An Overview of the Biological Effects of Focused Ultrasound

*Note: the descriptions of individual biomechanisms in this document also appear on the Focused Ultrasound Foundation’s website (www.fusfoundation.org)

Abstract
Focused ultrasound is a medical technology platform that can produce a variety of biological effects in tissue that enable its potential use in many clinical indications. With an emphasis on the clinical applications of focused ultrasound currently under investigation and those planned for the near future, this manuscript provides an overview of focused ultrasound-induced bioeffects and their underlying thermal and mechanical mechanisms. It is these bioeffects that are the foundation for new focused ultrasound therapies with the potential to provide alternative or complementary treatments to improve the quality of life for millions worldwide.

Introduction
Focused ultrasound is a platform technology that produces a variety of biological effects in tissue that enable noninvasive treatment of a wide range of clinical conditions [1]. As represented in Figure 1, a specific bioeffect may enable treatment of multiple conditions. Similarly, a specific condition may benefit from multiple different bioeffects. When assessing focused ultrasound to address a given clinical need, it is important to evaluate the role of many bioeffects, including the synergism between multiple bioeffects, to best optimize the treatment.

These localized bioeffects are produced by either thermal or mechanical mechanisms of ultrasound interaction with the targeted tissue. These thermal and mechanical effects and their biological outcomes – bioeffects – are determined by the type of tissue (i.e. muscle versus bone) and the acoustic parameters (power, transmission duration, and mode – continuous versus pulsed).

Thermal Effects
The continuous transmission of acoustic energy raises tissue temperature at the focal point in the body [2]. The magnitude and duration of this temperature elevation is quantified as the “thermal dose” delivered to the tissue. Ultrasound energy can be used to create either a low level thermal

Figure 1. Focused ultrasound is capable of inducing 17 different biological effects when it interacts with tissue. Some of these bioeffects can be used in the treatment of many different diseases, and some diseases may benefit from the combination of several different bioeffects. (Click to enlarge)
rise over several minutes or hours (local hyperthermia) [3] or, conversely, a short (seconds), highly localized high temperature rise that destroys the tissue via protein denaturation (thermal ablation) [4]. The graph in Figure 2 illustrates different levels of thermal dose and their biological outcomes.

**Figure 2.** The threshold for thermal necrosis depends on temperature reached in the tissue. As the temperature decreases the exposure time needed to achieve thermal necrosis increases exponentially. Temperature and exposure times generally used for both local hyperthermia and thermal ablation are marked. Tissue boils above the threshold of 100°C regardless of the exposure time. (Click to enlarge)

*Mechanical Effects*
Ultrasound application using high power and very short pulses leads to a low energy deposition in the tissue and thus a minimal thermal rise. However, this type of application will create a large pressure change in the tissue that can induce various mechanical effects, from vibration of the target to cavitation [3,5].

As ultrasound waves propagate through tissue, they interact with dissolved gases in a process known as cavitation [5]. This is the most prominent mechanical effect of focused ultrasound. Depending on the clinical application, these dissolved gases can either be in the form of injected microbubbles or generated by the ultrasonic peak negative pressure itself [6,7]. In both cases the dissolved gas will be referred to as microbubbles in this review.

There are two types of cavitation: stable and inertial. Stable cavitation describes the steady oscillation of the size of microbubbles as the pressure changes at the focal point. It can induce moderate changes at the cellular level, such as increasing cell membrane permeability to drugs and other molecules [8]. Inertial cavitation occurs when the transmitted power is high enough to cause a violent collapse of microbubbles that destroys the tissue [3,5]. This type of cavitation can be used to create mechanical “lesions” such as those created by histotripsy [9].

Other physical phenomena like acoustic streaming and radiation forces also contribute to mechanical-based bioeffects [10].
Biological Effects

Applying focused ultrasound to living tissue results in one or more of the mechanisms listed in Figure 1. These diverse bioeffects may be categorized as tissue destruction, localized drug delivery, or a range of other effects.

Tissue Destruction

Tissue destruction is one of the most common applications of focused ultrasound [2]. By utilizing either thermal energy to induce the denaturation of proteins [4] or mechanical energy to destroy tissue using stress [3], focused ultrasound can be used to treat many diseases.

Thermal ablation

Thermal ablation, the most clinically advanced bioeffect of focused ultrasound, produces cell death in a targeted area with minimal damage to the surrounding tissue, as shown in Figure 3 [3,5]. Tissue damage can be accurately controlled using a range of focused ultrasound transducers with different sonication sizes. Magnetic resonance imaging allows for the monitoring of temperature rise in real time, allowing quantification of the therapeutic dose [11]. Alternatively, ultrasound imaging and tissue characterization techniques (e.g. elastography) can be used for treatment monitoring for many clinical applications [12]. Depending on the equipment and parameters used, the volume of focused ultrasound lesions can be as small as a grain of rice (10 cubic millimeters) [5]. This allows for an extremely localized treatment and a sharp border between treated and untreated areas.

