transition temperature (T_m) of nanoparticles (Grüll and Langereis, 2012). Accordingly, utilization of the thermal effects of ultrasound in thermo-responsive drug delivery systems can increase drug concentrations at target sites and improve therapeutic efficacy (Figure 2B).

Recent advances in ultrasound-triggered drug delivery based on thermal effects

Low temperature sensitive liposomes (LTSLs) represent the most used thermal-based drug delivery systems due to outstanding thermal sensitivity and high biosafety. Previous studies have reported various methods of inducing thermal effects to trigger drug release from thermosensitive liposomes, including the use of radiofrequency (RAF), photothermal, and microwave techniques (Paulides et al., 2020; Yang et al., 2017). However, each of these methods has limitations. RAF ablation is an invasive technique and homogeneous heat distribution is difficult to achieve, while both photothermal and microwave are only suitable for superficial lesions due to limited tissue penetration and uneven heat distribution. Conversely, ultrasound has excellent tissue penetration with homogenous heat distribution allowing treatment of both superficial and deep lesions. The use of pulsed high-intensity focused ultrasound (HIFU) with shorter duty cycles decreases the average temporal intensities of ultrasound to produce mild hyperthermia for drug delivery in contrast to strong hyperthermia or thermal ablation (Table 2). Dromi et al. (2007) first reported the use of the thermal effects of ultrasound to deliver drug treatments for cancer. They found the use of LTSLs with ultrasound exposure led to more rapid release and higher concentration of doxorubicin (DOX), which significantly decreased tumor growth. Wu et al. (2014) demonstrated mild hyperthermia induced by ultrasound combined with liposomes increased DOX delivery to brain tumors and inhibited tumor growth. de Smet et al. (2011) reported the development of a technique termed magnetic resonance-guided HIFU (MR-HIFU) allowing homogenous heating of tissues and temperature monitor during drug delivery. They developed LTSLs co-encapsulating DOX with a magnetic resonance imaging (MRI) contrast agent and demonstrated good correlation between DOX uptake and gadolinium concentrations. Therefore, DOX release could be monitored by measuring longitudinal relaxation time. Hijnen et al. (2017) used MR-HIFU to trigger and monitor DOX delivery and demonstrated HIFU combined with LTSL resulted in deeper cellular uptake of DOX and increased drug accumulation in the

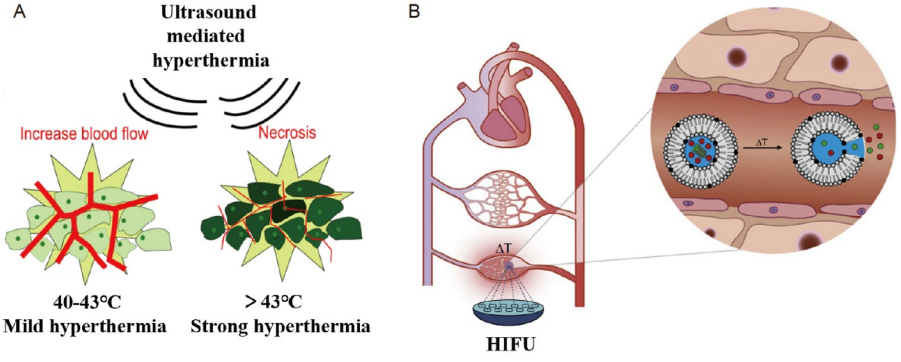

Figure 2 Mechanisms underlying ultrasound-induced thermal effects for drug delivery. A, Thermal effects on cells and tissues can enhance drug delivery (from Boissenot et al., 2016). B, Schematic of high-intensity focused ultrasound-induced thermal effects on drug release in thermo-responsive drug delivery systems (from de Smet et al., 2011).

Table 2 Ultrasound-triggered drug delivery based on thermal effects[a)]

Author/year/ reference	Type of liposome (composition/molar ratio)	US parameters					Tumor model	Results
		Frequency	Intensity/peak negative pressure/ power/voltage	PRF (Hz)	Pulse/duty cycle	Duration		
Dromi* 2007 (Dromi et al., 2007)	LTSLs	1 MHz	I_{SATA}=1300 W cm^{-2}	1	10%	2 min	Breast cancer	LTSL combined with mild hyperthermia induced by ultrasound enhanced drug delivery and reduced tumor volume
Hijnen* 2017 (Hijnen et al., 2017)	LTSLs (DPPC:HSPC: Chol:DPPE-PEG2000 =50:25:15:3, molar ratio)	1.44 MHz	35 W	NA	NA	15 min	Rhabdomyo-sarcoma	HIFU combined with LTSL led to deeper cellular uptake and higher DOX concentrations in the interstitial space
Wu* 2014 (Wu et al., 2014)	LTSLs (DPPC:MPPC: DSPE-PEG-2000=90:10:4, molar ratio)	500 kHz	0.97 MPa	NA	NA	10 min	Brain metastasis of breast cancer	LTSL combined with mild hyperthermia induced by ultrasound increased DOX delivery to brain tumors and inhibited tumor growth
Gray# 2019 (Gray et al., 2019)	LTSLs (DPPC:MPPC: DSPE-PEG-2000= 90:10:4, molar ratio)	0.96 MHz	50–140 W	NA	42%–100%	60 min	Patients with liver tumor	Demonstrated safety and feasibility of lyso-thermo-sensitive liposome combined with focused ultrasound for drug delivery
Park* 2013 (Park et al., 2013)	STLs (DPPC:DSPE-PEG-2000:cholesterol:modified ELP=55:2:15:0.4125, molar ratio)	1 MHz	12 W, I_{SATA}= 1,981.6 W cm^{-2}	5	50%	15 min for each spot, 4 spots	Squamous tumor	Tumor regression at 2 d with combined STLS and mild hyperthermia induced by ultrasound
Liang* 2015 (Liang et al., 2015)	HTSCs (CFL:DPPC: MSPC:DSPE-PEG-2000=43.25:43.25: 9.7:3.8, molar ratio)	0.5 MHz	190 mV	5,000	30%	5 min	Breast cancer	HTSCs combined with mild hyperthermia induced by ultrasound enhanced drug delivery and reduced tumor volume

