

# High Intensity Focused Ultrasound Technology, Its Scope and Applications in Therapy and Drug Delivery

Christopher P. Phenix<sup>1,2,3</sup>, Melissa Togtema<sup>1,4</sup>, Samuel Pichardo<sup>5,6</sup>, Ingeborg Zehbe<sup>1,3,4</sup> and Laura Curiel<sup>1,6</sup>

<sup>1</sup>Thunder Bay Regional Research Institute, Thunder Bay, Ontario, Canada; <sup>2</sup>Department of Chemistry, Lakehead University, Thunder Bay, Ontario, Canada; <sup>3</sup>Medical Sciences Division, Northern Ontario School of Medicine, Sudbury and Thunder Bay, Ontario, Canada; <sup>4</sup>Department of Biology, Lakehead University, Thunder Bay, Ontario, Canada; <sup>5</sup>Imaging Research, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada; <sup>6</sup>Department of Electrical Engineering, Lakehead University, Thunder Bay, Ontario, Canada

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**ABSTRACT** - Ultrasonography is a safe, inexpensive and wide-spread diagnostic tool capable of producing real-time non-invasive images without significant biological effects. However, the propagation of higher energy, intensity and frequency ultrasound waves through living tissues can induce thermal, mechanical and chemical effects useful for a variety of therapeutic applications. With the recent development of clinically approved High Intensity Focused Ultrasound (HIFU) systems, therapeutic ultrasound is now a medical reality. Indeed, HIFU has been used for the thermal ablation of pathological lesions; localized, minimally invasive ultrasound-mediated drug delivery through the transient formation of pores on cell membranes; the temporary disruption of skin and the blood brain barrier; the ultrasound induced break-down of blood clots; and the targeted release of drugs using ultrasound and temperature sensitive drug carriers. This review seeks to engage the pharmaceutical research community by providing an overview on the biological effects of ultrasound as well as highlighting important therapeutic applications, current deficiencies and future directions.

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## INTRODUCTION

In 1880, the Curie brothers reported that certain crystalline materials generated an electric potential when subjected to mechanical pressure (1), a phenomenon later named the piezoelectric effect. Practical applications of piezoelectric properties enabled the development of transducers which could generate ultrasound waves for different uses, of which submarine sonar was one of the earliest (2). Since then, ultrasound has been used in every day devices such as alarms, vaporizers, medical imaging and important industrial processes like plastic welding, material cleaning, and non-destructive testing.

In the pharmaceutical and chemical industries, the application of ultrasound to promote chemical processes is broadly known as sonochemistry (3). Sonochemistry, or the physical and chemical interaction of ultrasound with molecular species, has been thoroughly studied (3-5) and is an important tool for promoting reactions used for synthetic and medicinal chemistry as well

as for improving drug extraction processes (6-9). Ultrasound is being explored to solve pharmaceutical manufacturing and formulation issues (10) as well as the dispersion of solids, the deagglomeration of solids in liquid and the preparation of colloids. In addition, ultrasound has been used in the development of novel catalysts, nanomaterials, nanocrystals and nanoscale catalysts among other applications (11-13).

Diagnostic imaging is the most widespread medical application of ultrasound used as a clinical tool for more than 40 years, mainly due to its non-ionizing nature and the ability to conduct real-time imaging (14). In addition, ultrasound energy is used clinically for thermal tissue ablation, haemostasis, thrombolysis and to promote tissue regeneration (15-17). More recent developments have focused on

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**Correspondence Author:** Christopher Phenix, Thunder Bay Regional Research Institute, 2321-290 Munro Street, Thunder Bay, ON, Canada, P7A 7T1; Email: phenixc@tbh.net.

the use of ultrasound for molecular imaging (18), as well as ultrasound-mediated therapeutic biomolecule and drug-delivery (15,19,20). This has highlighted the value of developing applications with contributions from physicists, medicinal chemists, biologists, material and pharmaceutical scientists. In this article, we intend to draw attention to the potential uses of high intensity ultrasound for therapeutic purposes and the need for multidisciplinary efforts required for expanding its clinical application.

## ULTRASOUND BIOLOGICAL EFFECTS

Initial reports on the biological effects of ultrasound appeared as early as 1928 when changes in living tissues caused by exposure to high intensity and frequency sound waves were reported (21). During the 1940s, the use of focused ultrasound for therapeutic ablation, or ultrasonic surgery, was first proposed (22) and was later used to treat patients with Parkinson's disease and other neurological conditions (23). Nevertheless, the therapeutic applications of ultrasound were held back by the lack of imaging guidance during the treatment process raising important safety issues. With the development of ultrasound imaging, the potential hazardous effects of ultrasound were thoroughly investigated and damage mechanisms, thresholds, and propagation properties through tissues were elucidated (24-28). The term ultrasonic dosimetry, which relates ultrasound intensity, acoustic pressure and other physical parameters with the likelihood of producing biological alteration, was created to guide the design of ultrasound imaging devices. Fortunately, ultrasound dosimetry studies intended for assessing the safety of diagnostic imaging have advanced our understanding of the effects of ultrasound on cells and tissues inspiring the development of more advanced therapeutic applications (26,29). Two main biological effects are observed when high intensity acoustic waves propagate through tissues: thermal and mechanical.

