Triggered Drug Release and Enhanced Drug Transport from Ultrasound-Responsive Nanoparticles

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1 Introduction

There have been major advances in the ability to discover and develop novel drugs across nearly all diseases and drug classes [1–3]. However, for most fatal diseases, such as cancers, cardiovascular diseases, and neurological disorders, the therapeutic agents typically used are effective in treating the diseased tissue, but are either excessively toxic [4–7] or poorly distributed within the diseased tissue [8–11]. These limitations in drug delivery have impacted all routes of transport, such as: oral, nasal, aerosol, transdermal, and systemic. Each of the aforementioned drug delivery routes has their own associated set of challenges and opportunities. However, the scope of this chapter is focused on systemic drug delivery because it is one of the most widely used means of delivering a drug.

Efficacious yet highly toxic and nonspecific drugs often have limited bioavailability and distribution within diseased tissue [12]. These physiological challenges are not unique to specific diseases, but are present across cancers, cardiovascular lesions and occlusions, and the brain [13], and are therefore drug-class-agnostic. The need to overcome these challenges has resulted in a substantial increase in research devoted to techniques that promote site-targeted delivery and enhanced distribution of therapeutics. Many research groups have approached this need through a variety of "passive" and "active" drug delivery techniques. In this chapter, we focus on active processes that promote drug delivery in response to an ultrasound field.

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2 Applications of Drug Therapies

2.1 Cancer

Solid tumors represent a highly challenging environment for drug delivery, because of the chaotic vasculature, enhanced intratumoral pressure, dense extracellular matrix, and increased distance between a cancerous cell and the nearest blood vessel [14]. Tumors also present an unusual drug delivery opportunity by virtue of the leaky endothelial gaps that are typically present: this implies preferential accumulation or passage of therapeutics in the range 100–300 nm. Active delivery mechanisms typically have three roles to play in this context: enable increased extravasation of the therapeutic from the blood stream into the tumor, permit triggered release of the therapeutic at the tumor site only, and mediate improved transport and distribution of the therapeutic throughout the tumor mass.

Conventional chemotherapeutics include small molecular drugs, such as taxanes (e.g., paclitaxel [15]), anthracyclines (e.g., doxorubicin [16, 17]), and cytosines (e.g., arabinoside [18, 19]), which typically circulate well and have considerable diffusivity in tumors. However, all of these drugs are nonspecific, and are therefore highly cytotoxic to both healthy and cancerous tissues. In this context, ultrasound-mediated delivery could enable site-specific triggered release, as well as potentially enhance the penetration distance of the therapeutic from the perivascular space into the tumor mass.

Beside small molecular drugs, there is now an increasing trend towards using biologics, such as oncolytic viruses, peptides, and antibodies, to achieve more targeted cancer therapy. Oncolytic viruses selectively infect and kill cancer cells: although there are relatively few in the clinic, the first candidate for melanoma was recently approved by the FDA and EMA (T-Vec, Amgen) [20]. Peptides are typically used to block the production of vascular endothelial growth factor (VEGF) and prevent the proliferation of blood vessels [21]. Antibodies act by a wide range of mechanisms, with the most recent developments focusing on targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) [22] and the programmed cell death protein 1 pathway (PD-1/PD-L1) [23, 24].

Increased specificity does, however, come at the cost of greatly increased size and possibly much faster clearance in the systemic circulation. Peptides are typically on the order of 5 nm, antibodies are on the order of 10 nm and viruses range in size from 100 to 200 nm. The challenge in delivering these agents is therefore twofold. Their increased size implies greatly decreased extravasation and penetration into the tumor mass. Secondly, the very short half-life of agents, such as viruses, means that there is a very short time (on the order of 10 min) over which to convert a systemically administered dose into a therapeutic dose in the tumor. Once again, active delivery by ultrasound could facilitate both of those aspects.

2.2 Cardiovascular Diseases

Currently, there are several means to treat cardiovascular diseases. Treatments typically rely on regular doses of statins. However, for more acute instances of cardiovascular disease, treatments rely on the protein tissue plasminogen activators (tPA) to dissolve the occlusion and allow increased blood flow [25]. As a result, tPA therapies are used to treat embolisms, myocardial infarctions, and stroke that result from clot formations [26]. The drug catalyzes the enzymatic degradation of fibrin, a primary protein within clots. This drug, however, is very potent and nonspecific, and often needs to be delivered to the target under conditions of low or no blood flow. As a result, off-site bleeding is a substantial problem [27] and often prohibits some patients from this therapy. Active delivery mechanisms thus have a significant role to play in terms of enhanced transport, and improved specificity through triggered release.

2.3 Neurological Disorders

There is a wide range of therapeutics currently under investigation for the treatment of neurological disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Viruses, peptides, and other biologics have been utilized to help break down damaging plaque formations, influence neurotransmission, or enable the innate immune system to play a role in AD therapy [28]. Likewise, small drug molecules [29–31] and adenoviruses [32] have also been applied to PD. These therapeutic agents have shown great promise in treating these neurological disorders, as indicated by many of them entering into phase 1 and 2 clinical trials. However, many of these strategies rely on the passage beyond the blood–brain barrier (BBB) [33]. This is the key challenge for delivery of these classes of agent, and ultrasound has a key role to play both in reversible opening the BBB and helping transport agents across it.

3 Ultrasound for Drug Delivery and Transport

Ultrasound is a non-ionizing, non-destructive sound wave operating at frequencies above 20 kHz. These mechanical waves easily propagate through the human body, but are obstructed by bones or large gas cavities. Furthermore, the acoustic wave can be focused similarly to lens focusing of light [34], which allows for diseased tissue-specific targeting without harming nearby healthy tissue. It is therefore ideal for many diagnostic and therapeutic applications [35]. In the context for ultrasound-mediated drug delivery with nanoparticles, we broadly characterize the mechanisms of action from ultrasound into thermal or mechanical effects (Fig. 1).

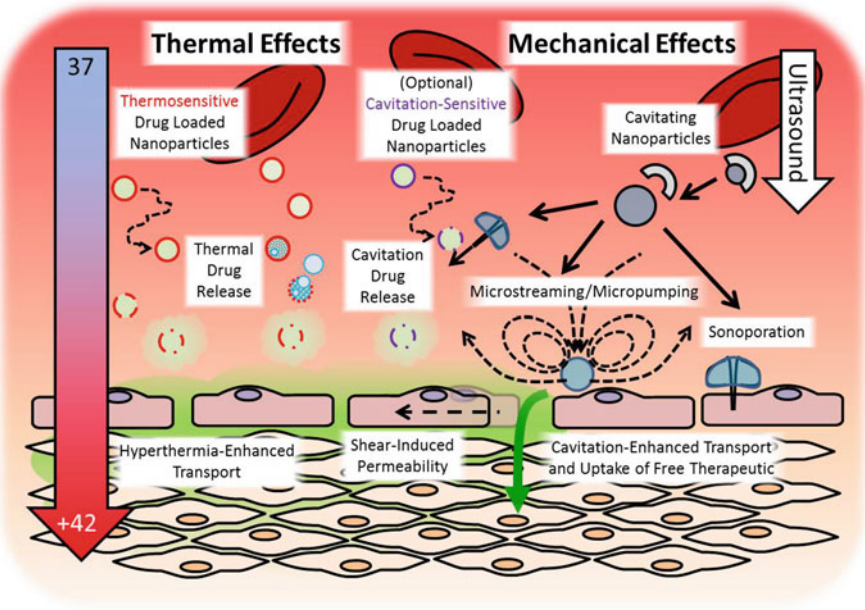

Fig. 1 Schematic of different mechanisms of ultrasound-mediated drug release, drug transport, and sonoportation from ultrasound-responsive nanoparticles

As an acoustic wave travels through a medium it is attenuated through reflection, scattering, and absorption [36]. Acoustic energy that is absorbed is converted to heat [35]. Ultrasound is thus one of the only modalities capable of generating highly localized mild hyperthermia (39–43 °C) at depth within the body. The resulting temperature rise can be monitored noninvasively using either ultrasound or MRI-based techniques, with MR-thermometry being most commonly used clinically in spite of its significant cost and limited spatiotemporal accuracy [37–40].

