Review

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Histotripsy: an innovative approach for minimally invasive tumour and disease treatment

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Abstract

Histotripsy is a noninvasive medical technique that uses high-intensity focused ultrasound (HIFU) to treat liver tumours. The two main histotripsy methods are boiling histotripsy and cavitation cloud histotripsy. Boiling histotripsy uses prolonged ultrasound pulses to create small boiling bubbles in the tissue, which leads to the breakdown of the tissue into smaller subcellular fragments. Cavitation cloud histotripsy uses the ultrasonic cavitation effect to disintegrate target tissue into precisely defined liquefied lesions. Both methods show similar treatment effectiveness; however, boiling histotripsy ensures treatment stability by producing a stable boiling bubble with each pulse. The therapeutic effect is ascribed to mechanical damage at the subcellular level rather than thermal damage. This article discusses the mechanisms, treatment parameters, and potential of histotripsy as a minimally invasive procedure that provides precise and controlled subcellular damage.

Keywords: boiling histotripsy, cavitation cloud histotripsy, complications, FDA, food and drug administration, high-intensity focused ultrasound, histotripsy, liver, tumour, ultrasonic cavitation

Introduction

In a recent article, the FDA approved histotripsy as a treatment for liver tumours[1]. Histotripsy is type of high-intensity focused ultrasound (HIFU) therapy which is an emerging noninvasive medical technique that involves directing a concentrated ultrasound beam within the body to specifically impact a designated area while preserving the surrounding tissues from harm.[2] This approach, known as "boiling histotripsy," employs extended ultrasound pulses with durations measured in milliseconds, as opposed to microseconds. These prolonged pulses swiftly create a small boiling bubble in the millimetre range rather than a larger cavitation cloud. This outcome is accomplished by heating the tissue, driven by the substantial reduction in ultrasound energy as shock fronts naturally develop in the acoustic waveform due to nonlinear propagation effects.[3] The collision between these shock fronts and the resulting vapour cavity leads to the breakdown of tissue into smaller subcellular fragments.

HIGHLIGHTS

- Histotripsy is a noninvasive medical technique that uses high-intensity focused ultrasound (HIFU) to treat liver tumours.
- The two main histotripsy methods are boiling histotripsy and cavitation cloud histotripsy.
- Boiling histotripsy uses prolonged ultrasound pulses to create small boiling bubbles in the tissue, leading to the breakdown of tissue into smaller subcellular fragments.
- Cavitation cloud histotripsy uses the ultrasonic cavitation effect to disintegrate target tissue into precisely defined liquefied lesions.
- Both methods show similar treatment effectiveness, but boiling histotripsy ensures treatment stability by producing a stable boiling bubble with each pulse.
- The therapeutic effect of histotripsy is ascribed to mechanical damage at the subcellular level rather than thermal damage.

Background

The term "Histotripsy" was derived from the Greek words "Histo," meaning "soft tissue," and "Tripsy," meaning "to break down." It was first demonstrated at the University of Michigan in 2004[4]. Advances in technology have prompted a shift towards less invasive procedures, as demonstrated by the progression from planar radiation therapy to stereotactic body radiation therapy. Examples of thermal-based ablations include radiofrequency ablation, microwave ablation, and cryoablation[5]. High-intensity focused ultrasound (HIFU) is a noninvasive thermal ablation

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method that employs an external ultrasound beam source to induce thermal necrosis of soft tissue, including fibroids, liver tumours, and kidney tumours[6]. Histotripsy is a noninvasive focused ultrasound technique that operates similarly to HIFU. Unlike HIFU, which relies on high-intensity sound waves to destroy tissue, histotripsy employs mechanical effects at the cellular level to achieve the same result[7].

Mechanism

The two main histotripsy methods are boiling histotripsy and cavitation cloud histotripsy. Although they use different ablation techniques, both methods show similar treatment effectiveness[8].