### Thermal Effects

When ultrasound waves propagate through tissues the wave amplitude decreases with distance. This phenomenon is called attenuation and is due to wave absorption and scattering (26). Absorption is the mechanism whereby a portion of the wave energy is converted into heat while scattering

results from the wave changing direction. As a result of acoustic absorption, tissue temperature increases at a rate greater than heat dissipation caused by conduction or blood perfusion. Tissue temperature increases caused by ultrasound energy can be calculated through the widely used bio-heat transfer equation (BHTE) (30). It is then possible to estimate the thermal dose (31) and evaluate if the dose is high enough to reach the threshold values at which tissue damage in the form of coagulation necrosis will appear (32-34).

By using these calculations, researchers can predict the tissue response to the thermal effects of ultrasound and therefore exert control by changing the exposure parameters such as time, power, frequency, geometry or distance. These techniques have been successfully used for a variety of ultrasound exposure conditions and tissue types for the design and planning of thermal treatments on bone (35,36), prostate (37), heart (38-40) and brain (36).

### Mechanical Effects

The mechanical effects induced by high intensity ultrasound include cavitation, microstreaming and radiation force. The phenomenon of acoustic cavitation begins when gas filled cavities, called microbubbles, which spontaneously form or are naturally present in a liquid medium oscillate under the influence of an acoustic wave. The term cavitation was first proposed as an explanation for rapid erosion on ship propellers caused from enormous turbulence, heat and pressure produced by bubbles in the water (41).

There are two forms of acoustic cavitation, inertial and non-inertial. Non-inertial cavitation is described as the stable oscillation of gas-filled bodies in an ultrasonic field whereas inertial cavitation results when a gas-filled cavity expands during part of the acoustic cycle and then collapses rapidly because of erratic oscillations and rapid growth of the cavity. This violent collapse produces high temperatures and pressures with important practical consequences such as light emission and the formation of reactive chemical species. In contrast, non-inertial cavitation causes bubble oscillation and solution microstreaming producing shear forces in the microenvironment. Bubble oscillation also produces mechanical effects caused by the viscous surrounding fluid which opposes the oscillation, creating what is known as radiation

force (42).

The complexity of the cavitation phenomenon depends precisely on the type of the media exposed to ultrasound, making it difficult to obtain a consistent response from living tissues which vary in composition. Even though multiple mathematical models have been proposed to predict and exploit cavitation generated by ultrasound, there is not a single widespread mathematical model currently used for predicting the mechanical bioeffects (43). Nevertheless, important models have been developed to allow for more controlled parameter choice and treatment planning for therapeutic applications that consider mechanical bioeffects (44-46).

### **ULTRASOUND IN THERAPY: HIGH INTENSITY FOCUSED ULTRASOUND**

High intensity focused ultrasound, widely known as HIFU, was born out of the medical application of the thermal effects of ultrasound waves. Whereas the maximum allowed time-averaged intensities of diagnostic ultrasound are  $0.72 \text{ W/cm}^2$  (47), HIFU has intensities in the range of 100 to  $10,000 \text{ W/cm}^2$  (15,48). The ultrasound wave is brought into a tight focus usually 1 mm in diameter and 10 mm in depth such that the thermal effects are localized. The temperature increases at the focus to more than  $60^\circ\text{C}$  for several seconds causing irreversible cell death via coagulation necrosis, without damaging the surrounding tissues where the energy density is significantly lower. The ability of HIFU to focus high intensity waves makes it an attractive non-invasive treatment option for ultrasound surgery (15). The optimal choice of ultrasound parameters is application-specific and represents a compromise between the target depth and the desired rate of heating. For superficial therapies such as intra-urethral prostate treatment the frequencies can be as high as 8 MHz, whereas frequencies as low as 500 kHz are used for deep tissue treatments or treatments through the skull (29).

As mentioned above, the first medical application of HIFU was proposed as an extracorporeal neurosurgery device (49). In the early 1980s, HIFU was used to treat glaucoma and intraocular tumours (50) but was eventually replaced by laser technology. However, there is renewed interest in HIFU for ophthalmological applications due to better focusing capabilities (48).

By the mid 1980s, multiple groups were engaged in HIFU for treating tumours either by inducing localized hyperthermia or tissue ablation leading to multiple clinical trials and the development of commercial devices in the 1990s. Recently, the number of clinical applications of therapeutic HIFU has expanded to include treatment of uterine fibroids (51), glaucoma (48), prostate (52-56), breast (57,58), heart (39,40,59), pancreas (60), liver and esophageal tumours (61,62). HIFU has also been proposed for thrombolysis (63), hemostasis (64,65) and the treatment of venous insufficiency (66).

Studies evaluating the pathological changes in normal and malignant human tissues following exposure to HIFU have shown that thermally ablated tissues undergo homogeneous coagulative necrosis with irreversible tumour cell death and severe damage to tumour blood vessels at the microvasculature level (67). In breast cancer patients, it was confirmed that HIFU-ablated tumour cells did not continue to express *cerbB-2* protein, estrogen and progesterone receptors, when compared to non-ablated tumour tissues (57,68). HIFU has also been found to elicit acute inflammatory responses increasing tumour tissue destruction through immune cell activation, which could enhance the treatment response by acting synergistically with other therapies (69-71).