For treatment of larger structures such as tumors, multiple lesions can be combined to encompass the entire volume [5,13]. A cooling period between sonications is often required to prevent unwanted heating of surrounding tissue. Therefore, the treatment of very large structures can be time-consuming. However, optimized scanning algorithms, the injection of microbubbles to increase the absorption of acoustic energy, and the use of spiral sonications are all techniques that have been employed to reduce the time of treatments [13].

Figure 3. Thermal ablation. When focused ultrasound is used in a continuous-wave (CW) mode, the temperature reached at the focal point can achieve a cumulative thermal dose great enough to kill the tissue by coagulative necrosis. This process works through the denaturation of the cellular membrane and can be focused to a volume as small as 10mm³. (Click to enlarge)
Focused ultrasound’s thermal ablation effect has been the most widely explored clinically, and may be used to non-invasively treat a variety of clinical conditions including symptomatic uterine fibroids [14,15]; tumors in the prostate, breast, and liver [4,5,16]; low back pain [17]; and brain disorders such as essential tremor, Parkinson's disease, and neuropathic pain [18–20] among many other conditions.

**Mechanical destruction**

The non-thermal effects of focused ultrasound can also be used for the precise destruction of tissue. At high enough acoustic intensities with a short pulse duration, inertial cavitation will release a shockwave capable of destroying cell membranes and even liquefying or annihilating cells as shown in Figure 4 [6,21]. The use of inertial cavitation to mechanically destroy regions of tissue is known as histotripsy, and is usually the compounded effect of multiple shockwaves. This technique can be very precise, causing minimal damage to surrounding tissue, and the bubbles used in cavitation are easily visible with ultrasound imaging, enabling accurate targeting and monitoring [9,22].

Clinically, histotripsy has a wide range of possible uses from cardiovascular disease [23] to various types of cancer [24]. For very sensitive regions such as the brain, more research is needed to confirm the safety profile of treatment with histotripsy. Using injected microbubbles, to lower the threshold for inertial cavitation only at the target, may help reduce damage to adjacent tissue in the brain [21,25].

**Targeted Drug Delivery**

Through various mechanisms, focused ultrasound can increase the precise delivery of drugs to targets in the body.

**Sonoporation**

Cell membranes often prevent large molecules such as drugs and genes from entering cells and taking effect. The mechanical force of focused ultrasound, via stable cavitation, can modify the permeability of cell membranes and enhance the absorption of these molecules. This effect,
known as sonoporation, can increase the efficacy of drugs and genes in precise areas in the body [8].

Stable cavitation can induce moderate and reversible changes at the cellular level, creating pores in cell membranes, allowing a greater volume of compounds to enter the cell, as shown in Figure 5 [8]. Additionally, stable cavitation produces acoustic streaming, which increases the flow of fluid in a cell’s environment. This increase in flow may assist in the opening of the pores, and it also directs the therapeutic molecules toward the cells, which enhances cellular uptake [26,27].

Enhanced drug delivery via sonoporation could enable treatment of tumors with dense stroma such as pancreatic tumors, and with less systemic toxicity (i.e. less circulating drug required) than with traditional chemotherapy. Focused ultrasound induced sonoporation is also an attractive option for delivery of genetic material when compared to the alternatives, because it can be used in vivo and can greatly increase the specificity of treatments [28]. Gene therapy can be used to treat a wide range of indications from immunodeficiency disorders to Parkinson’s disease and even certain types of cancer [29–31].

**Increased vascular permeability**

Physiological barriers exist between the interior of blood vessels and their surrounding tissue, which can limit delivery of drugs to their intended targets. Focused ultrasound can reversibly increase the permeability of blood vessel walls, thereby temporarily allowing drugs to pass through them and into the surrounding tissue [7].
The delivery of drugs across vessel walls is limited primarily by a network of endothelial cells joined by tight junctions [32]. The mechanical effects of focused ultrasound disrupt these tight junctions to increase permeability, as shown in Figure 6 [33]. Microbubbles can also be used to better control this process to reduce the risk of damage to the vessel [34].

This same effect has been used to open the blood-brain barrier, a particularly dense barrier of cells that severely inhibits the diffusion of many drugs and gene therapies into the brain (see Figure 7). In a pre-clinical setting, focused ultrasound coupled with injected microbubbles has been used to transiently open this barrier and enable delivery of various compounds into the brain [7,34,35]. The blood-brain barrier has been shown, via both MRI and histological analyses, to revert to its original structure without permanent damage within four hours after the end of the sonication [34].

