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# Ultrasound Therapy

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# 1 Introduction

The therapeutic applications of ultrasound are many, and varied. This chapter outlines the concepts and techniques involved in current therapeutic applications of ultrasound, and provides extensive references for further reading. Ultrasound is a propagating pressure wave, and, as such, it affects the medium through which it travels, transferring energy to it from the wave. This energy manifests itself as a radiation force and as heat, both of which are used to therapeutic effect, depending on the application.

The effects of ultrasound on tissue, and the mechanisms behind them, are described in section 2, which leads on to identify the therapeutic benefits of these effects in different parts of the body. The following three sections introduce those applications that primarily use heat to produce therapeutic benefit, ranging from the subtle temperature rises associated with physiotherapy, to the rapid localized heating that results in protein denaturation, in a process known as thermal ablation. Within each of these sections, the relevant technologies will be described, as well as their clinical application and any emerging research. Techniques used to monitor treatments will be introduced where applicable.

Sections 6 and 7 describe different therapeutic techniques, which take advantage of the mechanical effects induced by ultrasound. Histotripsy relies on high-pressure amplitudes to cause destructive effects, whilst sonoporation and sonophoresis are techniques, which work subtly at a cellular level, using oscillatory and streaming effects to aid the delivery of drugs to target tissues.

Finally, section 8 introduces the important concept of characterization and calibration of therapy acoustic fields. Although primarily a description of measurement techniques, this information is key to establishing the potential effects of ultrasound that may be generated by a particular device, and therefore describes the most important step involved in assessing whether a transducer is fit for therapeutic purpose.

In a field that is developing as rapidly as that of therapy ultrasound, it is inevitable that some techniques and applications have been missed in what follows. However, it is hoped that the main techniques have been covered, and that a useful overview of this important area of ultrasound is provided.

## 2 Therapeutic effects of ultrasound on tissue

Ultrasound propagating through any medium will undergo a number of interactions including its reflection, refraction, absorption and scatter. These all lead to a loss of energy that is exponential with distance, that is, it is attenuated. This loss has two main origins – absorption and scatter. The amount of energy lost to the surroundings depends on the properties of the medium. For example, an ultrasound beam is attenuated more in soft tissue than in water. Absorption is particularly important for therapeutic ultrasound applications, which rely on tissue heating, whereas scatter is essential for the formation of images. The processes by which ultrasound energy is converted to heat in the body are described in this section, as is the effect this heat may have on tissues. This is followed by an introduction to the mechanical effects caused by the forces exerted on tissue.

### 2.1 Thermal effects

Ultrasound waves (with frequencies in the MegaHertz (MHz) range) lead to compression and decompression of the medium through which they travel. Soft tissues are comprised of cells surrounded by an extracellular matrix, which can be pushed and pulled at the frequency of the incident ultrasound wave. If these compressions and decompressions are sufficiently rapid, and the power in the ultrasound wave high enough, the motion of the tissue cannot keep up with the rate of change of pressure in the wave. Some parts of the tissue therefore move at slightly different rates from their surroundings, resulting in frictional forces, which act to convert some of the kinetic energy into heat. This process is known as viscous heating (Bamber in (1)). Depending on the application, ultrasound fields can be focused to concentrate the energy in the beam within a specified volume, thus increasing the amount of local energy absorption.

If the intensity of a plane travelling wave is $I_0$ at the point of origin x = 0, it has reduced intensity I(x) at a distance x from the origin, given by the expression

$$I(x)= I_0 e^{-\mu x} \qquad (1)$$

where μ is known as the intensity attenuation coefficient.

Thus, the energy lost from a unit cross-section of the primary sound beam in travelling unit distance is given by μ I. As discussed above, μ is made up of two components: that due to absorption, $\mu_a$, and that due to scattering, $\mu_s$ such that $\mu = \mu_a + \mu_s$. It is important to note that energy scattered out of the main beam may be absorbed elsewhere in the tissue.

It is possible to obtain an estimate of the temperature rise that might occur in tissue from attenuation of an ultrasonic beam by assuming that all the energy removed from the primary beam leads to local tissue heating, i.e. that the proportion scattered is negligible. (This may be a reasonable approximation because quoted values for $\mu_a$ in soft tissues vary between 0.6μ and 0.9μ).

The rate of heat deposition per unit volume, $\dot{Q}$, is given by the equation

$$\dot{Q} = \mu I \qquad (2)$$

If no heat is lost from this volume by conduction, convection or radiation

$$\dot{Q} = \rho C \frac{dT}{dt} \qquad (3)$$

where ρ is the density of the medium, C is heat capacity and dT/dt is the rate of temperature rise.

Consider, for example, typical values for liver, at room temperature, and at 1 MHz: μ = 0.2 nepers $cm^{-1}$, ρ = 1 g $cm^{-3}$ and C = 4:18 J $g^{-1}$ $^oC^{-1}$. For such tissue, these give a rate of temperature rise dT/dt of 0.048 $^oC$ $s^{-1}$ (2.88 $^oC$ $min^{-1}$) at an intensity of 1W$cm^{-2}$.

Using simple heat diffusion equations, it is possible to calculate the temperature rise that would be expected in a tissue composed of a range of tissue types. The advantage of such mathematical modelling is that it allows one to predict the influence of ultrasonic and tissue parameters on the temperature profiles that can be obtained.

The temperature distributions that are achieved in an irradiated volume of soft tissue can be modified greatly if that volume contains, or overlies, bone since ultrasound is strongly reflected at the bone surface, and any energy that is transmitted into the bone is rapidly absorbed.

Bender et al. (2) described the effect of ultrasonic irradiation on dog femora into which thermocouples had been implanted. They used 800 kHz ultrasound for 2min, with 5W of power being emitted from a 5$cm^2$ irradiating area. Temperature measurements were made in

the bone cortex, in bone marrow and in the soft tissue lying between the transducer and the femur. The greatest temperature rise was found in the cortical bone. Similar differential heating has been described by others (3, 4). They indicated that heating at bone interfaces might define the pain tolerance limits for ultrasonic treatment. They showed that patients with less than 8 cm of soft tissue cover over bone reached their pain tolerance limit more quickly (in terms of treatment time at a given intensity) than those with more soft tissue cover. In a comparative study of the effects of short wave, microwave and ultrasonic diathermy for the treatment of hip joints, it has been found that short wave and microwave heating (at the maximum tolerated dose) gave rise to first-degree burns in skin and subcutaneous tissue without appreciable heating of the hip joint. Ultrasound, however, produced an adequate temperature rise at the bone without skin heating (5).

There are a number of ways in which cells can respond to temperature rises. A low power ultrasound exposure which gives rise to temperature rises of ~1-2°C, has been shown, for example, to aid in wound healing and muscle regeneration. It is therefore commonly used in physiotherapy. However, to this day, it is poorly understood whether it is the temperature rise that causes any beneficial effect seen. If temperatures are raised further, (by approximately 5-15°), and if these temperatures are maintained for many minutes, cells can be permanently damaged, even killed. Ultrasound hyperthermia (see section 4) is a therapy in which large tumours are heated to 43-45$^{o}$C for times up to an hour, in combination with chemo- or radiotherapy. The mechanism for this type of killing is reproductive cell death – the cells are unable to divide successfully. In contrast, High Intensity Focused Ultrasound (HIFU) (section 5) is a technique in which cells are heated very rapidly, to temperatures in excess of 56$^{o}$C for a few seconds, resulting in thermal necrosis and instantaneous cell death.

A quantity has been defined which relates temperature rises in tissue to their therapeutic effect, and accounts for the time for which cells are at elevated temperature. This is known as the thermal dose (6). Empirical evidence has indicated, for example, that if cells are raised to 43°C, it requires 240 minutes at this temperature before tissue ablation occurs. The thermal dose relationship roughly halves this time for each 1°C increase in temperature from this level. Thus, in order to cause rapid cell death in 1-2 seconds, the temperature required is 56°C (ter Haar in (7)). Whereas hyperthermic temperatures cause cells to undergo programmed cell

death over longer time periods or sensitise cells to other insults, ablation causes immediate denaturation of proteins, and thermal coagulation within a small volume of tissue.

A general expression of thermal dose is given by equation 1, and is expressed in Cumulative Equivalent Minutes at 43°C ($CEM_{43}$), that is the time it takes to produce a given biological effect at a temperature of 43°C.

$$CEM_{43} = \sum_{t=t_{initial}}^{t=t_{final}} R^{43-T} \Delta t \qquad (4)$$

Here $R$ is a constant which has been determined empirically to be equal to 0.25 for temperatures less than or equal to 43°C, and 0.5 for temperatures greater than 43°C (8). $T$ is the temperature of tissues over a given time interval $\Delta t$. Although there is a slight dependence on tissue type, a value of 240 minutes is generally accepted as the threshold for denaturation of proteins in mammalian cells (8, 9).