Three main categories of HIFU devices are currently used in the clinical setting and are usually classified according to the ultrasound energy delivery path: extracorporeal, intracavitary or interstitial. Extracorporeal devices are used for targeting organs that are readily accessible through an acoustic window on the skin such as uterine fibroids or breast (51,58); intracavitary devices are used for transrectal and transurethral treatment of prostate cancer (52,72) or for intraesophageal treatment (38,62); and interstitial devices are used for treating the biliary duct and other difficult to access targets (73,74).

Commercial HIFU devices have been in the market since 1995, when Ablatherm<sup>®</sup> (EDAP Technomed S.A., Vaulx-en-Velin, France) (72,75) and Sonablate 500 (Focus Surgery Inc., Indianapolis, USA) (55,56) started clinical treatment of the prostate. In October 2004, the FDA approved a HIFU device for the treatment of uterine fibroids, ExAblate (Insightec, Haifa, Israel) (76), which uses magnetic resonance imaging (MRI) for

treatment guidance, targeting and monitoring. Recently, the FDA classified HIFU systems as Class II (special controls) devices, in order to provide a reasonable assurance of safety and effectiveness of the equipment (77). Advanced devices such as Sonalleve (Phillips, The Netherlands) and ExAblate 2100 (Insightec, Haifa, Israel) are currently approved for clinical use on uterine fibroids and for relief of pain from bone metastases.

## HIFU FOR NON-INVASIVE DRUG DELIVERY

Enhanced response to therapeutic agents after ultrasound exposure has sparked interest in HIFU as a drug delivery tool (15). Acoustic cavitation and the associated microstreaming effects from confined and localized forces are believed to be the mechanisms responsible for ultrasound-mediated drug delivery. While the physical mechanisms behind the enhanced delivery are similar, applications have been divided according to their therapeutic goal. We will describe each delivery application commonly associated with HIFU devices as they are either performed at high energy levels attainable only by HIFU or they are combined with an ablation therapy using HIFU.

### Sonophoresis

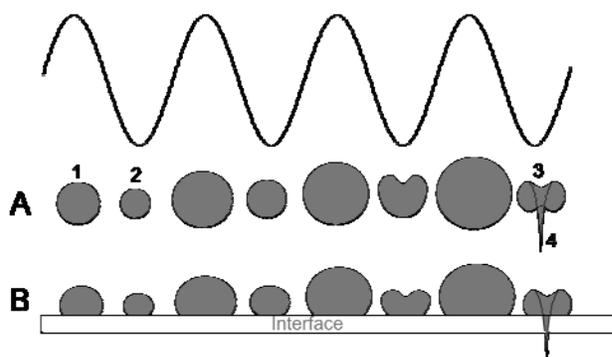
Transdermal enhanced delivery of drugs using ultrasound was first reported in 1954 when hydrocortisone was used to treat polyarthritis in conjunction with ultrasound. This delivery method is known as sonophoresis and is currently used as a powerful tool to enhance transdermal drug delivery and achieve needle-free drug administration (78). For a detailed review on the mechanisms of action, current uses and trends in the field of transdermal sonophoresis see Escobar-Chavez et al. (79).

The technique works by the shock waves generated from collapsing cavitating bubbles found naturally in the skin, that introduce small openings in the intracellular spaces allowing for the passage of small molecules (80). Since cavitation is more common at lower frequencies, sonophoresis is performed by devices that work under 100 kHz (78). As HIFU is usually performed at higher frequencies and depths, it is typically not associated with sonophoresis. However, the energy levels required for sonophoresis as well as the reported

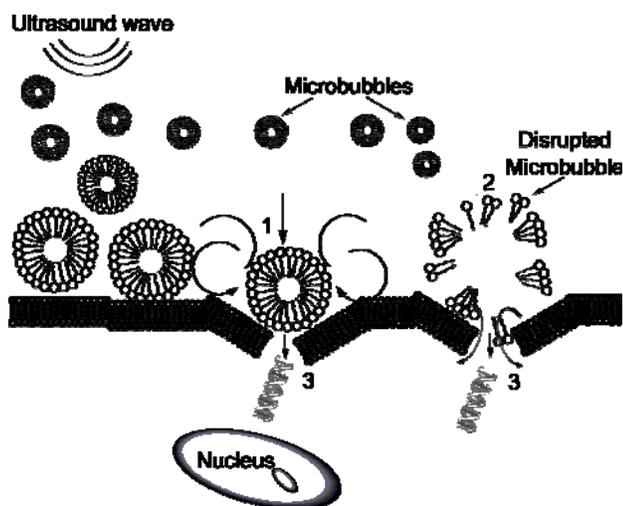
bioeffects are compatible with HIFU devices. The delivery facilitated by sonophoresis induces dispersion of the drug throughout the epithelial layers, but has not yet been proven to enhance intracellular delivery (79).