Cavitation cloud histotripsy (CH)

Cloud cavitation histotripsy is based on the ultrasonic cavitation effect, in which ultrasonic waves cause microbubbles to dynamically expand, compress, oscillate, collapse, and close[8]. This procedure produces both transient and steady-state cavitation, the latter of which produces shockwaves at gigapascal (GPa) levels that precisely target subcellular lesions. Using cavitation clouds made of microbubbles produced by transient cavitation, cavitation cloud histotripsy disintegrates target tissue into precisely defined liquefied lesions[9]. Intrinsic threshold mechanisms or shock-scattering mechanisms, in which the tissue cavitation threshold is exceeded or the peak negative pressure of ultrasonic pulses interacts nonlinearly with later-arriving waves, respectively, can produce cavitation clouds[8]. Cavitation cloud histotripsy, in contrast to extracorporeal shock wave lithotripsy (ESWL), uses pulses of on–20y cycles, each of which is a rarefactional phase and microbubbles that last a few seconds. A number of factors, including pulse length, operating frequency, pulse repetition frequency, number of pulses, output power, duty cycle, and transducer f-number, affect the effectiveness of cavitation cloud histotripsy[10] The cavitation threshold is also influenced by the properties of the tissue, such as its fibrous connective tissue content and stiffness, which necessitates higher positive pressure shockwaves for effective cavitation. The cavitation threshold is not significantly affected by tissue stiffness or ultrasound frequency, but the intrinsic threshold is unaffected by changes in the mechanical properties of the tissue. These results demonstrate the complex interactions between parameters in cavitation cloud histotripsy and provide information about its potential as a flexible and minimally invasive tissue ablation method[11].

Boiling histotripsy (BH)

Within the field of histotripsy, boiling histotripsy is a unique technique that uses shockwaves with nonlinear propagation effects to heat the target lesion quickly enough to form millimetre-sized boiling bubbles in milliseconds[12]. Tissue tearing is facilitated by the shear stress created around these bubbles, which is further enhanced by interactions with incoming shockwaves that encourage mechanical tissue separation. The tadpole-shaped lesions of boiling histotripsy in soft tissue have a head and tail, with the head oriented toward the HIFU transducer[8]. In contrast to cavitation cloud histotripsy (CH), boiling histotripsy ensures treatment stability by producing a stable boiling bubble with each pulse. Interestingly, because the heat remains in the focal area and does not spread to the surrounding tissues, the therapeutic effect is ascribed to mechanical damage at the subcellular level rather than thermal damage. Boiling histotripsy can be distinguished from cavitation cloud histotripsy by its treatment parameters, which include higher frequency (1–3 MHz), longer pulses (3000–10 000 cycles), and lower pulse repetition frequency (1–2 Hz)[13]. The time required to produce boiling bubbles depends on the transducer output power and operating frequency, among other factors that affect the effectiveness of boiling histotripsy. The size of the lesion is dependent on the number of pulses and the ultrasonic focal point size, with the focal point size dictating the maximum extent[14]. The degree of thermal damage depends on the duty cycle and pulse duration, highlighting the importance of precisely regulating these parameters for efficient mechanical destruction. In conclusion, boiling histotripsy has the potential to be a minimally invasive procedure that provides precise and controlled subcellular-level damage that is adapted to therapeutic needs because of its distinct mechanisms, stable treatment profile, and unique sound pressure characteristics[15].

BH lesions were larger and had a characteristic tadpole shape, whereas CH lesions were smaller and had a regular shape. Despite this, BH generated a larger treatment zone than CH in the same amount of time[16]. Maxwell *et al*.[17], highlighted a unique characteristic of CH technique in contrast to BH, which is the formation of fluid vortices near the bubble cloud. These vortices have been demonstrated to attract and erode millimetre-sized thrombus fragments when induced in large blood vessels.

Histotripsy procedure: an overview

Histotripsy employs focused, short-duration ultrasound pulses lasting less than 20 ms at high negative pressures exceeding 10–25 megapascals (MPa). This controlled acoustic cavitation is utilized to mechanically dismantle tissue at the cellular level, all while preserving the integrity of the surrounding tissue[12]. This leads to the creation of a well-defined treatment area characterized by an acellular homogenat that is, clearly distinguished from adjacent non-target tissue. Histotripsy has been applied in animal studies for the treatment of non-tumour-bearing tissues in various organs, including the brain, thyroid, liver, kidney, and prostate. Additionally, it has been employed in tumour models related to liver, pancreas, and bone cancer, as well as for addressing cardiac tissue and blood clots[7,18–26].