Ultrasonic waves are also capable of imposing mechanical effects, such as acoustic radiation force or cavitation. Acoustic radiation forces are the time averaged net force in the direction away from the ultrasound source [41]. Acoustic cavitation is the dynamic response of a gas and/or vapor cavity (i.e., a bubble) to an oscillating acoustic pressure amplitude [42]. However, bubble nucleation using ultrasound alone requires large pressure amplitudes [43]. In order to reduce the pressure amplitudes necessary for cavitation, cavitation nuclei are typically introduced via intravenous injection, and take the form of either microbubbles, also known as ultrasound contrast agents, or nanoscale cavitation nucleation agents [44, 45]. Occurrence of cavitation can be detected and monitored remotely through a technique known as passive cavitation detection, or passive acoustic mapping, whereby narrowband or broadband acoustic emissions arising from cavitating bubbles are remotely sensed [46, 47]. Acoustic cavitation has had significant impact on ultrasound based therapies [48–50] such as ultrasound-enhanced drug delivery, which is the focus of this chapter.

Non-inertial cavitation is the periodic oscillatory radial motion of a bubble, and is dependent on the size [51] and composition of the bubble [52]. This periodic motion perturbs the surrounding fluid over microsecond time scales, generating micro-streaming that results in convective transport of particles trapped in the currents [53–55], open up tight junctions between endothelial cells [56], disrupt cell membranes [57], and induce intercellular and intracellular bioeffects [58, 59]. Inertial cavitation occurs when the peak negative pressure amplitude becomes large enough to cause the bubble to unstably grow, and subsequently collapse during the positive pressure phase due to the inertia of the surrounding liquid. During the collapse phase of the bubble, jets, bubble fragments, and other asymmetric bubble shapes are often formed. The collapses emit shock waves that are detectable as broadband signals [60], which are useful for imaging techniques, such as passive acoustic mapping [61, 62]. As the collapses themselves can be periodic [63], inertial cavitation is also capable of generating micostreaming, along with the associated convective mass transport and bioeffects. As a result, inertial cavitation is a key enabler and facilitator in drug delivery.

We now describe nanoparticulate strategies that exploit the thermal and cavitational effects of ultrasound for ultrasound-triggered release, enhanced transport into biological targets, or improved delivery of therapeutics across the cellular membrane.

4 Ultrasound Triggered Drug Release from Nanoparticles

Drugs contained within biocompatible materials (e.g., lipids and polymers) to make drug-loaded nanoparticles increase disease specificity and reduce systemic toxicity of the encapsulated drug [64–67]. These drug delivery strategies are dependent on the size and composition of the drug-loaded nanoparticle [68]. However, it has also been shown that despite increased accumulation of drug-loaded nanoparticles, there is no associated increase in drug delivery within the tumor even when attempts to normalize the tumor environment are made [12, 69]. Without an external trigger to release the contents of the drug-loaded liposome, there remains a diffusive barrier of the encapsulation that prevents the bioavailability of the drug. In order to overcome these barriers, there has been a quest to develop external triggers to spatially and temporally release drugs from these nanoparticles, in order to achieve reduced systemic toxicity, enhanced drug accumulation, and improved bioavailability. Here we look at several designs of nanoparticles that release their payload in the presence of ultrasound.

4.1 Hyperthermia-Triggered Drug Release with Ultrasound

One of the key attributes for a well-designed drug delivery vehicle is incorporating a means to trigger the release of its contents. Encapsulating strategies that degrade in the presence of elevated temperatures have thus attracted considerable effort

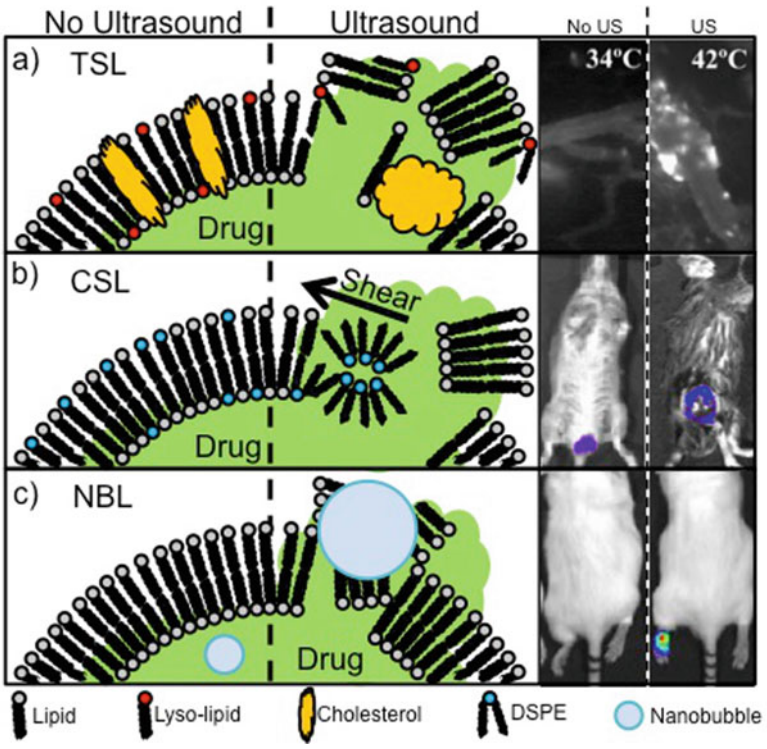

Fig. 2 Schematics and application of different ultrasound-mediated mechanisms for drug release from liposomal nanoparticles. (**a**) A cartoon of thermally sensitive liposomes (TSL) is shown before and after ultrasound exposure. Snapshots of blood vessels that indicate extravasation of a chemotherapeutic from thermosensitive liposomes before and after exposure to ultrasound-induced hyperthermia indicate presence of drug [74]. Image adapted with permission. (**b**) The mechanism of drug release from cavitation sensitive liposomes (CSL) upon exposure to ultrasound. In the presence of cavitation localized in a tumor demonstrates enhanced luciferin expression [100]. Image adapted with permission. (**c**) Drug release from nanobubble liposomes (NBL) is shown. Xenografted tumors in the foot of a mouse treated with NBL is shown to have increased luciferin expressions upon exposure to ultrasound [134]. Image adapted with permission

and interest. These thermosensitive drug delivery vehicles utilize heat-sensitive lyso-lipids, polymers, peptides, reactions, or a combination thereof in the shell of the vesicle (Fig. 2a). Here, we give an overview of the recent advances in thermosensitive drug delivery strategies that utilize ultrasound as the heat source.

Early work on thermosensitive liposomes primarily relied on the phase transition of the encapsulating material. For example, liposomes comprised of a combination of lipids with acyl chain lengths between 16 and 18 carbons will result in a leaky membrane between 41 and 54 °C, the phase transition temperatures of the respective lipid [70, 71]. Such mild hyperthermia is easily achievable with an extracorporeal ultrasound device. However, the effectiveness of such liposomes is not evident until more severe degrees of hyperthermia (>45 °C).

A key innovation towards liposomal drug delivery was the incorporation of temperature-sensitive lyso-lipids into the lipid bilayer shell [72]. These lyso-lipids begin to break down at temperatures around 39–40 °C, achieving maximum release at 41 °C [73]. A lyso-thermosensitive liposome (LTSL) formulation has been used to encapsulate doxorubicin [74] and is now in human trials under the trade name Thermodox (Celsion, USA) [75]. Subsequently, there has been continued work to develop other formulations [76–80] as well as demonstrate the capacity to trigger release from ultrasound-induced hyperthermia [81].

Other researchers have successfully encapsulated other chemotherapeutics into a thermally sensitive liposome. For example, cisplatin (a potent therapeutic for solid tumor present in head and neck, genitourinary, and lung cancers) was encapsulated in a formulation comprised predominately of hydrogenated soybean phosphatidylcholine and cholesterol [82]. The addition of a polyethylene glycol lipid enabled the liposome to be shielded from the innate immune response [83]. Unfortunately, this particular formulation has shown poor therapeutic efficacy despite the long circulation time, which has been shown to preferentially accumulate in tumors that have leaky blood vessels [84, 85]. Schroeder et al. [86] showed that when exposed to low frequency ultrasound (20 kHz), the STEALTH liposomal cisplatin released their contents in an in vivo murine model. Though no temperature or cavitation measurements were taken, Schroeder attributed their results to the large quantities of cholesterol in the shell of the liposome. Researchers have also utilized this shell composition to encapsulate other chemotherapeutics, such as 5-fluorouracil [87].