### Sonoporation

The transient permeabilization of cell membranes through ultrasound-induced pores in the lipid bilayer is known as sonoporation. Inertial cavitation induced by ultrasound at an interface, such as the membrane of a cell or tissue barrier, causes microbubbles within the focal point to collapse in a non-spherical manner driving high-speed jets of liquid into the interface (Figure 1). These jets are believed to produce temporary pores in the cell membrane as well as cause microstreaming in the extracellular environment. The passage of therapeutic agents occurs through the pores propelled in part by the mechanical effects of the microstreaming (81-83) (Figure 2) with additional effects from ultrasound induced endocytosis (84). Cavitation can be achieved through the natural formation of microbubbles under the influence of the high intensity ultrasound waves or it can be potentiated and controlled by exogenous systemic administration of microbubbles.



**Figure 1. Acoustic cavitation.** An ultrasound wave generates changes in pressure in the propagation medium where microbubbles are present. The microbubbles can be floating in the medium (A) or pushed against an interface (B) by the radiation force. When an inertial cavitation regime is present the microbubbles expand (1) and contract (2) in response to the pressure changes and eventually implode (3) leading to the generation of liquid streaming or jetting (4). The force generated by a high pressure jet of surrounding liquid may be sufficient enough to dent a solid surface or introduce pores in a nearby cellular membrane.



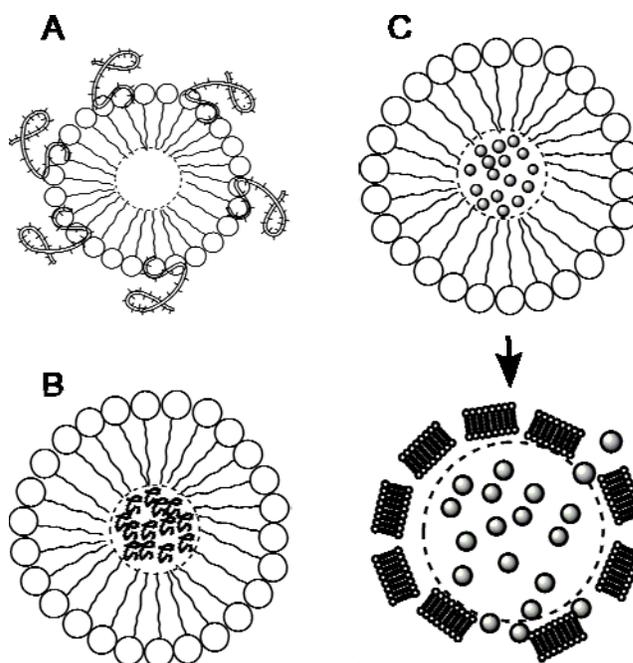
**Figure 2. Sonoporation mechanism.** The ultrasound-irradiated gas-filled microbubbles resonate under the influence of the pressure change and are pushed by the radiation force toward the cell membrane. Transient pores appear when the cell membrane is disrupted by the expansion of the bubbles (1) or bubble collapse (2). The collapse and expansion of the microbubbles generate shear forces around the cell membrane that cause microstreaming, propelling the agents through the temporarily formed pores.

Microbubbles typically used for sonoporation are clinically approved diagnostic ultrasound contrast agents (85,86) and are currently available in the form of either microcrystalline or microbubble emulsions. Table 1 highlights the nature and application of currently available ultrasound contrast agents used for sonoporation. Ultrasound has been successfully used in preclinical studies to introduce membrane impermeable agents into cells or tissues (87,88) including small interfering ribonucleic acid (siRNA) (89,90), peptides (91,92), plasmid DNA (93-95), nanoparticles (88) and antibodies (87,96) (see Table 2 for a summary). The application of sonoporation for the treatment of cancer (87,91,96-98), cardiovascular disease (99,100) and for gene therapy (101) is currently being explored.

In addition to the therapeutic effect caused by enhanced drug delivery into the cells, sonoporation has been suggested to enhance the cytotoxicity of anticancer therapeutic molecules (102,103) and promote ultrasound-induced apoptosis (92,104,105). Ultrasound-induced apoptosis is observed as a delayed biological effect in tissues

exposed to high intensity ultrasound, especially in cell types that regenerate poorly such as neurons. Glioma cells exposed to ultrasound were shown to have increased caspase-3 expression and decreased expression of anti-apoptosis factors such as Bcl-2 and survivin leading to ultrasound induced cell death (106).

Efforts to improve sonoporation-based therapies or the diagnostic specificity of ultrasound have led to the development of chemically modified microbubbles that possess either receptor targeting ligands or carry a drug payload (Figure 3). Advanced microbubbles that target a specific cell receptor have been successfully developed for ultrasound imaging and include microbubbles that bind to the P-selectin of activated platelets for atherosclerotic plaque detection (107,108), lipopeptides incorporated into the bubble membrane to target vascular endothelial growth factor (VEGF)



**Figure 3. Microbubbles as delivery agents.** Gas-filled microbubbles can be used as delivery agents by incorporating ligands on the hydrophobic ends (A), encapsulating ligands within the microbubble that would be delivered upon bubble collapse (B), or encapsulating drugs or nanoparticles within the microbubble which would be delivered when the bubble collapses (C). These microbubbles can act as carriers of drugs, gene therapy, small interfering RNA (siRNA) or other nanoparticles.