a) PRF, pulse repetition frequency; NA, not available; *, preclinical studies; #, clinical studies.

interstitial space. Recently reported results of a phase I clinical trial have demonstrated the feasibility and safety of the combination of focused ultrasound (FUS) induced mild hyperthermia with LTSLs for enhanced drug delivery, with increased drug delivery with FUS exposure compared with non-FUS exposure (Gray et al., 2019).

Although LTSLs have been used in the majority of previous studies, the stability of LTSLs can be increased by incorporating other materials to further increase drug delivery efficiency. For example, Park et al. (2013) incorporated a short chain elastin-like polypeptide (ELP) into LTSLs (STLs) to enhance stability, which resulted in enhanced DOX and significant tumor regression. Liang et al. (2015) developed hybrid thermosensitive cerasomes (HTSCs) with siloxane surface framework by combination of cerasome-forming lipid (CFL) with conventional LTSLs to enhance membrane stability and optimize the efficiency of DOX delivery based on thermal effects. This study further demonstrated tumor growth was significantly inhibited by HTSCs combined with HIFU compared with traditional LTSLs combined with HIFU. In addition, Ma et al. (2020) recently developed a new material named, Cerasomal Perfluorocarbon Nanodroplets, which allowed simultaneous delivery of chemotherapeutic drugs and release of oxygen dissolved in perfluorocarbon (PFC) in response to mild hyperthermia induced by ultrasound (Figure 3A and B). The release of oxygen in response to ultrasound allowed imaging-guided drug delivery with improved delivery efficiency and therapeutic outcomes (Figure 3C and D).

Ultrasound parameters, including peak negative pressure (intensity), frequency, and duration, influence delivery efficiency. An ultrasound frequency of approximately 1 MHz is typically used as this frequency allows simultaneous deep penetration and thermal effects on targeted tissues (Boissenot et al., 2016). Various peak negative pressures have been used in previous studies; however, ultrasound-induced hyperthermia is typically detected by MRI to avoid excessive heating above 43°C. As temperature increases are correlated with ultrasound duration, longer durations are typically used to induce mild hyperthermia. There is a lack of studies regarding the relationship between ultrasound parameters and drug delivery efficiency. Therefore, more detailed studies are required to accurately evaluate the effects of ultrasound parameters on drug delivery efficiency.