This technique could enable more effective pharmacological treatment of tumors throughout the body, and through blood-brain barrier opening, the treatment of various neurological disorders such as Parkinson’s disease, Alzheimer’s disease, and glioblastoma [7,36–38].
Local hyperthermia

Elevating tissue temperature to a mild 42°C (107°F) and maintaining for several minutes can increase blood flow and drug absorption in the targeted region without causing permanent damage [39]. Using the body's response to this localized mild hyperthermia, drug delivery and uptake can be enhanced [40]. Additionally, more oxygen is delivered to hyperthermic targets, enhancing their metabolic activity and sensitivity to drugs. This method has been used in clinical settings to enhance the delivery and efficacy of drugs in targeted areas with restricted blood flow, especially tumors (see Figure 8).

**Figure 8. Local hyperthermia.** Mild hyperthermia can be achieved at specific targets inside the body using focused ultrasound. The body’s natural response to hyperthermia increases the delivery and uptake of drugs and oxygen to the target site, enhancing the local effects of the drug. (Click to enlarge)

Focused ultrasound is an optimal technology for inducing hyperthermia because of its precise focus and its ability to deposit energy in various shapes and sizes. Tissue temperature can be monitored in real time using magnetic resonance imaging, ultrasound imaging or interstitial thermocouples, which allows for the accurate control of the treatment [41,42]. The effects induced by local hyperthermia are temporary and precise, and hold potential to make focused ultrasound an excellent complement to drug therapy [40,43,44]. Because of focused ultrasound’s ability to penetrate deep into the body, there are numerous and wide ranging potential clinical uses for hyperthermia. Many drugs would benefit from the enhanced delivery and efficacy, and mild hyperthermia has even been shown to induce an immune response against some tumors [45].

**Drug delivery vehicles**

Focused ultrasound can be used to release encapsulated drugs (e.g. genes, chemotherapeutics), delivering them in high concentrations to a precise point while minimizing their systemic effects. In this process, a drug is encapsulated in or bonded to a carrier vehicle (e.g. microbubble, liposome), that is sensitive to either elevated temperatures or pressures [46]. These carrier vehicles are then injected into the bloodstream. This encapsulation prevents the drug from interacting with its surroundings as it circulates throughout the body. Next, ultrasound is focused on the targeted area, causing the carriers to release the drug or to decouple from the drug which is then quickly absorbed by the surrounding tissue. Although the encapsulated drug is present throughout the entire body, it is only released in the area targeted by focused ultrasound. In this
way, the drug can circulate harmlessly throughout the body, and only be activated where desired. See Figure 9 for details on this mechanism. [39,47–49]

Figure 9. Drug delivery vehicles. Focused ultrasound is an ideal modality to combine with drug delivery vehicles, because it has two methods that can be used to release the encapsulated drugs: heat and pressure. Using delivery vehicles reduces the systemic toxicity of the encapsulated drug and increases the concentration of the drug at the target site, which is especially useful for chemotherapeutics. (Click to enlarge)

A significant amount of recent scientific work has been devoted to optimizing the various types of carrier vehicles such as microbubbles, liposomes, and nanoparticles. Release of drugs from microbubbles (i.e. ultrasound contrast agents) can be readily monitored in real time using ultrasound imaging. If drugs are coupled to MRI contrast agents, MRI can also be used for monitoring. With the use of low temperature sensitive liposomes (LTSLs), high intensity focused ultrasound is used to induce mild hyperthermia in a targeted location, characterized by a temperature elevation to approximately 40ºC [43,47]. As LTSLs pass through the hyperthermic region, the increased temperature causes their decomposition and the subsequent release of encapsulated drugs [47,49]. For acoustic pressure-sensitive carriers, ultrasound induced cavitation and radiation forces can “pop open” the carrier or decouple the drugs from the carriers, allowing for their release in the targeted area [50].

Hyperthermia [43], stable cavitation [8], and radiation forces [5] from focused ultrasound have all been shown to increase local drug absorption from the bloodstream. In heated tissue, blood flow and the rate of chemical diffusion are both enhanced [43], leading to more efficient uptake of drugs into the surrounding tissue. Stable cavitation induces acoustic streaming and increases cell membrane permeability [8], which also locally increases drug bioavailability.