## 2.2 Mechanical effects

### *2.2.1 Radiation force*

The application of a pressure causes a force to be exerted on a medium. This is no different for ultrasound. When an ultrasound beam is activated, it exerts a force on whatever lies in its path. This is known as the radiation force. This concept is used to help determine the output power of therapeutic ultrasound devices in water, as described in section 8.

### *2.2.2 Streaming*

Tissues are comprised of cells and extracellular spaces, which contain fluids, which the radiation force can set in motion. This is known as streaming. Small currents, which can cause changes in cell membranes, are set up between cells. As described in section 3, some of the observed biological effects of low power physiotherapy ultrasound exposure are attributed to streaming rather than to temperature rise.

### *2.2.3 Cavitation*

As an ultrasound wave propagates, the positive pressure components of the wave compress tissue, and the negative (rarefactional) pressures pull the tissue apart. If negative pressures are strong enough, they can also pull dissolved gas out of solution within tissue, causing bubble formation. The subsequent oscillation of these bubbles in response to the incident ultrasound field is known as cavitation(10-12). Bubbles can oscillate stably, in phase with the ultrasound field, with gas diffusing in and out of the bubble as it grows and shrinks. In the presence of more rapidly changing pressures, e.g. at higher frequencies or greater pressure amplitudes, more gas is pulled into the bubble during rarefaction than can diffuse out during compression. In this case, the bubble grows rapidly over a short number of cycles of the pressure wave, and, when its resonant size is reached, a violent collapse ensues. This is known as inertial or collapse cavitation. Stable cavitation is commonly also referred to as being “non-inertial”.

Both stable and inertial cavitation bubbles emit an ultrasound signal as they oscillate, and upon collapse. Cavitation can therefore be detected by listening for these ultrasonic signals. In tissue, the cavitation phenomenon is mainly associated with therapies, which use very high powers, such as HIFU, lithotripsy and histotripsy. In particular, the latter two rely on creating large populations of violently collapsing bubbles to break up tissues or calcifications (see section 6). In HIFU, the presence of cavitation bubbles may help with treatments due to the increased local energy absorption, which may enhance the heating effect of the ultrasound(13). Unfortunately their effects may also be detrimental, as they may scatter the incoming sound in an unpredictable way, diverting the ultrasonic energy away from the desired target (14). Although cavitation is not usually monitored during clinical treatments, the ability to do this during HIFU and histotripsy may help to determine whether a treatment is being delivered in an optimum way (15).

Stably oscillating cavitation bubbles can also have a therapeutic effect. As they oscillate within extracellular spaces, for example, the fluids surrounding them begin to move in in a fashion similar to that described in section 2.2.1. This process is known as microstreaming. Stable cavitation bubbles exert forces on their surroundings, due directly to their oscillation, or to microstreaming flow. If cavitation occurs close to the wall of a blood vessel, the force may be enough to stretch the vessel wall, making it more permeable to therapeutic agents. Similarly,

forces on nearby membranes may increase their permeability, allowing drugs to enter cells for a number of therapeutic applications (16-18). These will be described in more detail in section 7.

# 3 Physiotherapy Ultrasound

### *3.1 Introduction*

One of the earliest areas of interest for the application of therapy ultrasound was physiotherapy. The aim here was initially the alleviation of pain and reduction of oedema in chronic and acute inflammatory disease. More recently, ultrasound at very low power levels has also been used to accelerate bone fracture healing, to break down scar tissue, and to increase the penetration of therapeutic agents through the skin (an application known as sonophoresis or phonophoresis)(19, 20).

The aim of physiotherapy ultrasound is primarily to induce beneficial changes in tissue. For this reason, low intensities, in the range up to 3 W $cm^{-2}$ are usually used, with the aim of producing subtle biological or functional changes. Low frequencies (0.5-1.5 MHz) are used for treatment of deep-seated problems, while higher frequencies (2.0 – 5.0 MHz) are used for more superficial problems.

### 3.2 Principles

Initially, the rationale for using ultrasound in physiotherapy was to produce biological effects using “micromassage” and/or gentle heating, and it was thought of as a diathermy treatment – an alternative to hot packs, microwave or radiofrequency methods. The ability of ultrasound to heat tissue is discussed in section 2.1. The lack of properly designed clinical trials in this area has meant that treatment regimes are usually empirically determined. More recently, as the understanding of the way in which ultrasound interacts with tissues improves’ treatment regimes that make use of the potential of ultrasound to produce non-thermal effects have also been devised (see section 2.2). These involve lower intensities and/or pulsed exposures.

A number of physiotherapy devices are available commercially. These are designed to be lightweight and portable, and usually operate in the frequency range 0.5 – 5.0 MHz. The choice

of frequency to be used depends on the depth of the tissue being targeted, with the lower frequencies being used for the deeper targets. The exposure can be delivered in “continuous wave” or “tone burst” mode, with typical pulsing regimes being 2 ms:2 ms (on time:off time) or 2 ms:8ms, equivalent to 50% or 20% duty factor respectively. Physiotherapy transducers most commonly consist of plane discs made from low loss piezo-electric material (e.g. the lead zirconate titanate PZT4), are encased in a waterproof housing, and are mounted on a handle to enable hand-held delivery of a treatment. By far the most frequent delivery method uses direct contact with the skin, with a coupling medium applied to ensure good sound transmission. Where awkward geometries are to be treated (such as, for example, the elbow), the region to be exposed is placed in a container of water into which the therapy head can be immersed. The water then couples the sound into the tissue. The transducer is either held still during a treatment (stationary head technique), or is moved continuously over the affected area. This latter movement is generally thought to be preferable as it averages out the incident intensity over the region of interest, thus “smoothing out” hot spots due to variations in the ultrasound field, and also avoids the formation of standing waves that can occur when the transducer is held still over a reflecting structure such as bone.

### 3.3 Applications

On reading the clinical literature relating to the use of ultrasound in physiotherapy, it is abundantly clear that there is an urgent need for well-designed clinical trials into its efficacy, with the majority of evidence for benefit being either anecdotal or coming from pre-clinical *in vitro* and *in vivo* studies. A few applications have, however, been the subject of more rigorous study, as described below. Despite this lack of evidence base, it appears that physiotherapists still put it to extensive use (21-24). In one survey conducted in Brazil (25) 83% of respondents assessed their use of ultrasound as being of high importance or essential to their practice, with 73% using it for more than 50% of their patients. Similar take up was reported in a larger Canadian survey (26) with 81% saying that ultrasound had an important role in clinical practice. The most frequent uses for ultrasound listed in these surveys were resolution of acute and chronic inflammation, remodelling of soft tissue and scar tissue, promotion of fibroblast proliferation, reduction in oedema and modulation of acute and chronic pain.

The most common use of ultrasound in physiotherapy is for the stimulation of tissue repair and wound healing. In pre-clinical studies, there is good experimental evidence that ultrasound can accelerate the “inflammatory” phase of wound healing (27-33), increase collagen and synthesis and stimulate the formation of granulation tissue in the “proliferative” phase (34, 35), and influence the way in which new collagen is laid down to give stronger and more elastic scar tissue in the “remodelling” phase (36, 37). A number of clinical studies have corroborated these clinical findings, indicating that non-contact, low frequency ultrasound aids in volume reduction of chronic wounds (including varicose ulcers), and speeds up the healing process (see, for example, (38-43).

One of the most extensively reported physiotherapy applications of ultrasound is for the acceleration of the repair of fractures. The ultrasound exposure regime used here is often referred to as LIPUS (low intensity pulsed ultrasound), with frequencies in the range 45 kHz - 3 MHz and spatial average, time average (SATA) intensities of 5-1000 mW $cm^{-2}$ being used. A typical treatment might use a frequency of 1.5 MHz, and a SATA intensity 30 mW $cm^{-2}$ in pulsed mode (200µs on:800µs off for 20 minutes daily (44-47). Ultrasound is delivered using either a planar handheld transducer moved in contact with the skin, or from a simple wearable device.

Following Heckman’s original paper (48) there have been a number of publications that have reported successful healing of acute fractures, delayed unions and non-unions (reviewed by (44, 49, 50)). As with all physiotherapy ultrasound applications, the evidence base for enhanced repair is weak, but this may, in part, be a result of the wide range of exposure conditions used.