Cavitation effects

During ultrasound, bubbles are compressed by positive

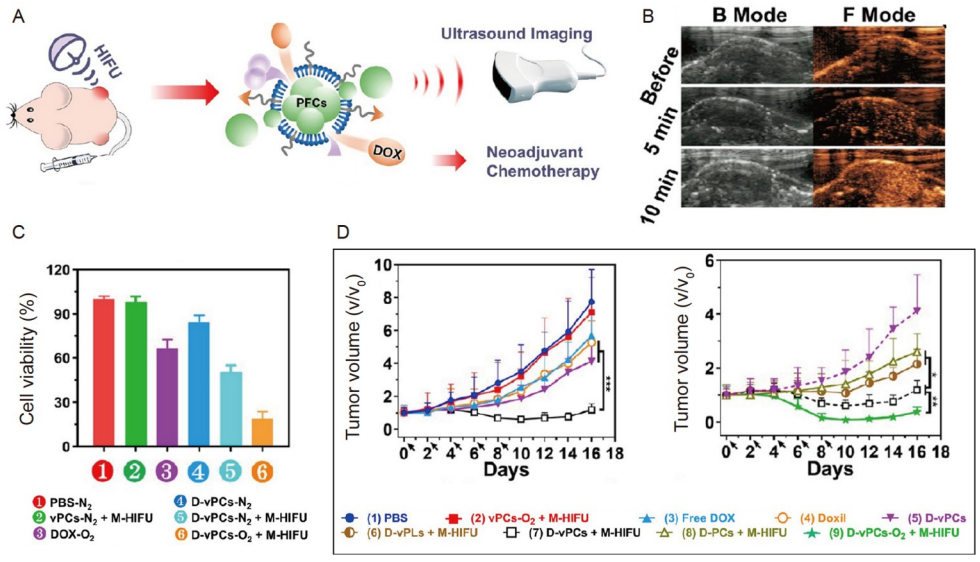

Figure 3 The application of ultrasound-triggered drug delivery based on thermal effects. A, Schematic of PFC combined with HIFU resulting in DOX and oxygen release in response to ultrasound. B, Tumors ultrasound imaging before, 5, and 10 min after M-HIFU demonstrating oxygen release. C, 4T1 cells viability detected by CCK-8 assay after different treatments. D, Changes in tumor volume of implanted 4T1 cells in response to different treatments (from Ma et al., 2020).

pressure and expand during negative pressure in a process termed cavitation. According to oscillation amplitude, cavitation can be divided into non-inertial cavitation and inertial cavitation. Non-inertial cavitation, termed stable cavitation, refers to the expansion of bubbles to a resonance size at low peak negative pressure followed by linear oscillation around this resonance size. In response to peak negative pressure increases, bubbles initially expand rapidly and then shrink to produce nonlinear oscillation, ultimately resulting in an explosion. This process is termed inertial cavitation. In the case of non-inertial cavitation (stable cavitation), oscillating bubbles generate microstreaming and Bjerknes secondary forces. During inertial cavitation, symmetrical shock waves are created when bubbles explode away from solids while unsymmetrical shock waves and liquid jet streams are created when bubbles collapse close to solids (Ahmadi et al., 2012).

Mechanism of drug delivery induced by cavitation effects

Microstreaming and Bjerknes secondary forces induced by non-inertial cavitation and shock waves induced by inertial cavitation can produce shear stress, which may be strong enough to change or disrupt the structure of micro- or nanodrug carriers allowing drug release (Chen et al., 2019) (Figure 4A). Price et al. (1998) were the first to demonstrate cavitation effects can cause microvessel rupture resulting in extravasation of erythrocytes into the interstitial space. These results indicate cavitation effects induced by ultrasound can disrupt endothelial membranes and enhance permeabilization of the cell membrane to improve drug delivery (Snipstad et al., 2018). This process can be explained by sonoporation, the mechanism by which shear stress induced by both stable and inertial cavitation effects can disrupt cell membranes and microvessels (Bekeredjian et al., 2007; Böhmer et al., 2010; Lentacker et al., 2014) (Figure 4B). Yudina *et al.* reported the duration of pore opening can be up to 24 h while other studies have reported durations ranging from seconds to minutes (Yudina et al., 2011). Other mechanisms underlying the effects of cavitation on cell membrane permeability have been posited. Intracellular reactive oxygen species may form in response to inertial or stable cavitation, which may contribute to increased cell membrane permeabilization (Lentacker et al., 2014) (Figure 4B). Local temperatures can transiently increase to 4,300–5,000 K when microbubbles explode, which may affect phospholipid bilayer fluidity and increase cell permeability (Didenko et al., 2000a; Didenko et al., 2000b; Kiesel et al., 2002). Active transportation, including endocytosis and phagocytosis, in response to cavitation effects may also enhance drug delivery (Lentacker et al., 2014) (Figure 4B).

Recent advances of ultrasound-triggered drug delivery based on cavitation effects

As microbubbles are particularly sensitive to ultrasonic mechanical forces, a large number of studies have evaluated the use of cavitation-triggered microbubbles for drug delivery (Bioley et al., 2012; Frenkel, 2008; Geers et al., 2013; Hernot and Klibanov, 2008; Meijering et al., 2009; Pitt et al., 2004; Sirsi and Borden, 2009). Microbubbles can be divided into endogenous and exogenous microbubbles, both of which have applications in drug delivery.

Endogenous microbubbles are naturally present within tissues such as gas bubbles within skin sweat ducts. When ultrasound waves exceed the cavitation threshold, micro-