Instead of using lyso-lipids for temperature sensitivity, other researchers have added thermosensitive polymers to encapsulate drugs [88–90]. One polymer in particular has garnered much attention owing to its sensitivity to temperatures around 40 °C. *N*-isopropylacrylamide (NIPAM) is a polymer that undergoes a reversible phase transition between 32 and 40 °C, depending on the polymer chain length. This phase transition changes the hydrogel structure of NIPAM to a collapsed dehydrated state, losing up to 90 % of its initial volume. As a result, this polymer allows a change in the shell morphology, opening up pores, and allowing for drug release.

An alternative to changing the properties of the liposome shell for drug release is to instead generate bubbles from within the liposome. To do so, researchers have added ammonium bicarbonate to the liposomal core [91, 92]. This chemical decomposes to form ammonia, water, and carbon dioxide bubbles at temperatures above 36 °C. However, within the liposome, researchers have shown that the temperature sensitivity of the bubble generating liposome does not occur until temperatures above 40 °C. Once a carbon dioxide bubble is generated, the sudden expansion in volume disrupts the lipid membrane. This disruption opens pores within the membrane or ruptures it entirely, releasing the payload. Furthermore, these bubbles can be imaged using ultrasound, giving a clear indication of delivery.

Another means of bubble generation from a heat source is the use of gases, such as perfluorobutane and perfluorpentane, which have relatively low boiling points. Researchers have shown that this bubble may be stored initially as a meta-stable liquid. Once a liquid droplet is formed, interfacial forces enable these chemicals to

remain as a liquid in elevated temperatures (such as those inside the body) [93, 94]. Heat from an ultrasound source is capable of temporarily disrupting this equilibrium, forcing the liquid to phase change into a gas. Because many of these gases are hydrophobic, researchers have dissolved hydrophobic drugs (such as many taxane-based chemotherapeutics [95–97]) into the nanodroplets. Upon ultrasound triggered phase-change, these droplets instantaneously release the therapeutic agents into the surrounding medium.

Though there are substantial advantages of triggered drug release from heat-sensitive nanoparticles, this technology suffers from the inability to monitor heat deposition. There are currently a limited number of techniques to noninvasively monitor temperature during treatment. The methods that are currently in use (MRI guided thermometry) are slow (i.e., not in real time) and expensive. Furthermore, current thermometry techniques are fairly inaccurate. Such inaccuracies may mean the difference between no-treatment and complete treatment. Thus the key challenge in heat-sensitive technologies lies not in the nanoparticle development, but instead with techniques to monitor the success of therapy in a safe and cost-effective way.

4.2 Mechanically Triggered Drug Release with Ultrasound

In order to avoid the imaging challenges presented by heat-based drug release therapies, there has been a surge in the utilization of mechanical means to disrupt drugs encapsulated by lipids, peptides, or polymers because these can potentially be more readily monitored by ultrasound. The goal of stimulus-responsive drug carriers is to release a drug in the presence of an externally triggered event. Similar to the heat-sensitive liposomes discussed earlier, researchers have also developed a mechanical energy analogue that exploits inertial cavitation to rupture the lipid bilayer.

In order to create a lipid shell that is sensitive to cavitation shockwaves, researchers used a lipid that has the propensity to change its solid phase structure in the presence of shear forces. Distearoyl-sn-glycero-3-phosphatidylethanolamine (DSPE) forms a lamellar gel structure in ambient conditions [98, 99]. In the presence of a shockwave, the structure of the solid phase transitions from a gel to an inverted hexagon. This change in structure destabilized the lipid bilayer, allowing for an abrupt release of the encapsulated payload. Cavitation-sensitive liposomes have recently been developed to break apart in the presence of a shockwave induced by inertially cavitating bubbles [100]. In the presence of artificial cavitation nuclei (in this case SonoVue microbubbles), these liposomes achieved close to 100 % release at peak rarefactional pressures on the order 1.5 MPa at 0.5 MHz. This represents a fraction of the pressure amplitude typically required to release thermosensitive liposomes (>4 MPa at 1 MHz) and is an operating regime that is potentially achievable by conventional diagnostic ultrasound scanners rather than highly specialized and expensive high intensity focused ultrasound systems.

Cavitation-sensitive liposomes require the proximity of artificial cavitation nuclei to generate the shockwave. These cavitation nuclei can range in size from as large as 5 μm to as small as 200 nm [101], with both types of nuclei having successfully

demonstrated release As a result, the source of cavitation may have different pharmacokinetics depending on the size of the cavitation nuclei. To avoid the need for secondary nanoparticles, researchers have developed echogenic liposomes.

Unlike cavitation sensitive liposomes, echogenic liposomes are hypothesized to house nanoscopic gas pockets in the hydrophobic layer, either in the lipid bilayer shell or a micelle within the liposome. Upon exposure the ultrasound, the nanobubbles reportedly cavitate and destroy the integrity of the shell. Once broken, the contents of the liposome are released. Suzuki et al. [102, 103] successfully demonstrated improved luciferase coding plasmid DNA transfection to tumors from echogenic liposomes only in the presence of ultrasound. In addition to gene transfection, echogenic liposomes have also encapsulated tissue plasminogen activator in order to improve thrombolysis therapies [104–107]. Other hydrophilic and lipophilic therapeutics have also been encapsulated by echogenic liposomes [108].

Others have reported a polymeric nanobubble with a coating of a gene-loaded micelle [109]. These nanobubbles are capable of scattering ultrasound similar to microbubbles. These nanobubbles also demonstrated sustained acoustic response greater to that of gas-core liposomes. Moreover, in vivo survival studies with murine tumor models indicated that tumor volumes treated with ultrasound and gene-loaded polymeric nanobubbles were significantly more effective at controlling or reversing tumor growth.

5 Ultrasound-Enhanced Drug Transport from Nanoparticles

One of the key limitations of many drug therapies is not only their nonspecificity (which was addressed earlier), but also their inability to access tissue far beyond blood vessels. Such a challenge exists across all drug classes and has hindered the capacity to treat diseases, such as neurological disorders and cancer. To combat this challenge, many treatments rely on elevated drug doses that often come with severe side effects. This is perhaps best reflected in cancer treatments whereby the tumor itself hinders the passage of drug beyond 20–50 μm from a blood vessel [12]. The stunted travel distance of even small drug molecules often results from the tumor physiology, despite the leaky vasculature of a tumor. Moreover, physiological barriers, such as the tight junctions of the blood–brain barrier, preclude the use of nearly any drug administered simply by intravenous injection. It is therefore crucial that new generations of nanomedicines address this concern, and focus on methods to promote not only drug specificity but also its distribution within the diseased tissue.

To address this challenge without surgical intervention, elevated drug doses, or resorting to palliative care, researchers have looked towards ultrasound as a modality to interact with deep tissue. As mentioned in the previous sections, we have shown that nanoparticles that respond to ultrasound have been implemented for site-specific drug release. Note that the methodologies to be discussed are applicable to freely circulating drugs. This is an important distinction because the encapsulation of a drug presents a substantial regulatory and financial challenge.

Moreover, we want to distinguish between cellular transport (i.e., sonoporation), which is discussed in another section, and transport to extravascular tissue. Below, we show the capacity and advantages for nanoparticles to enhance the transport of therapeutics into a targeted tissue beyond blood vessels.

5.1 *Improved Transport from Ultrasound-Induced Hyperthermia*

There has been considerable effort in using ultrasound-induced hyperthermia to remotely trigger drug release from nanoparticles (as seen in our earlier section). Furthermore, there is evidence that hyperthermia itself enhances cell permeability, improving the efficacy of cellular drug transport [110–112]. It has been suggested that heat improves circulation in tissue with a dense microvasculature such as tumors, thereby improving local drug concentrations. However, recent work has demonstrated that hyperthermia does the opposite; blood-flow decreases due to the arteriolar–venular pressure gradient [113]. Thus, the exact mechanisms for enhanced transport of therapeutics from ultrasound-induced hyperthermia are still unclear.

Because the source of the heat is irrelevant, ultrasound provides a noninvasive means to locally increase temperatures in deep tissue, allowing for increased uptake of drugs such as monoclonal antibodies [112]. Beyond enhanced cellular uptake, it has been shown that hyperthermia from high-intensity focused ultrasound will disrupt the BBB, allowing easier passage of drugs into the brain [114, 115]. However, in these studies it was difficult to delineate the extents to which cavitation or hyperthermia disrupted the endothelial tight junctions. More recently, others [116] have shown that mild hyperthermia from ultrasound opens up the tight junctions of the BBB, allowing for increased drug uptake into the brain.