There have been reports of clinical studies which indicate that low intensity pulsed ultrasound can improve the quality of life of patients with arthritis of the knee (24, 51-53), reduce pain following whiplash injury, and improve mobility (54, 55). There is, however, some controversy as to the efficacy of these treatments, with other authors being unable to find a therapeutic advantage when ultrasound is used (e.g. (52)).

Several reviews have addressed this question of lack of evidence base for physiotherapy ultrasound, despite its very widespread use (21, 56-61). In a well- argued article, Speed (56) points out that the divergent results may be the result either of a lack of real effect, or of a number of confounding factors such as technical variables, the complexity and variety of

underlying pathologies and methodological limitations. An important aspect may be a lack of good knowledge about the ultrasound exposures used, due to inadequate calibration and Quality Assurance protocols for these therapy units.

# 4 Hyperthermia

## 4.1 Introduction

Hyperthermia treatments involve heating a tissue target to a constant, and uniform, elevated temperature, in the range 40 – 45$^{o}$C, for times up to ~ 60 minutes. It is generally recognized that hyperthermia on its own is not a curative option, but when applied in combination with chemo- or radiotherapy it can give significant improvements in clinical outcome. Thermal treatments have been used for many years, in fact, the first known record of heat based cancer therapy appears to have been recorded in the Edwin Smith papyrus which describes the treatment of a breast tumour by the Egyptians some 5000 years ago (62). There have been numerous accounts of tumour regression in patients with fever, including fevers deliberately induced using Coley's toxin (63, 64). Westermark (65) was probably the first to induce hyperthermia in a systematic fashion. He treated inoperable uterine carcinomas with circulating hot water.

The biological effects of hyperthermic temperatures have been studied extensively, both in vivo and in vitro (8, 66, 67). While there is no evidence that malignant cells are more easily killed by heat than cells in normal tissues, those in an acidic environment, such as those in necrotic regions of tumours, are more thermally sensitive than those held at a normal pH. The phenomenon of thermo tolerance has been identified, with cytotoxicity to cells reducing for a second, higher temperature exposure (68).

While heat induced changes to cell membranes may enhance their permeability to cytotoxic drugs, and changes in local blood flow may improve their accessibility, perhaps the most promising use of ultrasound hyperthermia is for triggering drug release from thermally sensitive vehicles (17, 69-82).

It has been known for some time that heat is an effective radiosensitiser (83-86). Cells appear to be most sensitive to thermal insult in S phase, the stage of the cycle that is most resistant to radiation damage. Tumour vasculature collapses at lower temperatures than that of normal

tissue, and normal tissue blood flow increases by a significantly greater amount in response to heat than does tumour blood flow, thus conferring some protection against thermal damage. The combination treatment works most effectively when heat and radiotherapy are administered simultaneously, although the effect is still seen when the treatments are given in quick succession (in either order) (86).

Clinical hyperthermia treatments can be delivered using a number of heat sources, with radiofrequency and microwave energy having been used most frequently to date (87). Ultrasound based methods confer many advantages, the most useful of which is probably the ability to heat selectively at depth, using weakly, or highly, focused beams. However, where uniform large volume heating is required, it is usually necessary to scan the beam mechanically or electronically, in such a way as to maintain the desired temperature profile (87-93). Since the synergistic effects being sought are temperature dependent, it is important that the target temperature distribution is known, especially where large vessels are present as these can act as heat sinks. Probe thermometry in ultrasound fields is problematic (94), and so MR thermometry may be the method of choice (95, 96). Thermal modelling may also play a useful role where the vascular pattern is known, and where some single point reference temperatures can be obtained (97-99).

4.2 Clinical applications

The literature on clinical applications of ultrasound hyperthermia is relatively sparse. Zhu et al (100) have reviewed the applications of the combination of ultrasound hyperthermia and radiotherapy. The most common uses have been for head & neck, breast & chest wall and prostate cancers using extracorporeal and trans-rectal devices. As with other heating methods, the efficacy of these treatments depends strongly on the thermal doses achieved. There were early reports of good response with radiotherapy and ultrasound hyperthermia in melanoma (101, 102), with tumours heated to 43.5 ± 0.5$^{o}$C for an hour immediately before radiotherapy giving a significantly better response rate than those exposed to a single modality. This work does not appear to have been followed up.

A small phase 1 study investigated the feasibility of treating malignant brain tumours with hyperthermia and radiotherapy (103). This required removal of skull bone to provide an acoustic window. It was shown that it was possible to maintain a temperature of 42.5 $^{o}$C at the measurement points within the tumour.

A recent study in Oxford has demonstrated that when ultrasound hyperthermic temperatures are combined with doxorubicin for the treatment of liver metastases, the concentration of drug was significantly increased in the heated volume (74, 104, 105).

# 5 High Intensity Focused Ultrasound

## 5.1 Introduction

As discussed in section 2, the concept of HIFU differs from that of hyperthermia in that it seeks to induce very rapid tissue heating over a short period of time, with the desired biological endpoint being coagulative necrosis. This is achieved by focusing the ultrasound field into a specified target volume within the body.

The first reported clinical application of HIFU was for the treatment of Parkinson's disease by producing small regions of ablated tissue (lesions) within the brain. This was demonstrated by Fry et. al. in the 1950s (106-108). However, the high attenuation of ultrasound by bone required removal of part of the skull in order to couple the ultrasonic energy effectively into the brain. The technique was therefore not widely used, and HIFU research for the treatment of neurological disorders slowed somewhat. In subsequent years, the concept of treating cancers in soft tissues has been explored, and new, varied and effective applications have been developed (109-113). These will be described in greater detail in the following paragraphs. Most recently, techniques that allow trans-skull focusing have been developed, and neurological HIFU has experienced a resurgence of interest, with the treatment of essential tremor in particular showing encouraging success. (114-117)

## 5.2 HIFU technologies

### *5.2.1 Transducers and focusing*

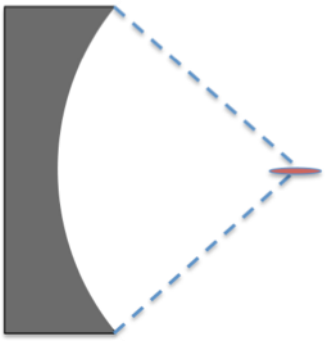
Therapy ultrasound devices use either single or multi-element transducers. An element may be a single crystal, or a composite material, in which smaller cylinders of crystal are set into a polymer. These may be cut into smaller elements as required.

There are a number of ways in which focusing of the beam can be achieved. Acoustic lenses act in an equivalent manner to optical lenses, and can be placed in front of a transducer to

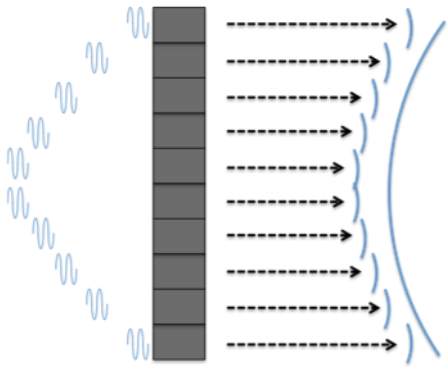
focus the beam. Alternatively, simple geometric focusing involves shaping the transducer such that its face is concave. Where the form is that of a spherical bowl, the focal peak of the acoustic field will occur (to a first approximation) at a distance in front of the transducer, which is equal to the radius of curvature of the sphere (Figure 1).

**Figure 1 Geometric (a) and electronic focusing of an ultrasound beam (b).**

a



b

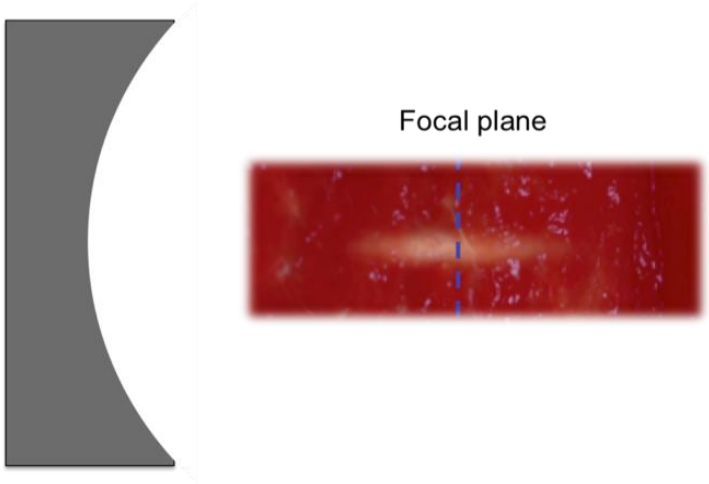


Alternatively, if the transducer is split into multiple elements, the voltage signals to these elements can be timed such that the wavelets from each element combine into a curved wave front, propagating in the same way as it would from a focused bowl. This is illustrated in figure 1b. The advantage of a multi-element device is that, in clinical practice, the applied signals to each component can be timed to sweep the beam electronically in different directions, thus allowing the transducer to treat different regions of tissue from a fixed position.