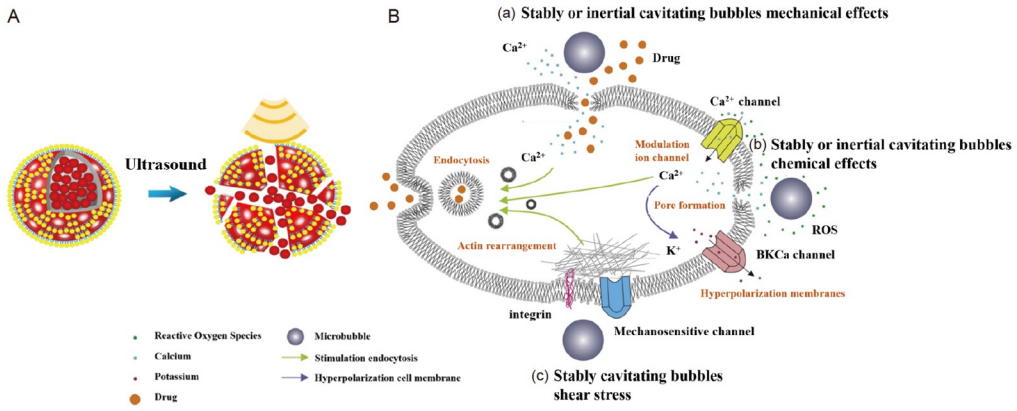

Figure 4 The mechanism of ultrasound-induced cavitation effects for drug delivery. A, Ultrasound-induced cavitation effects increase permeabilization of drug carries to enhance drug delivery (from Chen et al., 2019). B, Different effects involve in affecting the permeability of cell membrane to enhance drug delivery (from Lentacker et al., 2014).

bubbles can burst to produce cavitation effects which can be utilized for drug delivery. However, the activation of endogenous microbubbles typically requires high acoustic pressure, which may be unsafe and damage normal tissues. Accordingly, endogenous microbubbles have only been used for gastrointestinal tract and transdermal drug delivery to date. In contrast, exogenous microbubbles consist of a gaseous core (e.g., SF_6, C_3F_8) and a shell (e.g., synthetic phospholipids, proteins, or polymers) and can be used as cavitation nuclei to lower the cavitation threshold. Lower acoustic pressure is required for drug delivery as exogenous microbubbles can concentrate acoustic energy more efficiently than endogenous microbubbles. Accordingly, there is increasing interest in the use of exogenous microbubbles for drug delivery in a range of disease settings (Table 3).

Generally, there are two methods of utilizing exogenous microbubble-induced cavitation effects for drug delivery. The first is physical mixing of free drugs with microbubbles followed by co-administration. The second approach is to load drugs into microbubbles prior to administration. Greenleaf et al. (1998) first developed the method of co-administrating microbubbles with DNA to improve gene transfection efficiency in 1998. Subsequent studies have evaluated drug delivery using this method in treatments for cancer and cardiovascular diseases (Table 3). Aryal et al. (2013) demonstrated significant inhibition of tumor growth after co-administration of DOX and microbubbles followed by the application of ultrasound, indicating enhanced delivery of DOX. Xie et al. (2013) reported tissue plasminogen activator-loaded microbubbles and ultrasound significantly increased microvascular reflow following acute myocardial infarction. The first clinical trial of the co-administration approach for enhancing drug delivery was reported in 2013. The study demonstrated ultrasound combined with microbubbles improved survival of patients with pancreatic cancer (Kotopoulis et al., 2013). Carpentier et al. (2016) used a combination of ultrasound and microbubbles to delivery drugs across the blood-brain barrier (BBB) in patients with recurrent glioblastoma. They found this method was safe and well-tolerated, indicating this approach had potential utility in optimizing chemotherapy delivery to brain tissues. Unger et al. (1998) first developed the drug-loaded microbubble approach for drug delivery in 1998. They developed paclitaxel-loaded acoustically-activated microbubbles and found paclitaxel was released selectively when microbubbles were exposed to ultrasound. This technique has subsequently been applied for the treatment of a range of cancer types including liver, breast, brain, and pancreatic cancer (Table 3). In addition, cardiovascular diseases can be treated by combination of drug-loaded microbubbles and ultrasound. Hua et al. (2010) reported increased clearance of intravascular thrombus using tissue plasminogen activator-loaded microbubbles combined with ultrasound. Liu et al. (2019) demonstrated FK506-MBs combined with ultrasound targeted microbubble destruction (UTMD) increased mean survival time, reduced graft rejection, T cell infiltration, and inflammatory cytokines secretion following heart transplantation (Figure 5).

This method of co-administration has entered clinic practice faster than the drug-loaded approach, as the microbubbles (such as Definity and SonoVue) and ultrasound in co-administration methods have already approved for clinical diagnostic use. And drug-loaded microbubbles are a more recent development, further safety verification is required as this delivery method is more complex than the co-administration approach. However, drug-loaded microbubbles may represent a more efficient method of delivery than the co-administration approach as drug degradation is lower and cavitation effect is higher, thereby increasing drug delivery efficiency.

In addition to microbubble preparation and delivery method, studies have demonstrated microbubble size,

Table 3 Ultrasound-triggered drug delivery based on cavitation effects[a)]