As mentioned earlier, phase-change nanodroplets have shown great promise in localized drug delivery activated by ultrasound-induced hyperthermia. Chen et al. have shown that these nanodroplets are capable of separating the tight junctions between the endothelial cells in the brain [117]. They demonstrated that there was preferential uptake of contrast agent into the brain following ultrasound exposure. Considering the beneficial effect of hyperthermia alone, it is difficult to distinguish drug transport induced by nanodroplets from hyperthermia alone.

5.2 *Cavitation-Enhanced Transport of Small Molecular Drugs and Biologics with Ultrasound*

Perhaps one of the most established means to promote transport of a drug beyond blood vessels is acoustic cavitation. As mentioned earlier, cavitation is typically generated by cavitation nuclei co-injected with the therapeutic. Upon exposure to

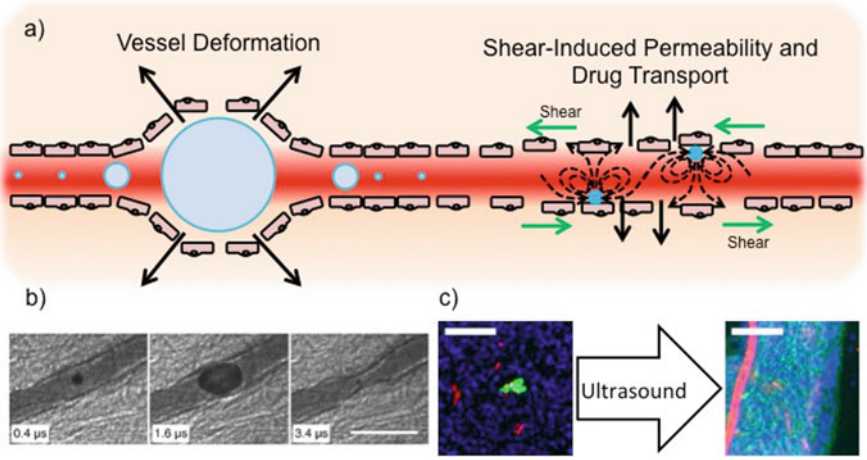

Fig. 3 (**a**) A cartoon of mechanical deformation and transport of a therapeutic beyond a microvessel from a cavitating bubble. Both vessel deformation and shear-induced permeability are shown (**b**) A microbubble inducing microbubble expansion and invagination as a direct result of cavitation in an ex vivo microvessel [118]. Image adapted with permission. The scale bar represents 50 μm. (**c**) Fluorescent microscopy images of a tumor treated with a co-injection of gas-stabilizing solid nanoparticles and a fluorescent antibody demonstrates the effect of ultrasound on drug-extravasation. Without ultrasound, antibody is co-localized with the blood vessel [45]. Image adapted with permission. *Blue, red*, and *green* represents the cancer cells, blood vessels, and antibody respectively

ultrasound, cavitation nuclei experience large volume changes that generate microstreams and pump drugs into the tissue, as well as open up endothelial junctions (Fig. 3) [118]. To date, these cavitation nuclei are typically of the micron size range and are primarily comprised of gas (i.e., a microbubble).

Microbubbles have long been established as the key cavitation agent used in biomedical technologies. However, many biomedical applications require submicron sizes and sustained cavitation response times. Microbubbles are suboptimal for these applications. As a result, there has been a surge in the development of submicron cavitation agents, such as nanobubbles and gas-stabilizing solid nanoparticles.

5.2.1 Gas-Stabilizing Solid Nanoparticles

Another means to generate bubbles from nanoscale nuclei is to partially stabilize a bubble on the surface of a solid nanoparticle. Much like bubbles in a champagne flute, nucleation of bubbles from solid surfaces requires defects (such as cracks and crevices on glass) that entrap gas. For bubbles on microparticles, these surface stabilized bubbles rapidly expand and detach from the surface when exposed to shockwaves. The expulsed bubble pushes the microparticle away, actively propelling it away from the cavitation site. This phenomenon has been well established on micron size particles, but there has been limited number of studies for gas-stabilizing nanoparticles, especially in the context of drug delivery.

Creating surface defects on nanoparticles capable of trapping gas is an immense challenge. Borkent et al. [119] have shown that a single well-defined cavity is able to trap a nanobubble. Upon exposure to a shockwave, the bubbles trapped within these nanopits expanded and detached from the cavity. However, surfaces such as those are impractical for drug delivery applications. Single "cup" shaped cavities that trap gas are nevertheless possible on nanoparticles [45]. Moreover, the cavities on these "nanocups" are tuneable [120]. Much like the nanopits on the surfaces presented by Borkent et al. [119], these nanocups are able to eject a cavitating bubble from their cavity. Once ejected, these cavitation bubbles rapidly expand and collapse, emitting a broadband signal indicative of inertial cavitation. The inertially cavitating bubble has been shown to promote drug delivery in both in vivo and in vitro experiments [45]. Because these particles exclusively emit broadband emissions, they are detectable with diagnostic ultrasound probes.

Others have also developed gas-stabilizing nanoparticles. In contrast to the nanocups, these nanoparticles contain gas within the pores of the nanoparticles. When exposed to ultrasound, gas from within the pores extends out, nucleating a cavitating bubble. Studies have shown that such cavitating bubbles are capable for diagnostic ultrasound [121]. However, there has not been any study that has evaluated the ability for these nanoparticles to promote drug transport.

In principle, these nanoparticles simply provide a source of nanobubbles. It is these bubbles that provide the means by which a circulating therapeutic extravasates beyond the blood vessel. The mechanism of action for enhanced extravasation is mechanical in nature, and as such is drug-class-agnostic. As a result, the key advantage of cavitation, inducing solid nanoparticles, is their capacity to promote the effectiveness of therapies across several clinical indications without the need to modify existing drugs.

6 Sonoporation

Gene therapies require the delivery of genes to the nucleus of the cell. However, there are substantial challenges in promoting cellular uptake of these genes. Sonoporation, therefore, is the use of ultrasound to temporarily permeate the cell membrane wall, allowing nucleic acid polymers (DNA, RNA, siRNA, etc.) to enter the cytoplasm.

Mechanistically speaking, sonoporation occurs from cavitation [122, 123]. Cavitating bubbles, as we discussed earlier, enable highly localized shear forces with shear rates on the 10^7 s^{-1} [124]. Such shear rates near the cell wall force the cell membrane to temporarily open. Alternatively, shock waves generated by collapsing bubbles are also capable of disrupting the cell membrane wall. In addition to shock waves, jets formed by an inertial cavitation bubble [125] have also been shown to temporarily form pores on the surfaces of cells [126, 127]. These temporary openings in conjunction with the enhanced transport associated with cavitation results in an effective means to transfect diseased cells.

6.1 Sonoporation from Ultrasound Induced Hyperthermia

Earlier in sections 4 and 5, we discussed the use of phase-change nanodroplets for both drug release and drug transport. It is therefore not surprising that these nanodroplets have also been used for gene delivery. For example, Burgess and Porter [128] demonstrated successful transfection of cancer cells. To do so, they utilized a green fluorescent protein expressing siRNA freely suspended with phase-change nanodroplets. Expression of GFP only occurred in the presence of high intensity focused ultrasound (5 MHz center frequency at 6.2 MPa). Others were able to bind DNA to phase-change droplets. Upon exposure to ultrasound and DNA bound to phase-change droplets, Gao et al. [129] demonstrated a substantial increase in transfection of HepG2 cells.

Other temperature-sensitive materials (such as poly-NIPAM) have also shown promise for gene therapy [130]. However, there have been few (if any) studies that have manufactured nanoparticles with these materials and applied ultrasound induced hyperthermia for cell transfection.

6.2 Mechanically Induced Sonoporation by Ultrasound-Mediated Cavitation

A more direct route to permeate the cell membrane is to utilize the innate mechanical responses of pre-formed bubbles (Fig. 4). As of late there has been a surge of interest in the development of nanobubbles. These nanobubbles either exist as a stand-alone gas bubble with various coatings [131, 132] or are encapsulated within a hydrophobic shell or are contained within a liposome [133]. In our earlier

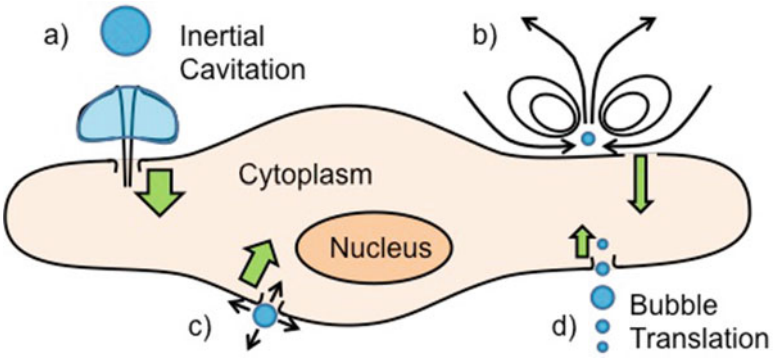

Fig. 4 A diagram of different mechanisms by which a cavitation bubble from an ultrasound-responsive nanoparticle can enhance permeation of a cell membrane and promote gene transfection is shown through (**a**) inertial cavitation, (**b**) microstreaming and micropumping, (**c**) membrane deformations, and (**d**) bubble translation as a result of acoustic radiation force

sections, we discussed the benefit of several of these nanobubble constructs for ultrasound-mediated drug release. Here we focus on their capacity to also promote cell transfection.