The high intensities involved in HIFU originate from constructive interference of different parts of the wave front as they move closer together. During treatment, the tissues within a

small ellipsoidal volume at the focus will be killed rapidly. The size of this 'lesion' (the name given to a region of thermally ablated tissue) will depend on how tightly focused the beam is, but is typically of the order of centimetres in length and millimetres in width. Figure 2 shows the effect of a single shot of HIFU on a piece of liver tissue, which has been cut along the sound beam axis.

**Figure 2 A typical HIFU lesion produced in ex-vivo liver tissue, and cut along the sound axis.**




In order to treat larger volumes of tissue, individual lesions can be stacked together, or the beam can be continually swept over a large region until the appropriate thermal dose has been imparted to the whole target volume. In order to determine whether tissues have been successfully treated, some form of imaging is required during therapy. The following paragraph introduces a number of clinical systems, which use either magnetic resonance imaging (MRI) or ultrasound imaging (US) for real-time guidance.

### *5.3 Clinical systems and applications*

#### *5.3.1 Introduction*

Although the concept of HIFU treatment is relatively straightforward, the ability to translate it into clinical application requires sophisticated equipment and techniques. The basic

transducers described above are used for extracorporeal treatments, where the transducer remains outside the body, but there are a number of alternative geometries, which allow the ultrasound source to be moved as close as possible to the treatment target. These include transrectal devices for prostate treatment, transvaginal systems for treatment of uterine fibroids, toroidal transducers for intra-operative use, and miniaturized interstitial transducers, which enable cardiac or blood vessel ablation, amongst other applications (112, 118-125).

The first commercial HIFU systems used ultrasound imaging for guidance (USg). Two USg systems are in clinical use for prostate treatments, the Sonablate® from Sonacare Medical (formed by the merger of Focus Surgery Inc and USHIFU), and the Focal One® (the successor to the Ablatherm®) from EDAP Technomed. These use transrectal ultrasound probes, which combine imaging and therapy, and interleave ultrasound-imaging frames with bursts of HIFU for treatment. This allows the tissue to be monitored between each HIFU exposure, ensuring that a lesion has been created at each location before moving the beam. JC HAIFU® systems developed by Chongqing HAIFU (HIFU) Technology Co. Ltd. rely on ultrasound imaging for guidance. Their devices are used for the treatment of many diseases, including cancers of the liver, pancreas, breast, kidney and other soft tissues, as well as bone cancers and uterine fibroids. They have separate, application specific systems for the treatment of cervical disorders and even allergic rhinitis(126-128).

During the 1990s, the concept of temperature measurement using MRI led to the development of new MR guided HIFU systems (MRg), such as the Sonalleve™ (from Profound, formerly a Philips product) and the ExAblate™ system from Insightec (95). Both have been used to treat uterine fibroids and metastatic bone disease. Clinical trials are in place to investigate the potential for MR guided prostate, breast and brain therapies.

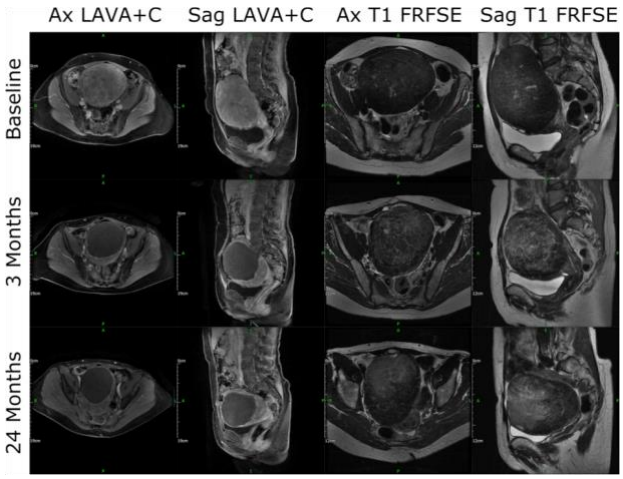
In the following paragraphs, the most common clinical applications will be reviewed, and methods for treatment guidance and monitoring discussed in more technical detail.

### *5.3.2 Uterine fibroids*

Uterine fibroids are common in women of childbearing age. Although benign, they cause a number of symptoms, which significantly reduce quality of life. The main desired outcome of treatment is to shrink the fibroids and reduce symptoms. HIFU treatment of uterine fibroids is a technique approved by FDA, EU and other regulatory authorities, with many of the most

recent clinical trials being performed using an MR-guided system (ExAblate™, Insightec, Israel) (129-134). Treatment involves extracorporeal treatment through the abdomen using a HIFU transducer situated in the bed of the MR scanner. As air/water and air/tissue interfaces cause.

**Figure 3 MRIs of a large uterine fibroid treated with a single session of HIFU using an ultrasound guided device (JC200, Haifu Medical Ltd). Baseline (first row), 3 month (second row) and 24 month (third row) follow-up MRI scans. Selected T1 axial and sagittal contrast-enhanced LAVA sequences (first and second columns) and T2 FRFSE axial and sagittal sequences (third and fourth columns). The follow-up sequences show a dramatic reduction in the non-perfused volume and a reduction in the volume of the treated fibroid, with subsequent (anatomical) descent of the fundus of the uterus into the deep pelvis (courtesy of Paul Lyon).**



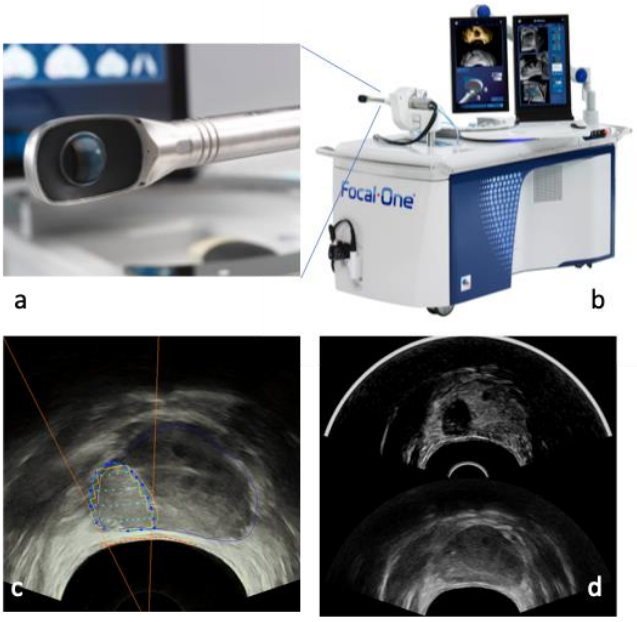

Significant reflection of the ultrasound energy, it is very important during any HIFU treatment to avoid air gaps in the beam path. MR imaging is first used to localize the fibroids and any adjacent sensitive structures (132). Treatment planning then involves computerized calculation of the number, position and length of ultrasound exposures (sonications) required to completely cover the fibroid. This can be performed under mild sedation, making it a minimally invasive outpatient treatment, which is convenient for the patient. Trials have shown good success in terms of treatment outcome, with shrinkage of 31% (135) or 33% (136)

on average at 6 months follow up, corresponding to the volume of the fibroids treated, no serious complications or adverse events (136, 137), and symptomatic improvement to 12 months (137). More recent systematic reviews support the finding that the procedure is fertility sparing, and has few adverse events (8.7%) (130, 138-141). The symptom severity score decreases (~-30.5 at 12 months) and is comparable with uterine artery embolization. The re-intervention rate is 13-14% at 12 months

### *5.3.3 Prostate treatments*

HIFU is commonly used to treat both Benign Prostatic Hyperplasia (BPH) and localized prostate cancers. Transrectal probes are used to deliver these treatments, as they allow close proximity of the ultrasound source to the treatment region (Figure 4).