Author/year/reference	Type of microbubble (composition/molar or quality ratio)	Approaches of delivery	US parameters					Disease model	Results
			Frequency	Intensity/ peak negative pressure	PRF (Hz)	Duty cycle	Duration		
Aryal* 2013 (Aryal et al., 2013)	Lipid MB (Definity)	Co-administration	690 kHz	0.55–0.81 MPa	1	1%	60 s	Glioblastoma tumor	DOX delivery via combination of US and microbubbles increased mean survival
Xie* 2013 (Xie et al., 2013)	Lipid MB (MRX 801)	Co-administration	1.6 MHz	MI=2 or 1	NA	NA	30 min	Acute myocardial infarction	Microvascular reflow was increased via combination of tissue plasminogen activator with microbubbles and ultrasound
Kotopoulis# 2013 (Kotopoulis et al., 2013)	Lipid MB (Sonovue)	Co-administration	1.9 MHz	0.27 MPa	5	1%	NA	Pancreatic cancer	Gemcitabine delivery via combination of US and microbubbles decreased tumor size and increased the quality of life of patients with pancreatic cancer
Carpentier# 2016 (Carpentier et al., 2016)	Lipid MB (Sonovue)	Co-administration	1.05 MHz	0.5–1.1 MPa	1	2.38%	2.5 min	Recurrent glioblastoma	Combination of microbubbles with ultrasound was safe for BBB-disruption and had potential to optimize chemotherapy delivery
Hua* 2010 (Hua et al., 2010)	Lipid MB (DPPC; DSPC; D-glucose; AT-PEG)	Drug-loaded microbubbles	2 MHz	1.8 W cm^{-2}	NA	95%	10 min	Thrombosis	Drug-loaded microbubbles combined with ultrasound resulted in greater thrombolysis with lower doses
Kang* 2010 (Kang et al., 2010)	Lipid MB (DPPC:DPPE: DDPA=5 mg: 2 mg:1 mg)	Drug-loaded microbubbles	3,000 kHz	2 W cm^{-2}	NA	50%	6 min	Liver tumor	Docetaxel-loaded microbubbles combined with US inhibited liver tumor growth and promoted apoptosis
Tinkov* 2010 (Tinkov et al., 2010)	Lipid MB (DPPC:DPPG: DPPE-PEG2000=1.74 mg: 0.45 mg: 0.08 mg)	Drug-loaded microbubbles	1.3 MHz	1.2 MPa (MI=1.6)	NA	NA	NA	Pancreatic cancer	DOX-loaded microbubbles combined with US increased DOX delivery and inhibited tumor growth
Eisenbrey* 2010 (Eisenbrey et al., 2010)	Polymer MB (PLA:camphor=0.5 g: 0.05 g)	Drug-loaded microbubbles	5 MHz	MI=1.0	1,000	NA	20 min	Liver tumor	Combination of DOX-loaded microbubbles and US resulted in decreased hepatic delivery of DOX but increased DOX delivery to tumor tissues
Ting* 2012 (Ting et al., 2012)	Lipid MB (DPPC: DSPE-PEG-2000=19:1, molar ratio)	Drug-loaded microbubbles	1 MHz	0.7 MPa	5	5%	NA	Glioblastoma multiforme	1,3-bis(2-chloroethyl)-1- nitrosourea (BCNU)-loaded microbubbles with US controlled tumor progression and improved mean survival
Li* 2012 (Li et al., 2012)	Lipid MB (DPPC:DPPA: DPPE-PEG2000=47:10:5, molar ratio)	Drug-loaded microbubbles	1 MHz	2 W cm^{-2}	NA	50%	6 min	Liver tumor	10-hydroxycamptothecin-loaded microbubbles combined with US resulted in higher drug accumulation and increased tumor inhibition compared with 10-hydroxycamptothecin-loaded microbubbles or 10-hydroxycamptothecin
Deng* 2014 (Deng et al., 2014)	Lipid MB (DSPC:DSPE-PEG2000:DSPE-PEG2000-biotin=9:0.5:0.5, molar ratio)	Drug-loaded microbubbles	1 MHz	1.65 W cm^{-2}	NA	20%	15 s	Breast cancer	Drug delivery, cell uptake, and decreased drug efflux with DOX liposome-loaded-microbubbles plus US leading to increased cytotoxicity
Liu* 2019 (Liu et al., 2019)	Lipid MB (DSPC:DSPE-PEG2000=9:1, molar ratio)	Drug-loaded microbubbles	1 MHz	2 W cm^{-2}	NA	50%	2 min	transplanted heart	Enhanced drug delivery, decreased graft rejection, and improved survival

a) PRF, pulse repetition frequency; NA, not available; *, preclinical studies; #, clinical studies.

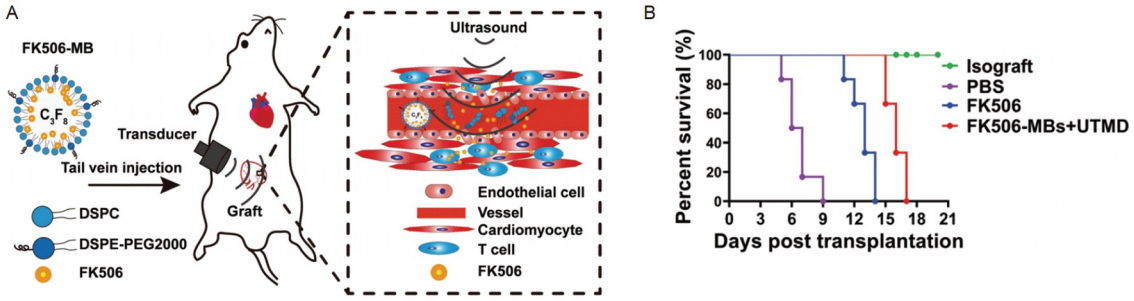

Figure 5 The application of ultrasound-triggered drug delivery based on cavitation effects. A, Schematic illustration for the structure of FK506-MBs and the therapeutic process using UTMD *in vivo*. B, Survival time of cardiac grafts. Mean survival was significantly longer in the FK506-MB+UTMD group (16.00 d±0.89 d) compared to the PBS group (6.66 d±1.36 d) and FK506 group (12.83 d±1.17 d;n=6) (from Liu et al., 2019).