**Table 1.** Proprietary agents used for contrast enhanced ultrasound (CEUS), compositions, indications and current market status

<b>Contrast agent</b>	<b>Composition</b>	<b>Imaging Indications</b>	<b>Sonoporation application</b>	<b>Market status</b>
Albunex	Air filled albumin microspheres suspended in 5% w/v human serum albumin	Echocardiography	(93,184)	Developed by Molecular Biosystems Inc Marketed by Mallinckrodt Inc.
Definity (formerly Aerosomes)	Perflutren (octafluoropropane gas) lipid microspheres	Echocardiography	(96,112)	Developed by ImaRx Therapeutics Inc Marketed by Lantheus Medical Imaging Inc
Echovist-200	Microcrystalline suspension of galactose	Female genital tract Echocardiography	See Levovist®	Developed by Schering AG New formulation currently marketed as Levovist®
Levovist	Microcrystalline suspension of galactose and palmitic acid in sterile water	Female genital tract Echocardiography	(184)	Developed by Schering AG Marketed by Bayer Schering Pharma AG
Optison	Perflutren protein-type A microspheres (human serum albumin) and perflutren (octafluoropropane gas)	Echocardiography	(91,184)	Developed by Molecular Biosystems Inc Marketed by Nycomed Amersham and Mallinckrodt, now GE Healthcare Inc
Sonovue	Phospholipid stabilized sulphur hexafluoride microbubbles	Echocardiography	(84)	Developed and marketed by Bracco Imaging

and image angiogenesis (109), as well as antibody-loaded microbubbles to detect prostate cancer by targeting the prostate-specific membrane antigen (PSMA) (110). Since gas microbubbles are typically prepared by high-shear mixing of the gas in liquid phase with the shell-forming material, stable targeting vectors such as peptides, some proteins, oligosaccharides and other small molecules can be attached to the shell forming component prior to the bubble formation. A modified procedure is important when incorporating proteins such as antibodies and enzymes, both of which can be unstable at high temperature and under the sonication conditions required for bubble formation. For such targeted microbubbles, a modified anchor amenable to protein attachment is incorporated into the shell forming agent and included during the formulation process. Conjugation of the protein to the preformed microbubble bearing the appropriate anchor can then proceed under mild conditions (111).

Sonoporation is an attractive tool for molecule delivery that can be applied non-invasively and in a localized manner by focusing

the ultrasonic energy to a specific area. Enhanced drug uptake has been shown to be localized, and multiple studies have indicated that large and small molecules can be successfully delivered (112). Others have optimized the ultrasound parameters and physical settings that achieve the best results (90,113), including the temporal window for successful delivery (114) and the biological conditions that can influence response (95). Sonoporation would benefit greatly from advanced targeted-microbubble formulations for therapy and the production of custom-developed microbubbles specific to a therapeutic target or an intended application.

#### **Blood Brain Barrier Disruption**

Other uses of ultrasound cavitation have been explored such as the treatment of gliomas through locally induced transient disruption of the blood brain barrier (BBB) produced by employing a combination of high intensity ultrasound, doxorubicin and Optison® microbubbles (115). Like sonoporation, cavitation is also believed to be the main mechanism behind the reversible opening of the BBB improving drug uptake in the brain.

**Table 2.** Application of microbubbles in sonoporation

Microbubbles used	Cells used	Molecules transfected into cells	Parameters tested	Reference
Albunex	Chinese Hamster Ovarian Cells	Luciferase plasmid, FITC-dextran	Gene and macromolecule delivery	(93)
Optison	HeLa and Epstein Barr Virus positive BJAB cells	Calcein, Bak BH3 peptide	Peptide delivery	(91)
Optison	Epstein Barr virus positive BJAB , SV-40 and HSV containing mouse C166 and C166-GFP cells	Calcein, eGFP siRNA, pEGF-C3	siRNA delivery	(89)
Soft (1A009) and hard (BG1766) shelled ultrasound contrast agents	Rat mammary adenocarcinoma MAT B III cells	FITC dextran, fluorescent latex nanospheres [25, 44, 75 nm sizes]	Nanoparticle delivery	(88)
Optison	Jurkat lymphocytes, human peripheral blood mononuclear cells	Anti-rabbit IgG-Alex Fluor®, anti-mouse IgD-FITC, Adriamycin hydrochloride	Antibody and Drug delivery	(87)
Optison	SV-40 and HSV containing C166 cells, C166-GFP cells	Calcein, eGFP siRNA	siRNA delivery	(90)
Optison	Chinese Hamster Ovarian cells	FITC-dextran	Macromolecule delivery	(185)
Sonovue	Primary bovine aortic endothelial cells, rat femoral arteries	Tetramethylrhodamine, isothiocyanate-dextran, FITC dextran, lysine fixable FITC dextran	Drug and macromolecule delivery	(84)
Optison	Rat KHT-C fibrosarcoma cells	FITC-dextran	Macromolecule delivery	(112)
Definity	Rat glial C6 cells	Sytox Green, Sytox Blue, TOTO-3 intercalating fluorophores	Macromolecule delivery	(114)
Sonovue	Human MCF-7 cells	Polyethylenimine:deoxyribonucleic acid	DNA delivery	(94)
Sonovue	HEK-293T cells	Branched Polyethylenimine, Vascular endothelial growth factor (165) peptide	Peptide delivery	(92)
Definity	HPV-positive CaSki and SiHa cells	Monoclonal anti-E6 oncoprotein (F127-6G6) antibody from Arbor Vita and monoclonal anti-tubulin antibody	Antibody delivery	(96)
Cationic liposomes and Sonovue	Rat carotid artery	Full-length cDNAs of rabbit Scavenger receptor class B member 1 (SR-BI)	SR-BI DNA expression	(186)