As already discussed, hydrophobic gases have been shown to preferentially reside in the hydrophobic regions of a lipid bilayer encapsulation or within a liposome encapsulation. Upon exposure to ultrasound, researchers have shown that the radial oscillations of the nanobubble will disrupt the lipid membrane, thereby releasing its payload. Thus, these echogenic liposomes have shown great promise in drug release. Beyond their capacity to release drugs, the cavitating nanobubbles also generate mechanical forces that affect the surrounding medium. For example, echogenic liposomes have been shown to improve gene transfection into cancer cells in a tumor, which requires the permeation of the cell membrane [102, 134–136].

7 Conclusion

In this chapter, we review the use of ultrasound-responsive nanoparticles, which cause either thermal or mechanical effects, to address the key clinical challenges of current drug therapies, and in particular (1) the triggered release of a drug from a nanoparticle, (2) the extravasation and enhanced transport of a free or recently released drug to diseased tissue, and (3) the enhanced cellular uptake of a gene therapy or other pharmacological agent.

There has been a substantial amount of work to demonstrate the effectiveness of thermally sensitive liposomes, polymer nanocapsules, and phase-change nanodroplets. Thus, we concluded that this technology has had and will continue to have a major impact in drug therapies, as further indicated by current clinical trials. In contrast, mechanical release via cavitation of therapeutics from nanoparticles remains a relatively young technology. However, there is much interest in further developing it due to its cost-effectiveness and ability to deliver and monitor the treatment using low-cost, quasi-diagnostic ultrasound systems.

Historically, micron-sized ultrasound contrast agents have been used to transport therapeutics beyond circulation and into tissue. However, there has been a recent surge of interest in utilizing ultrasound-responsive nanoparticles to overcome many limitations present with these microparticles, including poor circulation, rapid depletion during ultrasound exposure and an inability to extravasate into tumor tissue. Yet, these technologies are still in their infancy as indicated by the literature. Currently, only thermally activated nanodroplets and mechanically driven nanocups have been put forward to specifically address the challenges related to drug transport. That being said, we believe that research on ultrasound-responsive nanoparticles for drug transport will increase substantially.

Much like drug transport, there are few instances of using ultrasound-responsive nanoparticles for sonoporation. Thermally activated phase-change nanodroplets have demonstrated promise in transfecting cells with siRNA, but this work has not been conducted with in vivo models to date. Gene-carrying nanobubbles have

shown that the cavitation generated from submicron gas particles is capable of temporarily perforating the cell membrane in both in vitro and in vivo models. It is clear that the use of ultrasound-responsive nanoparticles for sonoporation is still in its early stages, and thus there are many opportunities that still remain.

Unlike triggered release strategies from encapsulating nanoparticles, the ultrasound-enhanced delivery strategies presented in this chapter are ultimately drug-class-agnostic and rely on broadly applicable thermal and mechanical effects, which can be applied to free therapeutic macromolecules without modification. This portability across drug classes and clinical indications represents one of the major advantages of nanoparticle-enhanced ultrasound-mediated delivery, and could in future achieve the greatest impact in increasing the efficacy of current and emerging drug therapies.

References

1. Krall N, Pretto F, Decurtins W, Bernardes GJL, Supuran CT, Neri D. A small-molecule drug conjugate for the treatment of carbonic anhydrase IX expressing tumors. Angew Chem Int Ed. 2014;53(16):4231–5.
2. Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. Nat Biotechnol. 2014;32(1):40–51.
3. O'Reilly LP, Luke CJ, Perlmutter DH, Silverman GA, Pak SC. C. elegans in high-throughput drug discovery. Adv Drug Deliver Rev. 2014;69:247–53.
4. Lipshultz. Long-term cardiovascular toxicity in children, adolescents, and young adults who receive cancer therapy: pathophysiology, course, monitoring, management, prevention, and research directions: a scientific statement from the American Heart Association (vol 128, p. 1927, 2013). Circulation. 2013;128(19):E394–E.
5. Kramer AH, Jenne CN, Zygun DA, Roberts DJ, Hill MD, Holodinsky JK, et al. Intraventricular fibrinolysis with tissue plasminogen activator is associated with transient cerebrospinal fluid inflammation: a randomized controlled trial. J Cerebr Blood F Met. 2015;35(8):1241–8.
6. Brott T, Broderick J, Kothari R, ODonoghue M, Barsan W, Tomsick T, et al. Intracerebral hemorrhage after intravenous t-PA therapy for ischemic stroke. Stroke. 1997;28(11):2109–18.
7. Goldstein LB. Acute ischemic stroke treatment in 2007. Circulation. 2007;116(13):1504–14.
8. Mitragotri S, Burke PA, Langer R. Overcoming the challenges in administering biopharmaceuticals: formulation and delivery strategies. Nat Rev Drug Discov. 2014;13(9):655–72.
9. Minchinton AI, Tannock IF. Drug penetration in solid tumours. Nat Rev Cancer. 2006;6(8):583–92.
10. Primeau AJ, Rendon A, Hedley D, Lilge L, Tannock IF. The distribution of the anticancer drug doxorubicin in relation to blood vessels in solid tumors. Clin Cancer Res. 2005;11(24):8782–8.
11. Baker JHE, Lindquist KE, Huxham L, Kyle AH, Sy JT, Minchinton AI. Direct visualization of heterogeneous extravascular distribution of trastuzumab in human epidermal growth factor receptor type 2 overexpressing xenografts. Clin Cancer Res. 2008;14(7):2171–9.
12. Tailor TD, Hanna G, Yarmolenko PS, Dreher MR, Betof AS, Nixon AB, et al. Effect of pazopanib on tumor microenvironment and liposome delivery. Mol Cancer Ther. 2010;9(6):1798–808.
13. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006;7(1):41–53.
14. Carlisle R, Coussios CC. Mechanical approaches to oncological drug delivery. Ther Deliv. 2013;4(10):1213–5. Epub 2013/10/15.