concentration, and shell composition can affect the drug delivery efficiency of microbubbles. Song et al. (2015) demonstrated small-diameter (2 μm) microbubbles allowed greater drug delivery than large-diameter (4 and 6 μm) microbubbles. Low concentrations of microbubbles may be more unstable and have lesser effects on tissues or cells while higher concentration of microbubbles can cause tissue damage (Song et al., 2015). Typically, 10^7–10^8 microbubbles/milliliter are used in preclinical studies. The shell composition of microbubbles is another important factor for drug delivery. Lipid shells are softer and more sensitive to oscillation and induce greater cavitation effects during ultrasound sonication compared to other shell components such as proteins and polymers. Böhmer et al. (2010) demonstrated ultrasound combined with lipid-shelled microbubbles improved Evans blue delivery compared with polymer-shelled microbubbles in murine colon cancer. Therefore, lipid-shelled microbubbles have more favorable characteristics for drug delivery via increased cavitation effects.

Ultrasound parameters, including the number of cycles per ultrasound pulse (pulse repeat frequency or duty cycle), peak negative pressure (intensity or mechanical index), and frequency and duration of ultrasound, also affect delivery efficiency. The wide range of ultrasound parameters used in previous studies limit the comparison among differnt techniques (Table 3). In most drug delivery studies utilizing cavitation effects, ultrasound with a frequency of approximately 1 MHz is often used for microbubbles with a diameter of 1–3 μm. This frequency corresponds to the resonance frequency of microbubbles which is dependent on size, thereby producing improved cavitation effects. Ultrasound pressures differ substantially in different studies. Some studies have used higher acoustic pressures (550–1,200 kPa) for drug delivery while others have used lower acoustic pressures (270–400 kPa). Microbubbles may undergo inertial cavitation at acoustic pressures above 500 kPa. In addition, some studies have reported ultrasound intensity rather than ultrasound pressure. Generally, ultrasound intensities for drug delivery are in the range of 1.65–2 W cm^{-2}. However, higher intensities may be applied when pulse length and/or pulse repetition frequencies are decreased, leading to lower duty cycles (pulse length×pulse repetition frequency) and temporal average intensity (duty cycle×ultrasound intensity).

Acoustic radiation force

Acoustic radiation force (ARF) refers to the physical phenomenon of momentum transfer to the transmitting medium during propagation of ultrasound waves (Dayton et al., 2002). Acoustic streaming produced by ARF can increase the efficacy of convective transport.

Mechanism of drug delivery induced by ARF

ARF can push drug carriers against tumor vessel walls resulting in longer retention times and higher tumoral drug accumulation (Palmeri et al., 2006; Tian et al., 2020) (Figure 6A). In addition, ARF causes tissue displacement leading to increased drug extravasation and interstitial penetration (Frenkel, 2008). Frenkel et al. demonstrated ARF can open the intercellular space between endothelial cells, indicating its ability to improve drug delivery by increasing the permeability of adjacent cells to allow penetration of drug carriers (Dasgupta et al., 2016; Frenkel et al., 2000) (Figure 6B). In addition, ARF can enhance microbubble delivery to the surface of target blood vessels, thereby decreasing the dose of microbubbles required (Dayton et al., 1999). Therefore, ARF and cavitation effects may have a synergistic effect on enhancing drug delivery while reducing damage to normal tissues.

Recent advances in ultrasound-triggered drug delivery using acoustic radiation force

In addition to thermal and cavitation effects, several studies have demonstrated acoustic radiation forces can push nanoparticles toward vessel walls and induce shear stress to open cell junctions, thereby increasing cell permeability and improving penetration into tight tissues (Lum et al., 2006).

Oerlemans et al. (2013) encapsulated lipophilic (Nile

Red, NR) dyes into TSLs and NTSLs (non-temperature sensitive liposomes) and evaluated release kinetics in response to FUS. Surprisingly, continuous wave-HIFU (CW-HIFU) exposure led to greater release of NR from TSL than from NTSL. NR release from NTSL increased from 30% to 70% after 30 min of pulsed wave-HIFU (PW-HIFU) exposure. However, adding microbubbles to liposomes before PW-HIFU exposure did not increase NR release. These results indicate thermal and cavitation effects do not represent the predominant mechanism underlying NR release. Therefore, the researchers posited the acoustic radiation force may explain the release of NR from the lipid bilayer.