Initial work revealed that short, high-intensity ultrasound waves above the cavitation threshold were found to produce temporary BBB disruption. Unfortunately, the therapeutic benefit was hindered by brain tissue damage occurring in some animals (116). This was resolved when BBB disruption was consistently produced using focused ultrasound with concomitant injection of intravascular gas

microbubbles as additional cavitation sites. Moreover, the use of microbubbles reduced the required ultrasound intensity to levels below the threshold causing thermal damage to adjacent brain tissue and was more compatible with the induction of focal points within the skull (117).

The physical mechanism for BBB disruption is attributed to microbubbles cavitation activity but

the bioeffects are different compared to sonoporation. When the microbubbles pass through the tissue volume exposed to ultrasound they expand and contract at the frequency of the propagating acoustic wave due to the cyclic pressure reductions producing mechanical forces and microstreaming. In addition, the bubbles are pushed by a radiation force that moves them towards the vessel wall. Above an intensity threshold, the bubbles collapse close to the vessel wall creating fluid jets that can puncture the BBB allowing the passage of molecules through the barrier (116). Studies on the cellular mechanisms of this disruption have shown that macromolecule permeability is caused by the mechanical forces inducing the formation of channels and fenestrations in the endothelial cell wall, the opening or widening of interendothelial clefts and free passage through injured endothelial lining when the pressure is sufficiently high (118).

When using microbubbles in combination with ultrasound, the disruption of the BBB has been proven to be reversible with minimal damage to the local surrounding tissues in animals (20,117,119). Magnetic resonance imaging revealed that the BBB appears to remain permeable up to 24 hours after ultrasound exposure with optimal brain uptake occurring within 6 hours (117). Investigations into the safety of BBB disruption demonstrated that permeability is induced at 690 kHz and pressure values of 0.4 MPa significantly below the 2.3 MPa required for tissue necrosis (120). Histological examination of adjacent tissues after BBB disruption demonstrated insignificant levels of apoptosis or ischemia, with no observable differences up to 4 weeks after the disruption (121).

Ultrasound-mediated BBB disruption has been validated on various animal models such as rabbits (117), rats (115), mice (122) and non-human primates (123). HIFU successfully delivered dopamine receptor antibodies (122), enhanced response of brain cancers to doxorubicin and trastuzumab (115,124,125), promoted uptake of therapeutic antibodies for Alzheimer's treatment (126) and DNA for gene therapy (127). New formulations for microbubbles that target the BBB may greatly increase barrier permeability and have enormous potential for improving the introduction of proteins and other impermeable therapeutic agents into the brain.

### **Sonothrombolysis**

The use of ultrasound to potentiate the breakdown of blood clots, known as sonothrombolysis, has been investigated for several decades. This technique was initially used for enhancing intravascular thrombus dissolution but, more recently, has also been proposed for treatment of stroke (128). Early reports described the use of low-frequency ultrasound through the temporal bone to enhance thrombolysis *in vitro* (129). An improved efficacy of thrombolytic agents has been observed when coupled with ultrasound exposure, as reported by multiple groups (15). The mechanism for the enhanced thrombolytic effect of ultrasound was proposed to be the cavitation and collapse of endogenous microbubbles that disrupt the fibrin network (129). Further improvements were observed when thrombolytic agents were used in combination with ultrasound contrast agents, supporting the proposed disruption of fibrin by cavitation (130,131). The mechanical lysis of the clot can potentiate the activity of thrombolytic agents by improving drug penetration and altering the accessibility of fibrin structures to clot-dissolving enzymes (131,132).

Sonothrombolysis is therefore usually performed in conjunction with thrombolytic agents, resulting in significant clinical improvements in clot lysis over the use of these agents alone (132). The advantage of using sonothrombolysis results from reduction in dose of thrombolytic agent required therefore reducing the risk of associated hemorrhage, hypotension and myocardial rupture (133). The procedure is frequently performed with commercial diagnostic devices capable of emitting higher ultrasound intensities, such as those used for Doppler imaging. Recent work on sonothrombolysis is centered on the development of specific devices that can provide more controlled and reproducible results without the need for specialized care teams or training (134). Having sonothrombolysis devices widely available in emergency centers is critical because of the need to maintain early and constant ultrasound exposure for improved clinical outcomes (132).