15. Crown J, O'Leary M. The taxanes: an update. Lancet. 2000;355(9210):1176–8.
16. Hurley LH. DNA and its associated processes as targets for cancer therapy. Nat Rev Cancer. 2002;2(3):188–200.
17. Lee K, Qian DZ, Rey S, Wei H, Liu JO, Semenza GL. Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells. Proc Natl Acad Sci U S A. 2009;106(7):2353–8.
18. Karahoca M, Momparler RL. Pharmacokinetic and pharmacodynamic analysis of 5-aza-2'-deoxycytidine (decitabine) in the design of its dose-schedule for cancer therapy. Clin Epigenetics. 2013;5.
19. Chu MY, Fischer GA. A proposed mechanism of action of 1-beta-D-arabinofuranosyl-cytosine as an inhibitor of growth of leukemic cells. Biochem Pharmacol. 1962;11:423.
20. Hans R, Andtbacka I, Collichio FA, Amatruda T, Senzer NN, Chesney J, et al. OPTiM: a randomized phase III trial of talimogene laherparepvec (T-VEC) versus subcutaneous (SC) granulocyte-macrophage colony-stimulating factor (GM-CSF) for the treatment (tx) of unresected stage IIIB/C and IV melanoma. J Clin Oncol. 2013;31(18).
21. Folkman J. Antiangiogenesis in cancer therapy—endostatin and its mechanisms of action. Exp Cell Res. 2006;312(5):594–607.
22. Salama AKS, Hodi FS. Cytotoxic T-lymphocyte-associated antigen-4. Clin Cancer Res. 2011;17(14):4622–8.
23. Brahmer JR, Tykodi SS, Chow LQM, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455–65.
24. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol. 2012;24(2):207–12.
25. Wardlaw JM, Murray V, Berge E, del Zoppo G, Sandercock P, Lindley RL, et al. Recombinant tissue plasminogen activator for acute ischaemic stroke: an updated systematic review and meta-analysis. Lancet. 2012;379(9834):2364–72.
26. Williams JM, Navin TJ, Levi CR, Jude M. Recombinant tissue plasminogen activator (rt-PA) utilisation by rural clinicians in acute ischaemic stroke: an audit of current practice and clinical outcomes. Int J Stroke. 2012;7:42–3.
27. Lapchak PA, Chapman DF, Zivin JA. Metalloproteinase inhibition reduces thrombolytic (tissue plasminogen activator)-induced hemorrhage after thromboembolic stroke. Stroke. 2000;31(12):3034–9.
28. Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M. Alzheimer's disease: clinical trials and drug development. Lancet Neurol. 2010;9(7):702–16.
29. Olanow CW, Rascol O, Hauser R, Feigin PD, Jankovic J, Lang A, et al. A double-blind, delayed-start trial of rasagiline in Parkinson's disease. N Engl J Med. 2009;361(13):1268–78.
30. Schapira AHV, Bezard E, Brotchie J, Calon F, Collingridge GL, Ferger B, et al. Novel pharmacological targets for the treatment of Parkinson's disease. Nat Rev Drug Discov. 2006;5(10):845–54.
31. Goetz CG, Poewe W, Rascol O, Sampaio C. Evidence-based medical review update: pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. Mov Disord. 2005;20(5):523–39.
32. Kaplitt MG, Feigin A, Tang C, Fitzsimons HL, Mattis P, Lawlor PA, et al. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. Lancet. 2007;369(9579):2097–105.
33. Landreth G, Jiang QG, Mandrekar S, Heneka M. PPAR gamma agonists as therapeutics for the treatment of Alzheimer's disease. Neurotherapeutics. 2008;5(3):481–9.
34. Cobbold RS. Foundations of biomedical ultrasound. Oxford University Press on Demand; 2007.
35. Ter Haar G, Coussios C. High intensity focused ultrasound: physical principles and devices. Int J Hyperther. 2007;23(2):89–104.

36. Szabo TL. Diagnostic ultrasound imaging: inside out. Boston: Academic Press; 2004.
37. McDannold N, Clement G, Black P, Jolesz F, Hynynen K. Transcranial MRI-guided focused ultrasound surgery of brain tumors: initial findings in three patients. Neurosurgery. 2010;66(2):323.
38. Salomir R, Vimeux FC, de Zwart JA, Grenier N, Moonen CTW. Hyperthermia by MR-guided focused ultrasound: accurate temperature control based on fast MRI and a physical model of local energy deposition and heat conduction. Magnet Reson Med. 2000;43(3):342–7.
39. Rieke V, Pauly KB. MR thermometry. J Magn Reson Imaging. 2008;27(2):376–90.
40. Bradley Jr WG. MR-guided focused ultrasound: a potentially disruptive technology. J Am Coll Radiol. 2009;6(7):510–3. Epub 2009/06/30.
41. Sarvazyan AP, Rudenko OV, Nyborg WL. Biomedical applications of radiation force of ultrasound: historical roots and physical basis. Ultrasound Med Biol. 2010;36(9):1379–94. Epub 2010/08/31.
42. Leighton T. The acoustic bubble. London: Academic Press; 2012.
43. Maxwell AD, Cain CA, Hall TL, Fowlkes JB, Xu Z. Probability of cavitation for single ultrasound pulses applied to tissues and tissue-mimicking materials. Ultrasound Med Biol. 2013;39(3):449–65. Epub 2013/02/06.
44. Stride EP, Coussios CC. Cavitation and contrast: the use of bubbles in ultrasound imaging and therapy. Proc Inst Mech Eng H. 2010;224(H2):171–91.
45. Kwan JJ, Myers R, Coviello CM, Graham SM, Shah AR, Stride E, et al. Ultrasound-propelled nanocups for drug delivery. Small. 2015. Epub 2015/08/25.
46. Arvanitis CD, Bazan-Peregrino M, Rifai B, Seymour LW, Coussios CC. Cavitation-enhanced extravasation for drug delivery. Ultrasound Med Biol. 2011;37(11):1838–52. Epub 2011/10/04.
47. Ammi AY, Cleveland RO, Mamou J, Wang GI, Bridal SL, O'Brien Jr WD. Ultrasonic contrast agent shell rupture detected by inertial cavitation and rebound signals. IEEE Trans Ultrason Ferroelectr Freq Control. 2006;53(1):126–36. Epub 2006/02/14.
48. Roberts WW, Hall TL, Ives K, Wolf Jr JS, Fowlkes JB, Cain CA. Pulsed cavitational ultrasound: a noninvasive technology for controlled tissue ablation (histotripsy) in the rabbit kidney. J Urol. 2006;175(2):734–8. Epub 2006/01/13.
49. Wang YN, Khokhlova T, Bailey M, Hwang JH, Khokhlova V. Histological and biochemical analysis of mechanical and thermal bioeffects in boiling histotripsy lesions induced by high intensity focused ultrasound. Ultrasound Med Biol. 2013;39(3):424–38. Epub 2013/01/15.
50. Schade GR, Keller J, Ives K, Cheng X, Rosol TJ, Keller E, et al. Histotripsy focal ablation of implanted prostate tumor in an ACE-1 canine cancer model. J Urol. 2012;188(5):1957–64. Epub 2012/09/25.
51. Bloch SH, Short RE, Ferrara KW, Wisner ER. The effect of size on the acoustic response of polymer-shelled contrast agents. Ultrasound Med Biol. 2005;31(3):439–44. Epub 2005/03/08.
52. Dicker S, Mleczko M, Siepmann M, Wallace N, Sunny Y, Bawiec CR, et al. Influence of shell composition on the resonance frequency of microbubble contrast agents. Ultrasound Med Biol. 2013;39(7):1292–302. Epub 2013/05/21.
53. Collis J, Manasseh R, Liovic P, Tho P, Ooi A, Petkovic-Duran K, et al. Cavitation microstreaming and stress fields created by microbubbles. Ultrasonics. 2010;50(2):273–9. Epub 2009/11/10.
54. Liu X, Wu J. Acoustic microstreaming around an isolated encapsulated microbubble. J Acoust Soc Am. 2009;125(3):1319–30. Epub 2009/03/12.
55. Won JM, Lee JH, Lee KH, Rhee K, Chung SK. Propulsion of water-floating objects by acoustically oscillating microbubbles. Int J Precis Eng Man. 2011;12(3):577–80.
56. Samiotaki G, Vlachos F, Tung YS, Konofagou EE. A quantitative pressure and microbubble-size dependence study of focused ultrasound-induced blood-brain barrier opening reversibility in vivo using MRI. Magnet Reson Med. 2012;67(3):769–77.
57. Qiu YY, Zhang CB, Tu J, Zhang D. Microbubble-induced sonoporation involved in ultrasound-mediated DNA transfection in vitro at low acoustic pressures. J Biomech. 2012;45(8):1339–45.