The mechanisms underlying the effects of the acoustic radiation force on drug delivery have yet to be fully elucidated due to a lower number of reported studies compared to those investigating thermal and cavitation effects (Ciancia et al., 2020; Kilroy et al., 2012; Lum et al., 2006). Accordingly, more extensive and detailed studies regarding the underlying mechanisms, optimal parameters, and most effective delivery strategies for ultrasound-triggered drug delivery using acoustic radiation forces are required.

Ultrasound can produce thermal effects, cavitation effects, and RAF, which can be regulated by adjusting various technical parameters (Table 4). Thermal effects are suitable for drug delivery using thermosensitive liposomes while cavitation effects are favorable for drug delivery using microbubbles. ARF can be combined with cavitation effects to enhance microbubble delivery to the surface of target blood vessels, thereby decreasing the dose of microbubbles required (Dayton et al., 1999). Therefore, particular effects or combinations of multiple effects may be required to meet specific clinical needs and maximize drug delivery efficiency.

Safety considerations

The use of ultrasound-triggered drug delivery has yet to be approved for clinical use. There are currently three clinical trials in progress, one utilizing thermal effects and two utilizing cavitation effects induced by ultrasound. Attempts should be made to limit unwanted biophysical effects that may be associated with worse clinical outcomes in order to expedite the development of these techniques for clinical practice.

Internal organs adjacent to target lesions may be damaged by scattered or reflected ultrasound energy. For example, sciatic nerve damage was observed after ultrasound treatment of the uterus. The lungs and digestive tract are not

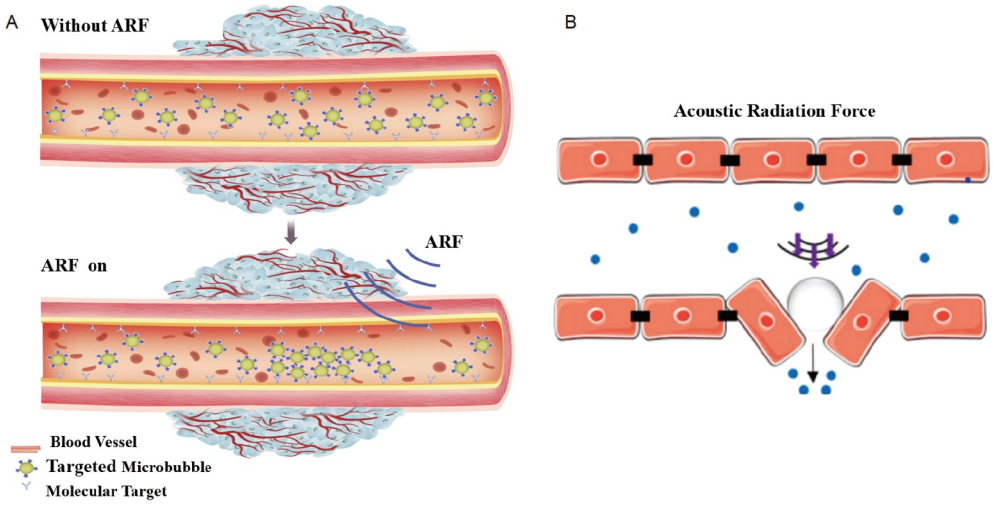

Figure 6 Mechanism underlying ultrasound-induced ARF for drug delivery. A, ARF can push drug carriers against tumor blood vessel walls (from Tian et al., 2020). B, ARF opens the intercellular space between endothelial cells to enhance drug delivery (from Dasgupta et al., 2016).

Table 4 Summary of effects induced by ultrasound for drug delivery

Type of effects	Type of applicable drug delivery systems	Characteristics of ultrasound parameters	Current status
Thermal effects	Thermosensitive liposomes	Prolonged duration of ultrasound (predominantly greater than 5 min)	One clinical study (Gray et al., 2019)
Cavitation effects	Microbubbles	Shorter duty cycle and duration of ultrasound	Two clinical studies (Carpentier et al., 2016; Kotopoulis et al., 2013)
ARF	Microbubbles	Low negative pressure	Preclinical studies (Ciancia et al., 2020; Kilroy et al., 2012)

suitable for ultrasound-induced drug delivery due to the risk of severe cavitation effects resulting from the presence of gas in these organs. There is concern regarding the risk of skin damage as air bubbles present in the skin may induce cavitation when exposed to ultrasound. Therefore, targeting superficial lesions such as breast cancer without injuring overlying skin is technically challenging. In addition, unwanted biophysical effects may also be induced in organs such as the skull due to acoustic shadowing from overlying bones. Inertial cavitation can cause significant temperature elevation in the range of 4,300–5,000 K causing thermal injury in adjacent tissues (Didenko et al., 2000a). Further, temperatures in this range may produce reactive free radicals (Duco et al., 2016). The observed variability in drug concentrations following mild hyperthermia is partly due to heterogeneity in tissue temperatures as drug release from thermosensitive carriers is particularly sensitive to small temperature changes. Accordingly, these issues may limit the clinical applications of this technique.