**Figure 4** **The medical device Focal One® (b) for treating prostate cancer with a transrectal HIFU probe (a). The treatment-planning phase allows definition of the positions of the focus that will be reached mechanically and electronically (c). Contrast enhanced ultrasound is used to assess treatment efficacy (d). Courtesy of EDAP TMS.**



In the case of BPH, the gold standard treatment is surgical resection of the whole prostate. Many clinical trials have been conducted into HIFU treatment of BPH as an alternative to surgery, with the aim of reducing side effects, but there are a number of disadvantages. The main issue is one of time. Following insertion of a dual modality imaging and therapy ultrasound probe, ultrasound images are used to determine the position of the treatment volume relative to the probe. Treatments are then planned using a number of single HIFU exposures, each creating a single necrosed lesion, between which the focus is moved to the next position, and a significant cooling time is allowed. Ablation of the whole prostate therefore takes longer than the alternative surgical procedure, but it may be performed as an outpatient procedure, significantly reducing the time spent in hospital. HIFU has not yet been shown to exceed surgery in terms of side effects and long-term recovery, although there is a lack of long-term follow-up data for analysis. Hegarty and Fitzpatrick (142) give a detailed review with trial data showing reduced symptoms up to 1 year post treatment. A small amount of data is available to suggest that this improvement continues to 2 years post-treatment, and is summarized in (143, 144).

Prostate cancer (PC) treatment options are highly dependent on the staging of the disease (145). For low-risk localized disease, cancer-specific survival is very high, and watchful waiting has now become the standard of care. For intermediate-risk and high-risk diseases, first line treatments are radical prostatectomy (RP) and radiotherapy (RT).

HIFU seems to have fewer side effects than RP and RT, but is nevertheless associated with incontinence, urethral fistulas, urethral obstruction, urinary tract infections and acute urinary retention (146). There are two main strategies used in the clinic for prostate cancer HIFU ablation. Either the whole prostate is ablated, or only the section in which the cancer is suspected to be contained. The latter is usually based on the results of biopsies and MRI and has lower side effects (147), especially concerning incontinence and urinary obstruction (148). HIFU is an alternative to watchful waiting for some low-risk cancers. No randomized controlled trial (RCT) has yet been published, but RCT are ongoing. Focal (149) and whole gland (150, 151) HIFU are also an alternative to prostatectomy and radiotherapy in intermediate-risk PC. No randomized controlled trial has been published as yet, but RCT are ongoing (152). The best evidence is a recent propensity score-matched study comparing focal therapy to radical

prostatectomy (153). Oncological outcomes over 8 years were similar between focal therapy and RP. Salvage HIFU is an attractive option for radio-recurrent prostate cancer (154-156). A recent meta-analysis found no difference on 5 year recurrence-free survival after several salvage treatments (stereotaxic radiotherapy, prostatectomy, HIFU, brachytherapy and cryotherapy) (157). Once again, this is not based on RCTs, but on adjusted retrospective studies.

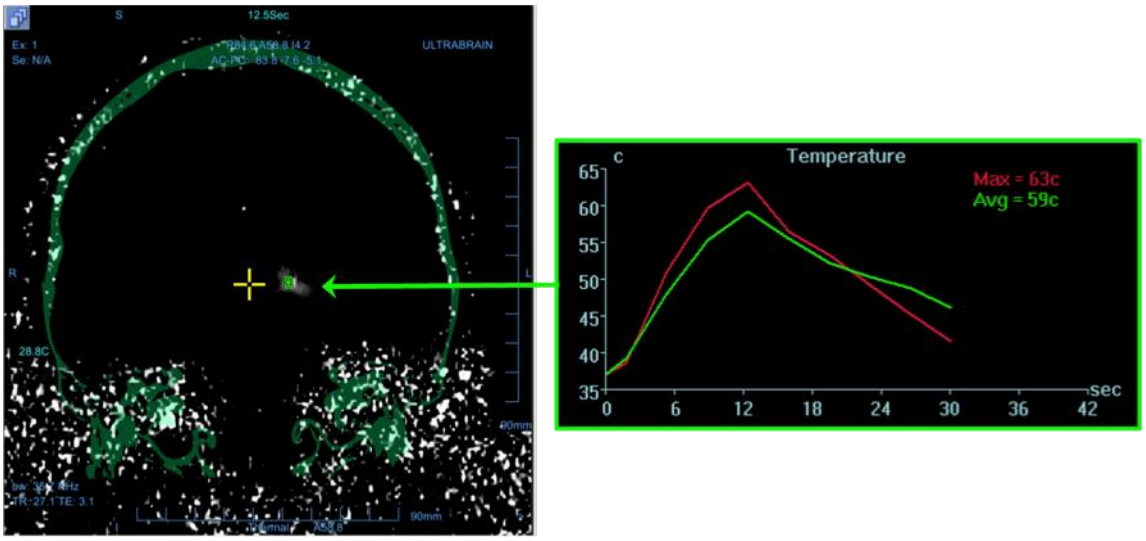
HIFU is a promising therapy for localized PC, and RCT are currently ongoing to prove its oncologic equivalence for standard of care.

### *5.3.4 Brain therapies*

As described in section 5.1, the first application of HIFU for treatment of Parkinson's disease became unpopular due to the necessity for removal of a part of the skull, making the treatment complex and invasive. In recent years, the problem of absorption of much of the ultrasound energy and distortion of the beam by the skull has been overcome. It was first found that by using a phased array transducer, where each of 64 individual elements could be fired independently, and using lower frequencies of 0.6 and 1.58 MHz to improve penetration through the skull, corrections could be applied to compensate for distortion, allowing accurate focusing through the skull (158). Larger arrays covering up to half of the skull have since been developed. These spread the energy over a larger area, thus minimizing the amount of skull heating (159-163). Due in part to developments in MRI guidance (see section 5.3), studies have indicated that localized brain therapies are possible with HIFU, both through ablation of malignant tissue (164), and by helping to open the barrier between blood vessels and tissues (known as the blood brain barrier, or BBB), in order to aid drug delivery (including (165, 166)). This latter technique relies on the presence of cavitation to increase the permeability of vessel walls, and will be discussed as a separate therapeutic application of ultrasound in section 7.

An exciting development of HIFU ablation in the brain is for the treatment of essential and Parkinsonian tremor (167-169). The focal beam can be used to perform selective thalamotomy under MR guidance. **Error! Reference source not found.** illustrates such a treatment. The thermal dose of one single 11s sonication is sufficient to induce permanent lesioning but most often several sonications are repeated to fully stop the tremor, based on the patient feedback.

**Figure 5 Thermal thalamotomy for the treatment of Essential Tremor: MRI thermography in gray scale (left) and time course of the temperature during the 11s sonication and the cooling time (right). Treatment performed at ICM (Paris France), as a part of the Ultrabrain project.**




The feasibility of modulating brain activity at low exposure levels without lesioning has been demonstrated in rodents (170-174), monkeys (175-180) and healthy volunteers (181, 182). This is being investigated clinically for psychiatric disorders such as severe depression.

### *5.3.5 Other applications*

A number of soft tissue cancers have been found to be good candidates for HIFU treatment, including cancers of the liver, pancreas, kidney and breast. There are a number of reviews of the clinical status of HIFU applications, which include these areas, and are recommended for further reading (143, 183-188). Treatment of solid soft tissue tumours within the areas specified above is generally performed using extracorporeal transducers, with a water bath to provide contact between the transducer face and the skin surface. Using the ultrasound guided Chongqing Haifu system, for example, Wu et. al. have presented encouraging results following treatments of areas including the breast, liver, pancreas and bone, in 1038 patients in 10 Chinese hospitals (189, 190). A small number of complications such as fever, skin burns and fracture following bone treatment were described, however the authors highlight the

need for longer-term follow-up to determine the true effectiveness and potential for cure. High frequency (> 3 MHz) devices have been developed specifically for superficial targets such as thyroid (191, 192), fibroadenoma (193-195), varicose veins (196-198)(6), or ciliary bodies for the treatment of glaucoma (199) (Figure 7).