58. Juffermans LJM, van Dijk A, Jongenelen CAM, Drukarch B, Reijerkerk A, de Vries HE, et al. Ultrasound and microbubble-induced intra- and intercellular bioeffects in primary endothelial cells. Ultrasound Med Biol. 2009;35(11):1917–27.
59. VanBavel E. Effects of shear stress on endothelial cells: possible relevance for ultrasound applications. Prog Biophys Mol Bio. 2007;93(1-3):374–83.
60. Zhong P, Cioanta I, Cocks FH, Preminger GM. Inertial cavitation and associated acoustic emission produced during electrohydraulic shock wave lithotripsy. J Acoust Soc Am. 1997;101(5):2940–50.
61. Salgaonkar VA, Datta S, Holland CK, Mast TD. Passive cavitation imaging with ultrasound arrays. J Acoust Soc Am. 2009;126(6):3071–83.
62. Farny CH, Holt RG, Roy RA. Temporal and spatial detection of Hifu-induced inertial and hot-vapor cavitation with a diagnostic ultrasound system. Ultrasound Med Biol. 2009;35(4):603–15.
63. Gyongy M, Coussios CC. Passive spatial mapping of inertial cavitation during HIFU exposure. IEEE Trans Biomed Eng. 2010;57(1):48–56.
64. Davis ME, Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer. Nat Rev Drug Discov. 2008;7(9):771–82.
65. Peer D, Karp JM, Hong S, FaroKHzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol. 2007;2(12):751–60.
66. Malam Y, Loizidou M, Seifalian AM. Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends Pharmacol Sci. 2009;30(11):592–9.
67. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream. Science. 2004;303(5665):1818–22.
68. Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov. 2005;4(2):145–60.
69. Andresen TL, Jensen SS, Jorgensen K. Advanced strategies in liposomal cancer therapy: problems and prospects of active and tumor specific drug release. Prog Lipid Res. 2005;44(1):68–97.
70. Yatvin MB, Weinstein JN, Dennis WH, Blumenthal R. Design of liposomes for enhanced local release of drugs by hyperthermia. Science. 1978;202(4374):1290–3.
71. Weinstein JN, Magin RL, Yatvin MB, Zaharko DS. Liposomes and local hyperthermia—selective delivery of methotrexate to heated tumors. Science. 1979;204(4389):188–91.
72. Anyarambhatla GR, Needham D. Enhancement of the phase transition permeability of DPPC liposomes by incorporation of MPPC: a new temperature-sensitive liposome for use with mild hyperthermia. J Liposome Res. 1999;9(4):491–506.
73. Needham D, Anyarambhatla G, Kong G, Dewhirst MW. A new temperature-sensitive liposome for use with mild hyperthermia: characterization and testing in a human tumor xenograft model. Cancer Res. 2000;60(5):1197–201.
74. Ponce AM, Vujaskovic Z, Yuan F, Needham D, Dewhirst MW. Hyperthermia mediated liposomal drug delivery. Int J Hyperther. 2006;22(3):205–13.
75. Needham D, Dewhirst MW. The development and testing of a new temperature-sensitive drug delivery system for the treatment of solid tumors. Adv Drug Deliver Rev. 2001;53(3):285–305.
76. Gaber MH, Hong KL, Huang SK, Papahadjopoulos D. Thermosensitive sterically stabilized liposomes—formulation and in-vitro studies on mechanism of doxorubicin release by bovine serum and human plasma. Pharm Res. 1995;12(10):1407–16.
77. Gaber MH, Wu NZ, Hong KL, Huang SK, Dewhirst MW, Papahadjopoulos D. Thermosensitive liposomes: extravasation and release of contents in tumor microvascular networks. Int J Radiat Oncol. 1996;36(5):1177–87.
78. Park SM, Kim MS, Park SJ, Park ES, Choi KS, Kim YS, et al. Novel temperature-triggered liposome with high stability: formulation, in vitro evaluation, and in vivo study combined with high-intensity focused ultrasound (HIFU). J Control Release. 2013;170(3):373–9.
79. Lindner LH, Eichhorn ME, Eibl H, Teichert N, Schmitt-Sody M, Issels RD, et al. Novel temperature-sensitive liposomes with prolonged circulation time. Clin Cancer Res. 2004;10(6):2168–78.

80. Hossann M, Wiggenhorn M, Schwerdt A, Wachholz K, Teichert N, Eibl H, et al. In vitro stability and content release properties of phosphatidylglyceroglycerol containing thermosensitive liposomes. Biochim Biophys Acta. 2007;1768(10):2491–9.
81. Mylonopoulou E, Bazan-Peregrino M, Arvanitis CD, Coussios CC. Exploitation of cavitation-enhanced heating for release of doxorubicin from thermosensitve liposomes by therapeutic ultrasound. J Acoust Soc Am. 2010;128(4):2418.
82. Peleg-Shulman T, Gibson D, Cohen R, Abra R, Barenholz Y. Characterization of sterically stabilized cisplatin liposomes by nuclear magnetic resonance. Biochim Biophys Acta. 2001;1510(1–2):278–91.
83. Torchilinl V, Papisov M. Why do polyethylene glycol-coated liposomes circulate so long?: Molecular mechanism of liposome steric protection with polyethylene glycol: Role of polymer chain flexibility. J Liposome Res. 1994;4(1):725–39.
84. Schroeder A, Sigal A, Turjeman K, Barenholz Y. Using PEGylated nano-liposomes to target tissue invaded by a foreign body. J Drug Target. 2008;16(7-8):591–5.
85. Dvorak HF, Nagy JA, Dvorak JT, Dvorak AM. Identification and characterization of the blood-vessels of solid tumors that are leaky to circulating macromolecules. Am J Pathol. 1988;133(1):95–109.
86. Schroeder A, Honen R, Turjeman K, Gabizon A, Kost J, Barenholz Y. Ultrasound triggered release of cisplatin from liposomes in murine tumors. J Control Release. 2009;137(1):63–8.
87. Al Sabbagh C, Tsapis N, Novell A, Calleja-Gonzalez P, Escoffre JM, Bouakaz A, et al. Formulation and pharmacokinetics of thermosensitive stealth(A (R)) liposomes encapsulating 5-fluorouracil. Pharm Res. 2015;32(5):1585–603.
88. Han HD, Shin BC, Choi HS. Doxorubicin-encapsulated thermosensitive liposomes modified with poly(N-isopropylacrylamide-co-acrylamide): drug release behavior and stability in the presence of serum. Eur J Pharm Biopharm. 2006;62(1):110–6.
89. Han HD, Choi MS, Hwang T, Song CK, Seong H, Kim TW, et al. Hyperthermia-induced antitumor activity of thermosensitive polymer modified temperature-sensitive liposomes. J Pharm Sci. 2006;95(9):1909–17.
90. Ta T, Convertine AJ, Reyes CR, Stayton PS, Porter TM. Thermosensitive liposomes modified with poly(N-isopropylacrylamide-co-propylacrylic acid) copolymers for triggered release of doxorubicin. Biomacromolecules. 2010;11(8):1915–20.
91. Chen KJ, Liang HF, Chen HL, Wang YC, Cheng PY, Liu HL, et al. A thermoresponsive bubble-generating liposomal system for triggering localized extracellular drug delivery. ACS Nano. 2013;7(1):438–46.
92. Chen KJ, Chaung EY, Wey SP, Lin KJ, Cheng F, Lin CC, et al. Hyperthermia-mediated local drug delivery by a bubble-generating liposomal system for tumor-specific chemotherapy. ACS Nano. 2014;8(5):5105–15.
93. Sheeran PS, Wong VP, Luois S, McFarland RJ, Ross WD, Feingold S, et al. Decafluorobutane as a phase-change contrast agent for low-energy extravascular ultrasonic imaging. Ultrasound Med Biol. 2011;37(9):1518–30.
94. Sheeran PS, Luois SH, Mullin LB, Matsunaga TO, Dayton PA. Design of ultrasonically-activatable nanoparticles using low boiling point perfluorocarbons. Biomaterials. 2012; 33(11):3262–9.
95. Wang CH, Kang ST, Lee YH, Luo YL, Huang YF, Yeh CK. Aptamer-conjugated and drug-loaded acoustic droplets for ultrasound theranosis. Biomaterials. 2012;33(6):1939–47.
96. Rapoport N. Phase-shift, stimuli-responsive perfluorocarbon nanodroplets for drug delivery to cancer. Wires Nanomed Nanobi. 2012;4(5):492–510.
97. Rapoport N, Nam KH, Gupta R, Gao ZG, Mohan P, Payne A, et al. Ultrasound-mediated tumor imaging and nanotherapy using drug loaded, block copolymer stabilized perfluorocarbon nanoemulsions. J Control Release. 2011;153(1):4–15.
98. Marsh D, Seddon JM. Gel-to-inverted hexagonal (L-Beta-Hii) phase-transitions in phosphatidylethanolamines and fatty-acid phosphatidylcholine mixtures, demonstrated by P-31-Nmr spectroscopy and X-ray-diffraction. Biochim Biophys Acta. 1982;690(1):117–23.