Clinically, combining focused ultrasound with drug delivery vehicles presents an attractive method for chemotherapy delivery. These drugs, which are typically delivered systemically, are very toxic to healthy cells. By taking advantage of drug delivery vehicles, chemotherapeutics can be delivered at high concentrations in the tumor without the systemic toxicities. These vehicles can also be used to deliver genes to specific targets in the brain to treat neurological disorders.
Vasodilation

Vasodilation—the widening of blood vessels—increases blood flow in a region. In tissue that is ischemic, vasodilation can be induced to enhance the effects of radiotherapy by increasing the delivery of oxygen and blood to the target. Vasodilation can also aid drug treatments by increasing the amount of the drug delivered to a target.

Focused ultrasound can create a pressure change at a precise location, triggering the endothelium of targeted blood vessels to release nitric oxide, the chemical signal that causes smooth muscle relaxation and the dilation of blood vessels (see Figure 10) [51]. This is a reversible process, and blood vessels revert to their original size shortly after the end of the focused ultrasound treatment with no permanent damage to targeted tissue [51,52]. Thermal effects are minimal when pulsed focused ultrasound is used, however, local hyperthermia will also cause localized vasodilation.

Certain drugs have been shown to more easily diffuse across dilated vessels, increasing the bioavailability of these drugs in surrounding tissues. Furthermore, dilated blood vessels carry a larger volume of blood, potentially providing a further increase in the amount of drugs absorbed by tissue [40]. By using focused ultrasound to noninvasively induce local vasodilation, the delivery of drugs to targeted tissues could be enhanced without causing permanent damage to blood vessels.

Due to the enhanced efficacy of radiotherapy after vasodilation, this treatment is perfectly suited to be used as a neo-adjuvant treatment prior to radiation therapy for many different cancers. Furthermore, there are numerous clinical indications that could be more effectively treated with the enhanced drug delivery effects of vasodilation.

Other Effects

Vasoconstriction

The mechanical and thermal effects of focused ultrasound can induce vasoconstriction, the reduction of blood vessel diameter, either temporarily or permanently [53,54]. Clinically, this could be used to treat vascular malformations or to block the downstream flow of blood in order to deprive tumors of nutrients [55,56].
When exposed to ultrasonic pulses of very short durations, blood vessels have been shown to constrict, though the mechanism is not well known (see Figure 11). In preclinical studies, this vasoconstriction slowly reduced over a period of up to two weeks after the vessels had been affected by ultrasound. This temporary vasoconstriction occurs with minimal thermal effects, indicating that the mechanical activity of the ultrasound is most likely responsible for inducing the physiological change [53].

Alternatively, by utilizing the thermal effects of focused ultrasound, it has been proposed that superficial venous insufficiency – a disorder caused by valvular incompetence in the venous system – could be treated by shrinking the intermediate fibers, namely collagen, in the endothelial lining. In one preclinical study, focused ultrasound was used to cause vasoconstriction in the great saphenous vein, correcting the insufficiency [54].

**Sensitization to chemotherapy**

Inducing hyperthermia in a tumor can allow treating physicians to enhance the effects of chemotherapy or achieve the same therapeutic outcome with lower doses of chemotherapy to minimize the treatment's adverse effects [57]. Focused ultrasound can bolster the effects of chemotherapeutics in a number of ways. First, through the effects of local hyperthermia, greater concentrations of drugs can be delivered to tumors [58]. Second, the increase of blood flow due to the hyperthermia also increases the amount of oxygen delivered to the tumors. This increases the metabolic activity of the tumor cells and enhances the cytotoxicity of the drug [59, 60]. In addition, it has been shown that cells that have acquired resistance to a particular drug can be made vulnerable again after ultrasound treatment [57, 61]. Finally, local hyperthermia also weakens the tumor due to its reduced ability to dissipate heat, making the tumor more susceptible to chemotherapy yet leaving healthy tissue unaffected [57, 62]. See Figure 12 for details on this mechanism.

Focused ultrasound provides a radiation-free, non-invasive method of inducing local hyperthermia [44]. The synergy between chemotherapy and focused ultrasound could allow for more effective chemotherapeutic treatments of cancer with diminished side effects [63]. Established methods of
coupling hyperthermia with chemotherapy have been proven effective in pre-clinical work with ovarian and cervical cancer, however, this mechanism may be beneficial for any cancer that is responsive to chemotherapy [62,64].

**Sensitization to radiotherapy**

Similar to sensitization to chemotherapy, tumors can be made more receptive to radiotherapy through local hyperthermia [63]. Hyperthermia triggers an increased flow of blood and delivery of oxygen to a tumor, enhancing the rate of its metabolic processes, which increases the efficacy of radiotherapy, especially in cases where the tumor tissue is hypoxic [60,65]. Sensitized tumors require a reduced radiation dose in order to induce cell death as shown in Figure 13, which mitigates the side effects of radiotherapy [40,57].