**Figure 6** **Clinical treatment of a varicose vein with the ultrasound-guided Sonovein® system. Courtesy of Pr Luis Izquierdo Lamoca, Hospital HM Montepríncipe.**

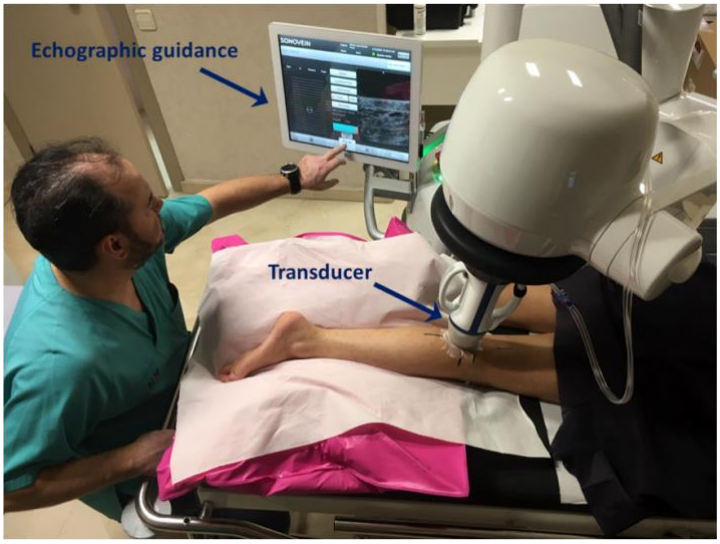
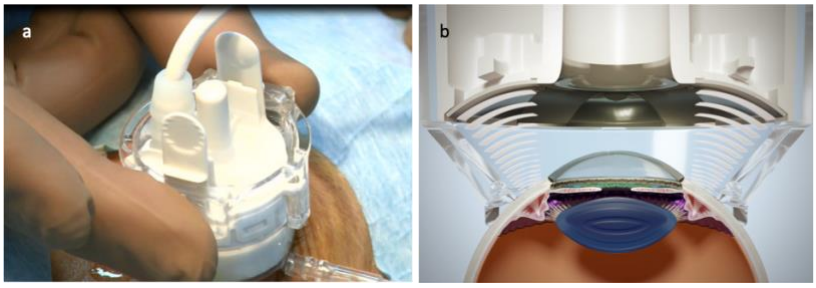



**Figure 7** **The ciliary body can be ablated thermally by HIFU with a disposable device for reducing the intraocular pressure and treating glaucoma – Courtesy of EyeTechCare.**



## 5.4 Treatment guidance and monitoring

As mentioned above, either MRI or ultrasound imaging can be used to monitor HIFU treatment as it is being delivered. This has the aim of confirming successful treatment and helping to avoid damage to healthy or particularly sensitive structures or tissues, giving feedback to the operator who can adjust the output power and/or position of the transducer accordingly. In this section, the techniques used to guide and monitor treatments are described in more detail.

### *5.4.1 MR vs. ultrasound guidance*

MRI is often regarded as the gold standard in HIFU treatment monitoring, primarily because of the high contrast available in the images compared with ultrasound, allowing detailed anatomical scans to aid in localization of regions to be treated, and because of the ability to monitor temperature changes in the body accurately and rapidly. Ultrasound imaging can also be used to detect temperature rises, but results obtained to date have been found to be limited in accuracy, and therefore has not yet found clinical usage (87, 200). Ultrasound imaging has two distinct advantages over MRI. Although the acquisition rate of MR images is increasing as techniques develop, MR thermometry is still realistically limited to ~ one 'frame', or image slice, per second, depending on the quality of the images required to deliver the treatment accurately. On the other hand, ultrasound systems can acquire images at rates of up to 20,000 frames per second with the most recent technologies, with typical general purpose clinical scanners currently working at around 20-40 frames per second. Additionally, ultrasound is very much cheaper than MRI, and its imaging technology can easily be combined with ultrasound therapy devices. The following paragraphs describe the concepts behind currently used monitoring techniques, and those in current research which show promise for future applications.

### *5.4.2 MR thermometry*

Protons within water molecules in the body are associated with a particular 'spin', which precesses under the influence of a magnetic field. Depending on its chemical environment the molecule's rate of precession varies. In an MR scanner, a strong magnetic field is applied,

causing these spins to precess, and pulsed fields are then applied. The response of the molecules is then detected, allowing the scanner to produce an image, in which different chemical environments, e.g., different tissue types, appear as different shades of grey. The frequency of precession is known as the proton resonance frequency (PRF), and is temperature dependent, and thus can be used to determine temperature changes in the body (201, 202). As well as a traditional 'magnitude' image, which is used to look at anatomical details, an additional type of image can be acquired which shows the phase of the spins. Spins precessing in-phase will have a bright appearance, and if they begin to dephase due to changes in the PRF, the signal from this area will be reduced. This is what happens when the temperature in a small region of tissue changes. The PRF will change, the spins will become progressively out of phase, and this change can be detected in these so-called phase images. By subtracting a reference phase image, obtained before heating, from one taken during heating, the phase change can be calculated in units of parts per million (ppm) of the applied magnetic field. From many measurements made in water and tissues, a generally accepted value of the PRF coefficient (α, the change in PRF for a given change in temperature) has been calculated to be -0.01 ppm/°C.

Equation 1 shows the relationship used to calculate temperature change (ΔT) from a change in phase (ΔΦ), which uses α.

$$\Delta T = \frac{\Delta \phi}{2\pi \gamma B_0 \alpha TE} \qquad (5)$$

The other quantities in the equation are known. The gyromagnetic ratio *γ* is a property of the proton; $B_0$ is the static magnetic field strength, and *TE* the echo time of the sequence being used for imaging, chosen by the user. In practice, a temperature map is acquired as rapidly as possible during HIFU heating, and overlaid onto an anatomical image, to indicate precisely where the heating has occurred. Calculations may then be made of the thermal dose as time progresses, and once a given part of the image has been shown to reach a sufficient thermal dose, this part of the anatomy is assumed to have been successfully treated. There is a trade off between the spatial and temporal resolutions achievable as these are determined by the voxel size.

### *5.4.3 Ultrasound imaging guidance*

Ultrasound imaging suffers from poor contrast compared to MRI, however there are certain features, which make it particularly suitable for HIFU guidance.

Many systems rely on the sudden appearance of bright echoes within the treated tissue. This phenomenon has been investigated in many research studies, and is thought to relate to the onset of boiling cavitation. Although in theory this does not give a direct indication of thermal ablation, studies relating ultrasound bright-ups to coagulated regions in tissue excised after treatment have shown a correlation between the treated volume and the bright region in the ultrasound image (203). Additionally, it has been shown that sub-ablative intensity exposures can also be visualized, indicating the possibility of localizing the ultrasound beam position prior to treatment (203, 204).

In addition to the above method of looking at changes in the ultrasound backscatter to indicate tissue changes, the other important effect that occurs on ablation is that the attenuation of the tissue increases suddenly. Through detailed signal processing, the combination of changes in both attenuation and backscatter has been shown to successfully indicate treated tissues (205).

### *5.4.4 Neuronavigation*

As described previously, current brain therapy devices are composed of hundreds of transducers, are capable of emitting more than 1000 acoustic watts(206) and are MR guided. Nevertheless, low energy applications such as BBB opening and neurostimulation require 10 to 100 times less power, and can be performed using small lightweight transducers that are compatible with neuronavigation guidance(207-209): optical cameras track the position of reflective spheres attached to the transducer in real time and the location of the geometrical focus of the transducer is displayed in real time on a set of 3D MR images acquired before the treatment.

One might suspect that single element transducers cannot compensate for the distorsions induced by the skull bone (time delays are adjusted on multielement arrays to compensate the phase shifts induced by the skull). However, acoustic lenses that shape ultrasound fields have recently been introduced t (210, 211), and have been adapted to perform transcranial

focusing with single element transducer(212-214), paving the way for neuronavigated deep brain therapy.

### *5.4.5 Motion compensation*

The movement of the organ often limits HIFU treatments, particularly in abdominal regions, during treatments of many minutes. Motion caused by the heartbeat, respiration and patient motion all contribute to an uncertainty in the exact target location relative to the HIFU focus. One advantage of ultrasound imaging during HIFU is that the fast frame rates allow motion to be monitored rapidly. Using obvious structures within B-mode images, it is possible to overcome the problem of tissue motion to some extent. One option is to gate the treatment, a technique whereby the HIFU beam is only switched on when the target region of tissue is within a specific region of the image. For the case of respiratory motion, for example, this would occur once during every respiratory cycle. The main disadvantage of this is that treatment times may be increased, although, as some cooling is required between sonications depending on the location and amount of motion, the time increase may not be significant. Alternatively, with developments in technology and image processing, researchers have shown that it is possible to compensate for motion by moving the focus of the HIFU beam as the tissue moves. This has been demonstrated in tissues moving at rates up to 40 mm per second (215). Tissue motion and deformation compensation has also been demonstrated during real time ultrasound thermometry in a rubber phantom
Motion compensation is not restricted to ultrasound guided HIFU. With rapid enough MR imaging, it has also been demonstrated using the Sonalleve HIFU platform (216)

## 6 Histotripsy

The term histotripsy, coined by Professor Charles Cain at the University of Michigan in 2003, derives from the Greek words ‘ιστός’ (soft tissue) and ‘τρυπσειν’ (to crush), refers to the mechanical destruction of tissue using the phenomenon of acoustic cavitation(217). This is achieved by using short (µs) bursts of ultrasound at high peak pressure amplitudes (>10MPa) and low duty cycles (<1%). Three types of histotripsy are often defined: ‘intrinsic threshold histotripsy’, ‘shock scattering histotripsy’ and ‘boiling histotripsy’. These each use slightly

different ultrasound exposure parameters to achieve the desired effect of producing localized damage in the form of acellular debris (218).