Currently, sonothrombolysis is also being explored as a standalone technique without the need for thrombolytic agents. Recent reports indicate that the use of ultrasound contrast agents are safe for sonothrombolysis and potentiate the therapeutic effect (130,131). It is therefore conceivable to use

only mechanical effects produced by ultrasound to induce clot lysis. In this context, HIFU may be the ideal approach that can provide the higher level of ultrasound energy required. Indeed, HIFU was successfully used to treat unconstrained clots *in vitro* (135) as well as constrained clots *in vitro* and *in vivo* (63). This work suggests that clot degradation is achieved by cavitation and can be monitored by changes in brightness of ultrasound images during treatment. New developments in biotechnology could advance sonothrombolysis therapy either by targeted thrombolytic agents (136,137), or by employing a combination of targeted thrombolytic agents with microbubbles to enhance the ultrasound effects.

### **Hyperthermia-triggered Drug Delivery**

The delivery or release of a drug at the desired site of action induced by the thermal effects of HIFU is known as hyperthermia-triggered drug delivery. The main purpose of this technique is to increase the therapeutic index of chemotherapeutics, which are often compromised by the distribution of the cytotoxic agent into normal organs and tissues leading to severe side effects. Additional benefits derive from enhanced serum stability and overcoming solubility issues compared to systemic administration of the parent drug.

Efforts to improve drug toxicity profiles while simultaneously protecting the drug from rapid metabolism and excretion has led to the development of temperature sensitive liposomes (TSL) (for a thorough review of TSL's see (138)). TSL encapsulate a water soluble drug within a hydrophilic core surrounded by a protective lipid bilayer (139). Injection of the nanosized liposome drug carrier into the patient results in the passive accumulation of the TSL into tumours through the enhanced retention and permeability effect. Site specific drug delivery is then fulfilled by mild hyperthermia causing the rapid and complete release of the drug into the tumour region. Mild hyperthermia of the tumour area and local vasculature is typically induced by microwave, radio or ultrasound waves (140). Although the spontaneous accumulation of drug containing liposomes typically occurs in tumour xenografts, mild local hyperthermia significantly enhances drug delivery into cancer cells and improves the therapeutic response (141,142). Additional benefits from local hyperthermia result from enhancing the

accumulation of the TSL in tumour tissue (143). In addition, increased blood flow to the tumour area coupled with enhanced cell permeability from hyperthermia induces improved delivery into cells of the tumour. However, it is important to acknowledge that hyperthermic drug release from the TSL in the tumour region is the dominant driving force leading to higher cellular uptake and improved therapeutic response (144). The success of the preclinical studies has led to a series of clinical trials evaluating a doxorubicin-loaded TSL called ThermoDox® (Celsion) for treatment of hepatocellular carcinoma (Phase III) and invasive breast cancer (Phase I) using microwave radiation to induce hyperthermia. Unfortunately, ThermoDox recently failed the phase III trial due to lack of patient benefit compared to the control group while the Phase I breast cancer study is still underway.

The application of HIFU to induce mild heating deep inside of tissues has considerable potential to improve the precision and clinical application of TSL-based chemotherapies. A significant benefit of HIFU hyperthermia results from the ability to focus and control heating by careful choice of the acoustic parameters including continuous or pulsed wave energy, frequency and intensity. Another significant advantage of using HIFU for hyperthermic drug delivery is its compatibility with MRI which enables real time thermometry monitoring of tissue temperature. Instantaneous feedback on the focal point coupled with accurate tissue temperature measurement through image guidance establishes HIFU as the most attractive device for hyperthermic drug delivery. Indeed, recent efforts to use MR guided HIFU to deliver ThermoDox was examined in rabbit muscle demonstrating increased Dox uptake in the area of hyperthermia (145). ThermoDox with HIFU was assessed as a complimentary therapy to thermal ablation of bone cancer with improved results (146). MR guided HIFU was used to treat rabbits bearing VX2 tumours with ThermoDox successfully sparing adjacent tissues from Dox uptake (147).

The application of molecular imaging to visualize and quantify HIFU-induced TSL drug release has recently gained attention. For example, TSL co-encapsulated with a Gadolinium contrast agent and Doxorubicin enabled imaging of TSL content release as demonstrated *in vitro* using squamous carcinoma cells (148) and later in a

tumour (149,150). This strategy was expanded to include the nuclear based SPECT imaging by Indium-111 radiolabeling of the TSLs co-encapsulated with Gadolinium and Dox enabling the researchers to determine blood kinetics and clearance of the TSLs as well as to monitor TSL content release (151).