Conventional methods for inducing sensitization to radiotherapy are the same as those used in the sensitization to chemotherapy processes, and thus focused ultrasound provides the advantage of being a non-invasive, local, and non-ionizing method of inducing hyperthermia [40]. In tumors where radiation therapy is a viable treatment option, focused ultrasound could be used as a neo-adjuvant to reduce the radiation dose needed to kill the cancer cells. The toxic effects of radiation accumulate in the body with each treatment, so reducing the dose needed in a single treatment affords the patient the ability to undergo additional radiation treatments later in their life if necessary [66].

![Figure 13. Sensitization to radiotherapy.](Click to enlarge)

**Neuromodulation**

Focused ultrasound can stimulate or suppress neural activity, depending on the parameters of the energy applied to neural tissue (see Figure 14). The mechanical effects of focused ultrasound are thought to dominate this mechanism [67–70], however, there is evidence of neuromodulation during various brain treatments at temperatures below the thermal ablation threshold [19].

Neuromodulatory effects could potentially enable a range of therapeutic benefits including:

- verifying targets in the brain prior to ablative procedures;
- suppressing epileptic seizures or symptoms of psychiatric disorders; and
- temporarily blocking nerves to treat pain;
Studies have shown that the mechanical effects of pulsed focused ultrasound can reversibly decrease the functionality of targeted neurons [68]. This allows for the temporary blocking of neural signals from targeted locations within the brain or spinal/peripheral nerves. Such techniques hold promise in the treatment of epilepsy or chronic pain [68,71].

Conversely, pulsed focused ultrasound can be used to stimulate targeted neurons [72]. Ultrasound energy with specific pulse parameters can trigger the activation and propagation of neural signals that could stimulate precise areas of the brain. This would enable the possibility of mapping neural networks and enhancing our understanding of the brain [73,74].

Finally, the thermal effects of focused ultrasound can also be used to induce neuromodulation. When brain tissue is raised to a slightly elevated temperature—lower than that required for thermal ablation—neural signals may be temporarily suppressed in that area [19]. This technique can be used to confirm the target in the brain during neurological treatments (e.g. essential tremor) before delivering the therapeutic dose of ultrasound energy to create a permanent lesion.

**Immunomodulation**

The treatment of tumors with focused ultrasound can stimulate the immune system and potentially enhance the body's ability to manage cancer. When a tumor is ablated, the exposed proteins and cellular debris can act as antigens that trigger an increased immune response to the tumor, both locally and potentially at distant metastases [63,75]. Furthermore, thermal ablation may cause local inflammation, stimulate the recruitment of immune effector cells, and activate anti-tumor adaptive immunity, all of which can increase the body’s resistance to cancer [76]. Immunomodulation through focused ultrasound could non-invasively augment and enhance existing chemotherapy and immunotherapy treatments for the management of tumors. Depending on the “mode” of focused ultrasound used – thermal ablation, mild hyperthermia, or mechanical destruction – there may be a slightly different immune response. Yet each of these mechanisms is capable of initiating an immune response by releasing antigens and danger signals from cancer cells (see Figure 15).

![Figure 14. Neuromodulation.](https://example.com/figure14.png)

Pulsed focused ultrasound is capable of both exciting and inhibiting neurons through its mechanical effects. The exact mechanism for this neuromodulation is still unknown. (Click to enlarge)
There are three proposed methods by which focused ultrasound may induce an immunotherapeutic response. First, tumor cell are stressed via one of the three focused ultrasound modes, causing the up-regulation of danger signals such as heat shock proteins (HSP60 and 70) and ATP, which can act as tumor vaccines and increase the immunogenicity of the tumor [77,78]. Second, focused ultrasound mitigates tumor-induced immunosuppression by decreasing levels of immunosuppressive cytokines (e.g. VEGF, TGF-β1, and TGF-β2) [79]. Third, cancer cell destruction creates tumor debris that act as antigens for antigen presenting cells, enhancing the immune response. Tumor debris can activate dendritic cells [80] and other anti-tumor adaptive immunity cells, such as CD8+ T cells [76].

However, based on the research to date, this effect may not be strong enough to control tumor growth on its own. Researchers are investigating the use of focused ultrasound in combination with other cancer treatments such as immunotherapy drugs. Because of focused ultrasound’s ability to penetrate the blood brain barrier and other dense stroma, it is an attractive modality to potentially boost the effectiveness of immunotherapies in notoriously difficult to treat cancers. Additionally, research into immunotherapies has shown that they are more successful in patients who have a baseline immune response to the cancer, which focused ultrasound is well poised to provide [81].