Strategies to optimize ultrasound-triggered drug delivery

Strategies to prevent unwanted biophysical effects and improve the efficacy and safety of ultrasound-triggered drug delivery include optimization to ultrasound parameters, ultrasound devices, and ultrasound-responsive delivery systems.

Ultrasound parameters can be optimized to avoid unwanted biophysical effects during therapy. The application of thermal effects for drug delivery requires uniform temperature within target tissues and temperatures to be monitored and maintained below 43°C. High intensities and long durations of ultrasound are typically required to generate thermal effects. When cavitation effects are used for drug delivery, the ultrasound frequency used should correspond to the resonance frequency of microbubbles, which is dependent on microbubble size. In addition, higher acoustic pressures can cause inertial cavitation while lower acoustic pressures induce stable cavitation. To avoid unwanted thermal effects when using cavitation effects, the mechanic index (MI) is usually set between 0.2 and 1.9. Regarding device design, FUS machines equipped with MRI functions can monitor temperature and may improve drug delivery by maintaining homogenous tissue temperatures below 43°C. Poor penetration of ultrasound through the skull can be mitigated by the development of new devices that can implanted directly into brain as reported by Carpentier et al. (2016). Ultrasound-responsive delivery systems should maintain constant circulating serum concentration and maintain responsiveness to ultrasound to control drug release at the target site. Accordingly, delivery systems should be carefully tailored to have specific physical or chemical properties that optimize ultrasound-induced drug delivery.

Conclusions and future perspectives

In conclusion, ultrasound can induce a range of biophysical effects, including thermal effects, cavitation effects, and acoustic radiation forces, which can affect drug carriers and tissues to enhance drug delivery. Numerous studies have investigated the utilization of the biophysical effects induced by ultrasound for drug delivery in cancer, cardiovascular disease, and neurological disorders. Although great progress

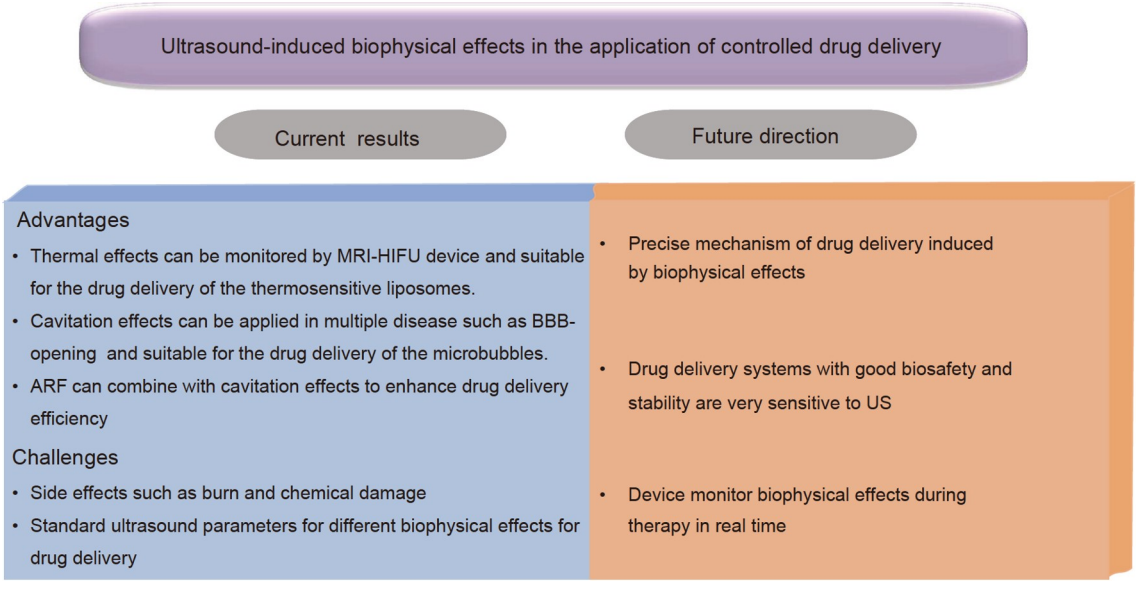

Figure 7 (Color online) Summary of recent advances and future research directions for ultrasound-triggered drug delivery.

has been made in ultrasound-triggered drug delivery in recent decades, significant progress is still required to establish the use of this technique in clinical practice. There is a need for new guidelines regarding balancing the risks and benefits of this novel technique in specific disease conditions. Further, the precise mechanisms underlying the biophysical effects induced by ultrasound for drug delivery have yet to be fully elucidated. For example, the duration and size of cell membrane defects induced by cavitation should be clarified to optimize therapeutic duration. Optimization of ultrasound parameters, ultrasound devices, and ultrasound-responsive delivery systems is required to maximize drug delivery efficacy. In addition, functional devices allowing monitoring of thermal effects, cavitation effects, and acoustic radiation forces in real-time are required to allow timely adjustment of ultrasound parameters to ensure patient safety and improve the efficiency of ultrasound-induced drug delivery (Figure 7).

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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