In order to translate HIFU into a routine drug delivery method used in the cancer clinic, several improvements are needed. Advanced heating algorithms for HIFU hyperthermia were investigated using a combination of mathematical modeling and *in vivo* experiments (152). Other groups have compared continuous wave versus pulsed wave HIFU in an effort to understand the mechanisms with which hydrophilic and lipophilic drugs are released from TSL (153). In addition, HIFU is an excellent preclinical tool to investigate the suitability of new formulations and compositions of TSL as well to evaluate new ultrasound sensitive drug carrier nanoparticles. For example, advanced nanosized “stealth” TSLs modified with the PEG polymer had high Doxorubicin loading capacity, enhanced physiological stability in circulation, faster drug release upon mild HIFU heating and improved efficacy compared to the traditional lysolipid TSL (154). Others have recently synthesized a novel TSL by using cholesterol and an elastin-like polypeptide as additives to produce liposomes having high serum stability and enhanced efficacy in tumour xenografts (155). Recent efforts have been made to expand the use of nanoparticles for encapsulating hydrophobic drugs using ultrasound sensitive micelles composed of hydrophobic polymers (156,157).

### HIFU IMAGING GUIDANCE TRENDS

Even though the therapeutic applications of ultrasound predated ultrasound imaging, it is the latter application which is universally known. The slow adoption of therapeutic ultrasound resulted from the lack of non-invasive targeting and temperature measurements. Eventually, advances in imaging methods during the 1980s and 1990s, particularly ultrasound imaging and MRI, helped further advance thermal applications for treating tumours (15,51,158,159).

The first clinical HIFU devices proposed in the 1990s used ultrasound imaging for guidance

(55,158,160). At the same time, more sophisticated techniques for HIFU guidance using MRI were proposed (161). The advantage of ultrasound imaging over MRI is the costs associated with both equipment and infrastructure, as MR requires shielded rooms. However, MRI is the only FDA-approved method for HIFU monitoring because real time temperature measurements are made during the ultrasound exposure (161-163). MR thermometry is particularly well-suited for HIFU providing the ability for closed-loop control of energy deposition, temperature measurement accuracy within 1 °C, spatial resolution of 1 mm, and temporal resolution of 1 sec or less. As a result, the thermal dose of the treatment can be controlled and superposed to anatomical information (164). Additionally, MRI is the only modality that can provide immediate post-treatment assessment of the necrotic area by using standard contrast agent imaging (29,161).

Multiple clinical applications of HIFU guided by imaging have been proposed, and some of them are commonly available in clinics worldwide (29). Overall, both ultrasound- and MR-guided HIFU devices have received wide spread acceptance. The choice of imaging guidance technology is not made by the user since it is integrated into the commercial HIFU device. The two devices more widely used for HIFU prostate treatment required ultrasound imaging for guidance, and it is for these devices that most clinical results have been reported (53,165,166). On the other hand, MR-guided HIFU devices have been approved and extensively used for gynecological applications, particularly uterine fibroid ablation (167-170) and pain palliation from bone metastases (171-173).

Clinical outcomes of HIFU treatments are generally satisfactory but have been reported to be related to practitioner experience (169,174). In particular for prostate cancer treatment using ultrasound-guidance, studies have shown that patients treated with HIFU can have post-therapy recurrence (53,174,175) suggested to be the result from off-target ablation sparing cancerous cells which was later confirmed by tissue biopsies following HIFU exposure (174).

Lessons from ultrasound-guided HIFU treatment of prostate cancer clearly show the value of precise tumour targeting and monitoring of the thermal treatment. Progress in ultrasound imaging techniques is still needed to monitor temperature or

tissue coagulation for ultrasound-guided HIFU devices (176-178). However, temperature control would not completely solve the clinical issues since the cancerous lesions and surrounding tissues are not easily distinguished.

Recent progress in MR or ultrasound molecular imaging could improve HIFU guidance by clearly defining tumour margins and more accurately identifying areas requiring treatment. A variety of these targeted contrast agents that recognize specific tumour biomarkers have been proposed and tested within *in vitro* and *in vivo* settings. MRI contrast agents rely on marker-specific affinity proteins conjugated to either gadolinium (Gd) or super paramagnetic iron oxide (SPIO) and could image tumours in animal models (179-182). Ultrasound contrast agents could be used for guidance by coating the microbubble shell with ligands that target specific cellular markers (19,111). Targeted ultrasound microbubbles have been reported to image cancerous cells *in vitro* and in animal models (110,183). Contributions to the development of new contrast imaging agents by the pharmaceutical and molecular imaging sciences will propel HIFU-based therapy into the world of personalized medicine by improving treatment guidance.

## SUMMARY

Ultrasound-based therapies, particularly high intensity applications, were inspired by efforts to understand the biological effects of ultrasound energy. Initial therapeutic applications relied purely on the thermal properties of ultrasound taking advantage of its minimally invasive nature and localized effects. Ultrasound has since been developed as a tool for drug delivery by sonoporation, the reversible opening of the blood brain barrier, and the release of drugs from protective carriers by localized heat. Other therapeutic applications of ultrasound have been suggested such as the lysis of clots and drug delivery through the skin using sonophoresis. HIFU therapy was advanced by merging clinical devices with medical imaging technology but also with substantial contributions by biotechnology, medicinal chemistry and the pharmaceutical sciences. The pace at which current ultrasound research is progressing coupled with renewed interest in ultrasound therapy has led to significant

investment in additional HIFU facilities throughout the world. New HIFU facilities providing access to multi-disciplinary research teams will establish high intensity ultrasound as an emerging clinical tool for advanced drug delivery and therapy.

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