**Clot lysis**

Focused ultrasound, either alone or enhanced by microbubbles and/or thrombolytic agents, can dissolve blood clots [82]. Ultrasound energy causes vibrations that can either break the clot apart directly (see Figure 16) —via disruption of the fibrin matrix—or make it more susceptible to the effects of thrombolytic agents [82–84]. In preclinical studies, researchers have shown the feasibility of treating both ischemic and hemorrhagic stroke [85–87] as well as inducing reperfusion of occluded blood vessels [82].

Conventional treatments for clots within the brain, particularly those due to intracerebral hemorrhage, require invasive action that can increase the risk of the procedure. Focused
ultrasound may enable minimally invasive or non-invasive treatment of clots that causes no permanent damage to the surrounding tissue or blood-brain barrier [87].

**Figure 16. Clot lysis.** Mechanical focused ultrasound is capable of disrupting the fibrin matrix of blood clots and restoring normal blood flow to the obstructed vessel. (Click to enlarge)

***Sonodynamic therapy***

Photodynamic therapy is a technique by which certain chemical agents, known to perfuse well to tumors, are activated by laser light to generate oxygen free radicals which in turn damage DNA and induce apoptosis (programmed cell death) of the tumor cells [88]. This procedure requires the insertion of a fiber optic laser probe into tissue, and is only effective against early stage and localized disease [89].

Alternatively, focused ultrasound may also be able to activate many of these same chemical agents (called sonosensitizers when they are activated by sound waves). In this sonodynamic therapy process, chemical agents such as 5-ALA, an innocuous dye that is absorbed preferentially by tumor cells, are injected intravenously [90]. Upon application of focused ultrasound to the targeted tumor, the agents induce the same toxic effect as in photodynamic therapy, causing apoptosis of targeted cancer cells (see Figure 17) [90–92].

Sonodynamic therapy could offer advantages as compared to photodynamic therapy by activating these chemical agents in a non-invasive manner. Focused ultrasound has the capability of treating regions deeper in the body where light would either be blocked or require more invasive delivery methods. Focused ultrasound can also provide

**Figure 17. Sonodynamic therapy.** Sonosensitizers injected intravenously will be preferentially absorbed by tumor tissue. When focused ultrasound interacts with these molecules, they are thought to release reactive oxygen species that induce apoptosis in the cancer cells. (Click to enlarge)
conformal dosage of energy, and thus induce apoptosis throughout the entire tumor. Furthermore, toxicity can be induced in a precise location while minimizing harm to other areas of the body [90]. While further research must be conducted on the mechanisms responsible for this phenomenon, it holds promise for non-invasive cancer treatment.

**Blood vessel occlusion and coagulation**

The heat generated by focused ultrasound can be used for hemostasis (e.g. to control internal bleeding) by thermally closing wounded blood vessels (see Figure 18) [93,94]. One pre-clinical study found that focused ultrasound was able to achieve hemostasis in a punctured femoral artery in all of their test animals with durable, long-term results [95]. Examination of the treated region has shown that the soft tissue surrounding the blood vessel hardens due to adventitia coagulation, creating a seal [96]. This mechanism has many clinical applications including stopping liver hemorrhage, which can be difficult to treat and is a leading cause of death in patients who have had liver trauma [97].

![Figure 18. Blood vessel occlusion and coagulation.](Click to enlarge)

Alternatively, it has been proposed that the mechanical effects of ultrasound can cause damage to vessel walls which exposes tissue factors. These tissue factors cause a cascade of biological reactions that result in the formation of a blood clot and eventual occlusion of the blood vessel [93,98]. Furthermore, blood vessel occlusion with focused ultrasound has potential for the treatment of esophageal and gastric varices, arteriovenous malformations, and varicose veins without the risk of distant clot formation or the use of a catheter [93,99,100]. It can also provide a noninvasive method to cut off the blood supply to benign or malignant tumors, effectively starving them of vital nutrients and making them more vulnerable to other treatments [55,94].
Amplification of cancer biomarkers

Low-frequency focused ultrasound can exert a mechanical force on a tumor to promote the release of biomarkers – chemical signals that are specific to the tumor – into the bloodstream (see Figure 19). These tumor biomarkers are typically in the blood at low levels that are difficult to differentiate from any noise, however, after focused ultrasound treatment, the increased levels may be more easily detected [101].

This bioeffect has a number of potential clinical applications. After the treatment of a known tumor, focused ultrasound could be used to determine the efficacy of the initial treatment by enabling periodic measurement of the biomarkers. If a potential tumor site has been identified, this mechanism could also be used to determine whether there in fact is a tumor or not. This can even potentially be used to confirm a cancer diagnosis rather than a minimally-invasive biopsy for certain types of cancer. Additionally, the presence or absence of a particular biomarker can be useful in determining the most effective treatment modality for a patient [101–103].