99. Evjen TJ, Nilssen EA, Rognvaldsson S, Brandl M, Fossheim SL. Distearoylphosphatidyleth anolamine-based liposomes for ultrasound-mediated drug delivery. Eur J Pharm Biopharm. 2010;75(3):327–33.
100. Graham SM, Carlisle R, Choi JJ, Stevenson M, Shah AR, Myers RS, et al. Inertial cavitation to non-invasively trigger and monitor intratumoral release of drug from intravenously delivered liposomes. J Control Release. 2014;178:101–7.
101. Graham S. Ultrasound-triggered drug release from liposomes using nanoscale cavitation nuclei. Oxford: University of Oxford; 2014.
102. Suzaki R, Takizawa T, Negishi Y, Utoguchi N, Sawamura K, Tanaka K, et al. Tumor specific ultrasound enhanced gene transfer in vivo with novel liposomal bubbles. J Control Release. 2008;125(2):137–44.
103. Suzuki R, Oda Y, Utoguchi N, Maruyama K. Progress in the development of ultrasound-mediated gene delivery systems utilizing nano- and microbubbles. J Control Release. 2011;149(1):36–41.
104. Shaw GJ, Meunier JM, Huang SL, Lindsell CJ, McPherson DD, Holland CK. Ultrasound-enhanced thrombolysis with tPA-loaded echogenic liposomes. Thromb Res. 2009;124(3): 306–10.
105. Tiukinhoy-Laing SD, Buchanan K, Parikh D, Huang SL, MacDonald RC, McPherson DD, et al. Fibrin targeting of tissue plasminogen activator-loaded echogenic liposomes. J Drug Target. 2007;15(2):109–14.
106. Tiukinhoy-Laing SD, Huang SL, Klegerman M, Holland CK, McPherson DD. Ultrasound-facilitated thrombolysis using tissue-plasminogen activator-loaded echogenic liposomes. Thromb Res. 2007;119(6):777–84.
107. Smith DAB, Vaidya SS, Kopechek JA, Huang SL, Klegerman ME, Mcpherson DD, et al. Ultrasound-triggered release of recombinant tissue-type plasminogen activator from echogenic liposomes. Ultrasound Med Biol. 2010;36(1):145–57.
108. Kopechek JA, Abruzzo TM, Wang B, Chrzanowski SM, Smith DAB, Kee PH, et al. Ultrasound-mediated release of hydrophilic and lipophilic agents from echogenic liposomes. J Ultras Med. 2008;27(11):1597–606.
109. Yin TH, Wang P, Li JG, Zheng RQ, Zheng BW, Cheng D, et al. Ultrasound-sensitive siRNA-loaded nanobubbles formed by hetero-assembly of polymeric micelles and liposomes and their therapeutic effect in gliomas. Biomaterials. 2013;34(18):4532–43.
110. Ning SC, Macleod K, Abra RM, Huang AH, Hahn GM. Hyperthermia induces doxorubicin release from long-circulating liposomes and enhances their antitumor efficacy. Int J Radiat Oncol. 1994;29(4):827–34.
111. Kinuya S, Yokoyama K, Hiramatsu T, Tega H, Tanaka K, Konishi S, et al. Combination radioimmunotherapy with local hyperthermia: increased delivery of radioimmunoconjugate by vascular effect and its retention by increased antigen expression in colon cancer xenografts. Cancer Lett. 1999;140(1–2):209–18.
112. Cope DA, Dewhirst MW, Friedman HS, Bigner DD, Zalutsky MR. Enhanced delivery of a monoclonal-antibody F(Ab')2 fragment to subcutaneous human glioma xenografts using local hyperthermia. Cancer Res. 1990;50(6):1803–9.
113. Jang SH, Wientjes MG, Lu D, Au JLS. Drug delivery and transport to solid tumors. Pharm Res. 2003;20(9):1337–50.
114. Vykhodtseva NI, Hynynen K, Damianou C. Histologic effects of high-intensity pulsed ultrasound exposure with subharmonic emission in rabbit brain in-vivo. Ultrasound Med Biol. 1995;21(7):969–79.
115. Mesiwala AH, Farrell L, Wenzel HJ, Silbergeld DL, Crum LA, Winn HR, et al. High-intensity focused ultrasound selectively disrupts the blood-brain barrier in vivo. Ultrasound Med Biol. 2002;28(3):389–400.
116. Cho CW, Liu Y, Cobb WN, Henthorn TK, Lillehei K, Christians U, et al. Ultrasound-induced mild hyperthermia as a novel approach to increase drug uptake in brain microvessel endothelial cells. Pharm Res. 2002;19(8):1123–9.

117. Chen CC, Sheeran PS, Wu SY, Olumolade OO, Dayton PA, Konofagou EE. Targeted drug delivery with focused ultrasound-induced blood-brain barrier opening using acoustically-activated nanodroplets. J Control Release. 2013;172(3):795–804.
118. Chen H, Kreider W, Brayman AA, Bailey MR, Matula TJ. Blood vessel deformations on microsecond time scales by ultrasonic cavitation. Phys Rev Lett. 2011;106(3):034301. Epub 2011/03/17.
119. Borkent BM, Gekle S, Prosperetti A, Lohse D. Nucleation threshold and deactivation mechanisms of nanoscopic cavitation nuclei. Phys Fluids. 2009;21(10).
120. Kwan JJ, Graham S, Myers R, Carlisle R, Stride E, Coussios CC. Ultrasound-induced inertial cavitation from gas-stabilizing nanoparticles. Phys Rev E Stat Nonlin Soft Matter Phys. 2015;92(2–1):023019. Epub 2015/09/19.
121. Chen Y, Yin Q, Ji XF, Zhang SJ, Chen HR, Zheng YY, et al. Manganese oxide-based multifunctionalized mesoporous silica nanoparticles for pH-responsive MRI, ultrasonography and circumvention of MDR in cancer cells. Biomaterials. 2012;33(29):7126–37.
122. Liang HD, Tang J, Halliwell M. Sonoporation, drug delivery, and gene therapy. Proc Inst Mech Eng H J Eng Med. 2010;224(2):343–61. Epub 2010/03/31.
123. Delalande A, Kotopoulis S, Postema M, Midoux P, Pichon C. Sonoporation: mechanistic insights and ongoing challenges for gene transfer. Gene. 2013;525(2):191–9. Epub 2013/04/10.
124. Nyborg WL. Ultrasonic microstreaming and related phenomena. Br J Cancer Suppl. 1982;5:156–60. Epub 1982/03/01.
125. Brujan EA, Ikeda T, Matsumoto Y. Jet formation and shock wave emission during collapse of ultrasound-induced cavitation bubbles and their role in the therapeutic applications of high-intensity focused ultrasound. Phys Med Biol. 2005;50(20):4797–809.
126. Prentice P, Cuschierp A, Dholakia K, Prausnitz M, Campbell P. Membrane disruption by optically controlled microbubble cavitation. Nat Phys. 2005;1(2):107–10.
127. Hu YX, Wan JMF, Yu ACH. Membrane perforation and recovery dynamics in microbubble-mediated sonoporation. Ultrasound Med Biol. 2013;39(12):2393–405.
128. Burgess MT, Porter TM. Acoustic cavitation-mediated delivery of small interfering ribonucleic acids with phase-shift nano-emulsions. Ultrasound Med Biol. 2015;41(8):2191–201. Epub 2015/05/17.
129. Gao D, Xu M, Cao Z, Gao J, Chen Y, Li Y, et al. Ultrasound-triggered phase-transition cationic nanodroplets for enhanced gene delivery. ACS Appl Mater Interfaces. 2015;7(24):13524–37. Epub 2015/05/29.
130. Zintchenko A, Ogris M, Wagner E. Temperature dependent gene expression induced by PNIPAM-based copolymers: potential of hyperthermia in gene transfer. Bioconjug Chem. 2006;17(3):766–72. Epub 2006/05/18.
131. Krupka TM, Solorio L, Wilson RE, Wu HP, Azar N, Exner AA. Formulation and characterization of echogenic lipid-pluronic nanobubbles. Mol Pharm. 2010;7(1):49–59.
132. Wang Y, Li X, Zhou Y, Huang PY, Xu YH. Preparation of nanobubbles for ultrasound imaging and intracellular drug delivery. Int J Pharm. 2010;384(1-2):148–53.
133. Nguyen AT, Wrenn SP. Acoustically active liposome-nanobubble complexes for enhanced ultrasonic imaging and ultrasound-triggered drug delivery. Wires Nanomed Nanobi. 2014;6(3):316–25.
134. Suzuki R, Takizawa T, Negishi Y, Hagisawa K, Tanaka K, Sawamura K, et al. Gene delivery by combination of novel liposomal bubbles with perfluoropropane and ultrasound. J Control Release. 2007;117(1):130–6.
135. Suzuki R, Namai E, Oda Y, Nishiie N, Otake S, Koshima R, et al. Cancer gene therapy by IL-12 gene delivery using liposomal bubbles and tumoral ultrasound exposure. J Control Release. 2010;142(2):245–50.
136. Negishi Y, Endo Y, Fukuyama T, Suzuki R, Takizawa T, Omata D, et al. Delivery of siRNA into the cytoplasm by liposomal bubbles and ultrasound. J Control Release. 2008;132(2):124–30.