Intrinsic threshold histotripsy uses 1-2 cycle pulses of very high negative pressures that exceed the tissue's inertial cavitation threshold (>26MPa) in order to form a bubble cloud at the beam's focus. The activity of the bubbles in this cloud liquefies the tissue. The number of pulses needed to produce the desired effect varies, with stiffer, collagen rich tissues being more difficult to damage than 'soft' tissue. 50-2000 pulses are required at duty cycles ≤ 1%. Shock scattering histotripsy regimes require slightly lower negative pressures (15-25 MPa) and somewhat longer pulses (3-10 cycles). This form of damage relies on the generation of an individual microbubble during the first couple of cycles. This is possible as cavitation is a probabilistic phenomenon. The nonlinear propagation that occurs in tissue at these high acoustic pressures leads to shockwaves with increased amplitudes (>50 MPa). When a positive shock front meets the bubble it is inverted in phase, and is reflected back towards the transducer with a peak negative amplitude that exceeds the intrinsic cavitation threshold, leading to a destructive bubble cloud. The third type of histotripsy relies on the generation of boiling bubbles by 1-20 ms pulses at high-pressure amplitudes (>70 MPa $P^+$, 10-20 MPa $P^-$). The damage mechanism here is thought to be tissue atomization. Since this technique relies on the production of microbubbles, the damage is visible on ultrasound images as it progresses.

Histotripsy induced damage in intact tissues such as the liver or prostate results in a liquefied homogenate of acellular debris. This is absorbed by the body over ~1-2 months, leaving a small scar`(219). When the bubble activity occurs at a tissue/fluid interface, tissue erosion is seen (220, 221). This can be useful for targeting the atrial septum, or dissolving a blood clot (217, 222).

Collagen rich structures such as large blood vessels, nerves and bile ducts are most resistant to histotripsy damage. This can be useful when treating near critical structures.

Since effects seen are solely due to bubble activity, damage is restricted to the region enclosed by the bubble cloud, and the boundary (in static tissue) is very sharp, on occasion bisecting a single cell.

There have been extensive pre-clinical studies of potential applications of histotripsy, including for hepatic, prostatic, renal, breast and pancreatic cancer. Of particular interest is

the possibility that the release of tumour specific antigens from the cavitation disrupted tissues might result in an anti-tumour immune response. A number of studies are now showing that this can occur. The potential use of histotripsy for treating brain tumours and intracerebral haemorrhage (ICH) has been investigated (223, 224). For ICH, transcranial histotripsy has been used in a porcine model in which large clots have been liquefied non-invasively under catheter based hydrophone guidance (225). Other applications currently under investigation include DVT clot dissolution (226), atrial septum perforation in neonatal hypoplastic heart syndrome (227) and treatment of calcified aortic stenosis (228).

Histotripsy is still in its infancy, and so only a few human clinical trials have been reported. A phase I trial for the treatment of benign prostate hyperplasia in 25 patients resulted in an improved International Prostate Symptom Score (IPSS) similar to that obtained with oral medication, but no tissue destruction was seen (229). The exposure regime used was that previously established in dogs. It is clear that although further work is required to optimize the treatment in order to improve its efficacy, the technique was shown to be safe.

Probably the most pioneering trial to date was undertaken by Vidal et al in Barcelona (https://www.fusfoundation.org/news/results-of-first-in-human-histotripsy-trial-for-liver-cancer-presented). They treated 11 liver tumours in 8 patients (one HCC, 10 metastases), without any adverse events. Although one tumour was mis-targeted due to poor imaging, the ablation zone (treated tumour and margin) contracted by an average of 71.8% in volume at 2 months. Two patients showed a post-procedural decline in tumour markers.

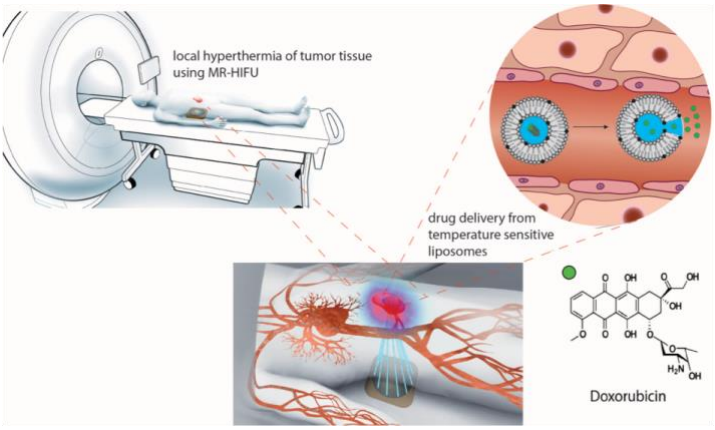
While still not widely used, histotripsy has been shown to be a useful tool for the non-invasive removal of tissue, providing highly localised damage, and tissue selectivity. The production of microbubbles allows real-time ultrasound monitoring of tissue damage using ultrasound, and the release of tumour-specific antigens may stimulate an immune response in cancer therapy.

## 7 Ultrasound mediated Drug delivery

There is increasing interest in the use of ultrasound-guided delivery of drugs and genetic material to tissues. There are a number of advantages to this approach, including the ability to minimize systemic dose and toxicity by calling on the spatial specificity available with focused beams. A number of excellent reviews on this, now extensive, topics have been written (17, 18, 230-235). Depending on the sonication protocol used, drug delivery can be

induced by local heating or by local mechanical effects acting on responsive drug delivery systems and tissue.

**Figure 8** **Concept of hyperthermia-induced drug delivery from Temperature Sensitive Lipsomes (TSLs) using MRgHIFU as a hyperthermia device. The TSLs can be loaded with hydrophilic drugs such as doxorubicin. At hyperthermic temperatures (40-43 ºC), TSLs become porous and release their payload within the heated target tissue.**




One of the first methods to be used in this field involved thermally sensitive liposomes (TSLs) in combination with ultrasound-induced hyperthermia (71). TSLs are nanoparticles in a size range of 100- 200 nm diameter with a lipid bilayer enclosing a water-rich inner compartment, which can be loaded with cytotoxic, hydrophilic drugs, such as doxorubicin, mitomycin, cisplatin, gemcitabine. The lipid bilayer is typically made up of a mixture of lipids that are at body temperature in sol-gel state and stably encapsulate the drug.

When exposed to hyperthermic temperatures, the lipid bilayer goes through a sol-gel to liquid-crystalline melting transition ($T_m$) triggering a rapid release of the drug. For drug delivery, the tumor tissue is heated with ultrasound to hyperthermic temperatures and TSLs are administered by infusion. Within the heated tissue, continuous drug release from TSLs is obtained as long as the hyperthermic temperatures are maintained (figure 9). Several preclinical studies performed with different doxorubicin-loaded TSLs have shown increased tissue concentrations of doxorubicin by a factor of 5 – 25 for various TSL formulations and

ultrasound protocols (236). One example for a doxorubicin-TSL formulation that reached clinical stage is Thermodox™ (Celsion Corporation, USA), which is designed to release its payload of doxorubicin at 42-45$^{o}$C. Thermodox has recently been used in a Phase I pilot study where the temperature in metastatic liver regions was raised using focused ultrasound (104, 105). The doxorubicin concentration is shown to be increased in the heated region. For ultrasound-induced hyperthermia, MR-guidance offers the advantage of non-invasive, near-real time thermometry, which can be used for a closed feedback to the ultrasound controller (237-239).

Mechanical effects of ultrasound can also be exploited for drug delivery applications. The ultrasound-induced increase in cellular permeability is often termed sonoporation, which depends on acoustic cavitation and on ultrasonically driven bubble activity. The bubbles involved may be endogenous or exogenous. Since acoustic pressures above the cavitation threshold are needed to draw exogenous bubbles out of tissues, the preferred method is to use introduced bubbles. The microbubble agents commonly used for ultrasound contrast imaging may be used, and increasingly, specifically designed carriers are being investigated. The oscillations of these bubbles in the ultrasound field generate mechanical stress on nearby cells. This may lead to cellular shape changes, which can cause small temporary openings in extracellular membranes. These membrane pores may be 10 – several hundred nm in size. If drugs are introduced simultaneously with these bubbles, their intracellular uptake may be enhanced. These pores may remain open up to 120s, with the smaller pores resealing most readily. The process of endocytosis can also be promoted by bubble activity. This increases the transport of macromolecules across the cell membrane. It has been suggested that small membrane pores can be sealed by endocytosis (240).