Stem Cell Homing

Stem cells offer a versatile treatment for the repair of damaged tissue in many organs. However, stem cell therapy is not targeted, and the cells often have trouble getting to the target site. The mechanical effects of focused ultrasound can stimulate the release of chemoattractant molecules as well as an increased expression of cellular adhesion molecules on endothelial cells, both of which improve the ability of stem cells to extravasate into the tissue (see Figure 20) [104]. Using focused ultrasound in combination with stem cell therapy could enable these cells to more specifically home to the damaged tissue.

Pre-clinical work has shown that focused ultrasound induced stem cell homing can be used to treat acute kidney injury [105], however, there is a wide array of potential clinical applications for this mechanism. Stem
cell therapy can be used in cardiovascular disorders (e.g. repair of cardiac muscle after a heart attack) [106], neurodegenerative disorders (e.g. generation of new neurons in Parkinson’s disease) [107], liver disease [108], osteoarthritis [109], and much more. In each of these cases focused ultrasound may be able to play a role in increasing the efficacy of the treatment by enhancing stem cell homing to the target site.

**Conclusion**

Interest in focused ultrasound technology has grown significantly in the past ten years, as has research into its clinical potential. Focused ultrasound is a precise tool that can be used as a stand-alone, non-invasive and non-ionizing alternative to surgery or radiation. It can also serve as a powerful adjuvant or enhancer to other treatments, including gene therapy, chemotherapy and immunotherapy. Focused ultrasound is unique amongst other ablative modalities in that it can also produce many other non-ablative bioeffects, enabling treatment of a wide array of clinical conditions with the same therapy platform. The bioeffects discussed in this paper allow for a number of treatments beyond noninvasive ablation for which the technology first gained attention, demonstrating the potential for focused ultrasound to increase the longevity and quality of life for millions of patients worldwide. Additional research is needed, however, before many of these bioeffects can be used clinically. Further development of the application of these effects could allow for noninvasive treatments as an alternative to many existing procedures that are sometimes very risky or uncomfortable for patients. Focused ultrasound could provide treatments with less trauma, faster recovery times, and ultimately less financial burden than the current standards of care.

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**References**


Figure 1.
Focused ultrasound is capable of inducing 17 different biological effects when it interacts with tissue. Some of these bioeffects can be used in the treatment of many different diseases, and some diseases may benefit from the combination of several different bioeffects. (View in document)
Figure 2.
The threshold for thermal necrosis depends on temperature reached in the tissue. As the temperature decreases the exposure time needed to achieve thermal necrosis increases exponentially. Temperature and exposure times generally used for both local hyperthermia and thermal ablation are marked. Tissue boils above the threshold of 100°C regardless of the exposure time. (View in document)
Figure 3. Thermal ablation.
When focused ultrasound is used in a continuous-wave (CW) mode, the temperature reached at the focal point can achieve a cumulative thermal dose great enough to kill the tissue by coagulative necrosis. This process works through the denaturation of the cellular membrane and can be focused to a volume as small as 10mm³. (View in document)
Figure 4. Mechanical destruction.
If focused ultrasound is used in a pulsed manner – as opposed to the continuous-wave mode of operation – the cumulative thermal dose will be low, and the effects on the tissue will be due to mechanical interactions. At high enough acoustic intensities microbubbles will form around cells and, through their oscillations, disrupt the cell membrane. The interaction of the ultrasound with microbubbles is known as cavitation, and when it is used to mechanically destroy tissue it is called histotripsy. (View in document)
Figure 5. Sonoporation.
The mechanical effects of focused ultrasound used at intensities lower than the threshold for tissue destruction can create stable cavitation near the targeted cells. The less violent oscillations of these microbubbles temporarily opens pores in the cell membrane, which allows for enhanced uptake of drugs while the pores are open. (View in document)
Figure 6. Increased vascular permeability.
Pulsed focused ultrasound can be used to open the tight junctions between endothelial cells that normally restrict the extravasation of drugs into nearby tissue. This effect of increased vascular permeability is temporary and lasts for only a few hours. (View in document)
Figure 7. Blood brain barrier opening. The effects of increased vascular permeability can also be used in the brain to open the blood brain barrier. This barrier is notoriously difficult for foreign objects to cross, which under normal conditions keeps toxic and infectious agents out of the brain. However, this also poses a large obstacle to beneficial drug delivery. Focused ultrasound, usually combined with injected microbubbles, has been shown to safely and temporarily open the blood brain barrier to enhance the delivery of drugs to the brain. (View in document)
Figure 8. Local Hyperthermia.
Mild hyperthermia can be achieved at specific targets inside the body using focused ultrasound. The body’s natural response to hyperthermia increases the delivery and uptake of drugs and oxygen to the target site, enhancing the local effects of the drug. (View in document)
Figure 9. Drug delivery vehicles.
Focused ultrasound is an ideal modality to combine with drug delivery vehicles, because it has two methods that can be used to release the encapsulated drugs: heat and pressure. Using delivery vehicles reduces the systemic toxicity of the encapsulated drug and increases the concentration of the drug at the target site, which is especially useful for chemotherapeutics.
(View in document)
Figure 10. Vasodilation.
The mechanical effects of focused ultrasound can interact with endothelial cells and induce the release of nitric oxide. Nitric oxide is a natural vasodilator that causes the widening of the blood vessels near the focal point. Vasodilation increases the blood flow through the vessel, which enhances drug delivery. (View in document)

Figure 11. Vasoconstriction.
The mechanical effects of focused ultrasound are capable of inducing temporary vasoconstriction through undetermined mechanisms. Alternatively, the thermal effects may be used to induce a more permanent vasoconstriction by shrinking the intermediate fibers in the endothelial lining. (View in document)
Figure 12. Sensitization to chemotherapy.
Focused ultrasound mild hyperthermia can be combined with chemotherapy to increase the toxicity of drugs. This occurs due to the amplified metabolic activity of the cancer cells after they are exposed to hyperthermia. (View in document)

Figure 13. Sensitization to radiotherapy.
Tumors can be treated with mild hyperthermia from focused ultrasound prior to radiation therapy to enhance the radiation’s toxicity by increasing metabolism. This allows for smaller doses of radiation to achieve the same clinical effect as the larger doses used with just radiation therapy alone. (View in document)
Figure 14. Neuromodulation.
Pulsed focused ultrasound is capable of both exciting and inhibiting neurons through its mechanical effects. The exact mechanism for this neuromodulation is still unknown. (View in document)
1. Baseline Tumor

Microscopic view of tumor cells and immature dendritic cells

Immature dendritic cell

2. Focused Ultrasound Applied

Thermal Ablation
- ATP
- Antigen
- Cancer cells die by coagulative necrosis

Mild Hyperthermia
- Cancer cells stay intact
- Heat shock proteins

Mechanical Destruction
- Cancer cells dying from cell membrane disruption

3. Dendritic Cells Mature

Immature dendritic cells recognize the tumor antigens and mature in the presence of danger signals such as heat shock proteins and ATP

Immature dendritic cell

Mature dendritic cell

4. Cytotoxic T Lymphocytes (CTLs) Activated

Lymph node

Mature dendritic cells move to the lymph nodes and activate cytotoxic T lymphocytes (CTLs)

Cytotoxic T lymphocyte

5. CTLs Target Remaining Cancer Cells

CTLs travel through the blood to the tumor

CTLs infiltrate the tumor and kill cancer cells

Antigens on tumor cell surface

6. Tumor Burden Decreased
Figure 15. Immunomodulation.

All three focused ultrasound regimes – thermal ablation, mechanical destruction, and mild hyperthermia – are capable of inducing a systemic immune response against cancer cells. Tumor associated antigens from the treated region are recognized by dendritic cells, which create a T cell mediated response against the cancer. (View in document)
Figure 16. Clot lysis.
Mechanical focused ultrasound is capable of disrupting the fibrin matrix of blood clots and restoring normal blood flow to the obstructed vessel. (View in document)
Figure 17. Sonodynamic therapy.
Sonosensitizers injected intravenously will be preferentially absorbed by tumor tissue. When focused ultrasound interacts with these molecules, they are thought to release reactive oxygen species that induce apoptosis in the cancer cells. (View in document)
Figure 18. Blood vessel occlusion and coagulation.
The thermal effects of focused ultrasound are capable of achieving hemostasis in a hemorrhaging blood vessel by inducing coagulation and hardening the adventitia around the vessel. (View in document)
Figure 19. Amplification of biomarkers.
When mechanical focused ultrasound interacts with cancer cells it promotes the release of biomarkers that are unique to the cancer type. The release of these biomarkers increases their concentration in the blood to levels that are detectable above the level of noise. (View in document)
Figure 20. Stem Cell Homing.
The mechanical effects of focused ultrasound increase the expression of cellular adhesion molecules and chemoattractants at the focal spot. These local tissue changes promote the attraction and extravasation of systemically injected stem cells. (View in document)