It has also been shown that the tight junctions between cells may be opened during bubble oscillation, allowing larger molecules, biologicals or nanoparticles to extravasate from the vascular system into the extravascular, extracellular space.

More efficient enhanced delivery can be achieved by loading the drug of interest on to the bubble shell. Local ultrasound disruption of the bubble ensures targeted delivery, thus reducing systemic side effects. This is particularly useful where target site access is difficult as may be the case for chemotherapeutic agent delivery to some tumours, and delivery of anti-

inflammatory drugs and genes. An added advantage is that the microbubbles allow imaging at the delivery site.

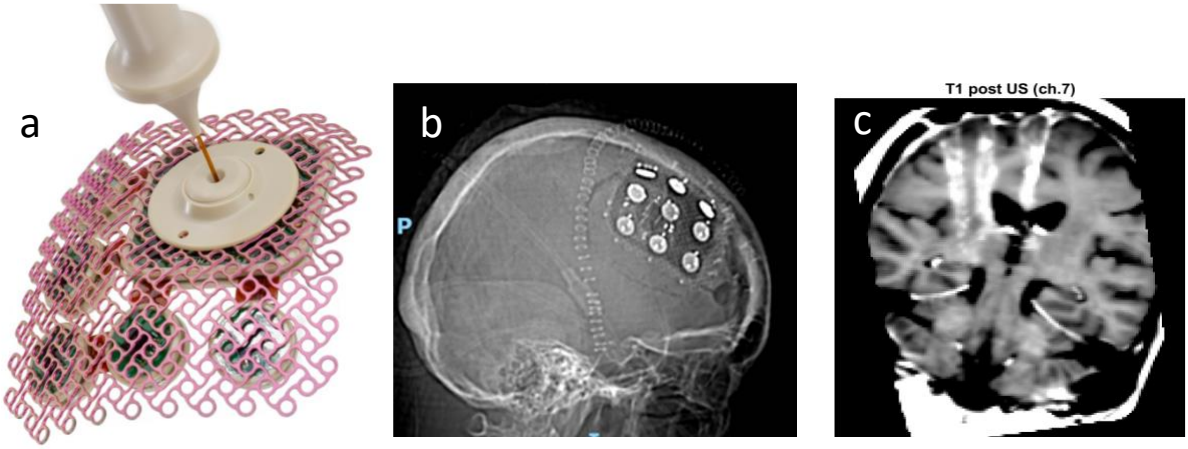
Drug delivery is at its most efficient when the bubble is driven at its resonant frequency. Since the bubble population is often comprised of mixed sizes, chirps (frequency sweeps) are used to drive them (16).

Drug loaded nanoparticles can be co-injected with, or attached to microbubbles. For example, poly(d,l-lactic-co-glycolide (PLGA) nanoparticles have been shown to be endocytosed rapidly into tumours, where they can slowly release their cargo, giving a sustained therapeutic effect (241, 242).

One growing area of ultrasound mediated drug delivery, and that is now being investigated in the clinic, revolves around the finding that the combination of ultrasound at exposure levels and microbubbles can temporarily open the blood brain barrier (BBB) (18, 243-247). The BBB forms a barrier between the capillaries in the brain and it parenchyma. It comprises tight junctions between the intravascular endothelial cells that serve to protect the brain from toxins and inflammatory proteins. However, this means that it also provides a barrier to drugs that might be used for a number of diseases such as cancer, and other neurological disorders. Stably oscillating bubbles can cause mechanical and physiological changes to BBB cells that allow trans-cellular and paracellular passage of molecules into the brain parenchyma (248-250). It has been demonstrated that the concentration of methotrexate in the rabbit brain can be increased by more than 3 times (251). In pre-clinical studies, ultrasound has been shown to enhance delivery of chemo-, immuno- and gene-therapy with subsequent improvement in treatment efficacy (127, 252-254).

The first clinical study of opening the BBB used an unfocused transducer implanted into the skull to enhance the delivery of carboplatin to glioblastoma (Sonocloud™ Carthera, France) (255-257). 19 patients were treated monthly for up to 6 months with increasing acoustic pressures (0.41 – 1.15 MPa). No adverse events were seen, and BBB opening was found in 56/65 treatments at acoustic pressures above 0.8MPa in combination with Sonovue bubbles (1.05 MHz). Interestingly, patients with no, or poor, disruption of the BBB visible on MRI had a median overall survival of 8.64 months that increased to 12.94 months for patients with significant disruption of the BBB. Further clinical studies are now ongoing with a larger implant and 9 implanted transducers (Figure 9).

**Figure 9 The Sonocloud® (a) is a medical ultrasonic device that is implanted in the skull (b) for transient opening of the BBB and delivering chemotherapy to treat cerebral tumours. Opening of the BBB can be observed on Gd-enhanced T1 weighed MRI (c). Courtesy of Carthera.**




While the Sonocloud™ operates without imaging guidance, Exablate neuro brain system™ (Insightec, Israel) uses MRI to guide the focal beam. This has been used to improve the uptake of drug in 5 patients with high-grade gliomas (258). Encouraged by these results, a multi-centre trial has been started (259). Clinical trials have also been initiated to investigate the role of ultrasound mediated BBB modulation in Alzheimer's disease (260) and Amyotrophic Lateral Sclerosis (ALS) (261).

The most recent innovation in this field is a neuronavigation-guided focused ultrasound system NaviFUS (Taiwan). This uses a hemispherical phased array transducer.(208). The advantage of this system is that it only involves semi-rigid fixation.

## 8 Calibration of therapeutic ultrasound devices

In delivering a therapy ultrasound exposure it is essential that the acoustic output from the therapy transducer is known and that it is regularly checked. The devices used for these measurements are, in the main, very similar to those for characterizing diagnostic ultrasound fields and are well described in chapter 28 of this course book. An additional device that is

used for characterizing the higher power fields used in therapy is the radiation force balance, which enables measurement of the total acoustic power, but gives no information as to the pressure (or intensity) distribution in space (262, 263). An ultrasound beam can generate a radiation force that is weak (equivalent to 0.67mN/W for an absorbing target) but can be used as the basis for measuring acoustic power. Under the right conditions, it is possible to measure acoustic powers of the order of 1mW using a target suspended on a precision chemical balance (264). This technique is widely used to measure the output power of a large variety of ultrasound devices for both imaging and therapy, with many measurement systems being available commercially. An attractive feature of the radiation force technique is the simplicity with which the measured force can be converted to a power by multiplication with the sound speed. These balances are relatively easy, and fast to use, lending themselves to routine QA protocols (265).

While it is energy deposition that it would be most useful to quantify for treatments, in the absence of agreement about a dose concept for ultrasound, there are a number of important parameters that should be quoted when properly describing an ultrasound exposure. These are listed in Table 1. It can be seen that the most relevant parameters depend on the type of therapy being employed (265, 266). It must be remembered that measurements can only be made under ‘free field’ conditions in a suitably designed water bath that is large enough to avoid reflections of the beam from its walls confusing the ‘true’ energy distribution from the probe. In order to calculate the actual exposure, and hence energy delivered, to deep seated tissues it is important to know the attenuation and/or absorption coefficient both for the target and for the overlying tissues.

**Table 1 Important parameters for describing therapy ultrasound fields.**

| Parameter | Measurement device | Therapy type | Rationale for its reporting |
|---|---|---|---|
| Acoustic frequency | Hydrophone/ Oscilloscope | all | Important for determining likely mechanisms, & treatment depths |
| Negative pressure amplitude (p-) at focus | Hydrophone | Histotripsy, bubble based therapies | Important for cavitation |
| Positive pressure at focus (p+) at focus | Hydrophone | All, especially bubble based therapies | Important for both heating and cavitation |
| Beam width | Hydrophone | All, especially thermal therapies | Tells you about the spatial extent of the ultrasound exposure<br>Ensures safety of critical structures that may lie in the beam path |
| Time averaged intensity | Hydrophone | Thermal therapies | Important for heating |
| Total acoustic power | Radiation force balance | All, especially thermal therapies | Important for heating |
| Exposure duration and pulsing regimes | Hydrophone | Both thermal & bubble based therapies | Important for both heating and cavitation<br>Important for determining safety & efficacy |
| Focal position | Hydrophone | All | Important for both heating and cavitation, and for ensuring safety of critical structures |
| Position & amplitude of extra-focal peaks | Hydrophone | All | Important for ensuring safety of critical structures |

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