The process of histotripsy operates by creating a controlled and accurate acoustic cavitation bubble cloud within the target tissue through a noninvasive approach[12]. In this procedure, non-thermal dismantling of the tissue by histotripsy does not result directly from the propagation of the ultrasound pulse. Instead, it occurs through mechanical strain and stress produced during the rapid expansion and collapse of cavitation bubbles generated at the focal point of the transducer. Histotripsy differs from HIFU, a thermal ablation technique. Unlike histotripsy, HIFU induces heating at the focal point by employing high-intensity continuous waves for extended periods with a high-duty cycle ($>20\%$)[27]. In contrast, histotripsy induces mechanical cavitation at the focal point by employing brief ultrasound pulses with extremely high pressure and a low duty cycle ($<1\%$). The cavitation bubbles formed in histotripsy originate from pre-existing endogenous cavitation nuclei, an inherent characteristic of the water present within the tissue[28,29].

The tissue outcomes produced by histotripsy differ from those of the existing cancer treatments. The non-thermal destruction of tissue in histotripsy is attributed to the action of cavitation bubbles formed within the tissue[30]. These bubbles, believed to originate in the water within the extracellular matrix of thee tissue, create concentrated and well-contained bubble clouds in areas of tissue hat surpass the histotripsy cavitation threshold. The swift expansion from tiny nuclei to large bubbles, measuring hundreds of microns in diameter, is achieved by forceful collapse of the bubbles. This entire sequence unfolds within a matter of hundreds of microseconds[30]. This sequence induces an intense local mechanical strain on tissue structures situated in close proximity to the bubbles, leading to the destruction of the tissue[30–32].

Histotripsy treatment results in liquefaction of the targeted tissue, leaving no viable cells. However, intact cell and membrane fragments, including proteins and damage-associated molecular patterns (DAMPs), appear to remain without undergoing denaturation[33,34]. Histotripsy operates as a binary process, causing tissue destruction only in regions where the negative pressure surpasses a cavitation threshold specific to the tissue. As a result, the treatment zones are remarkably precise, with a narrow surrounding margin (< 1–2 mm) of tissue that is partially treated[23,35].

Histotripsy benefits: exploring advantages and positive outcomes

Histotripsy relies on externally administered transcutaneous ultrasound and eliminates the need for surgical incision or puncture. This quality lowers the potential risks typically linked with percutaneous devices, such as bleeding, harm to organs, and the possibility of tumour spread along the path of needle insertion[11].

Histotripsy disrupts the intended tissue mechanically, transforming it into cell-free fragments that the body can absorb and effectively remove. This makes histotripsy suitable for situations requiring tissue elimination, such as thrombosis. Findings from pre-clinical studies involving animals and a preliminary human trial indicate that the treated tissue volume is typically absorbed by the body within a period of 1–2 months[9,36].

Significant studies involving large animals that have been published show that there is no risk of post-histotripsy bleeding in vascular organs, even in individuals who are receiving anticoagulant treatment[37].

Histotripsy applications: targeted indications

Histotripsy is indicated and have pre-clinical studies and application of diseases involving tumours of liver[22,23,38–40] kidney[36,39,41,42] prostate[43,44] neurological diseases[25,45] thrombosis, haematoma[16,46,47] neonatal and foetal congenital heart disease[7,48] Valvular diseases[49,50] Kidney stones[51,52] tendons[53] biofilm[54–56].

Histotripsy: adverse effects and limitation

Complications associated with histotripsy involve certain organs, such as the lungs and gastrointestinal tract, which are unsuitable for histotripsy treatment owing to the presence of gas. Histotripsy requires extremely high ultrasound pressures, and the attainable pressure is determined by the size of the transducer aperture unobstructed by bones (such as ribs) or gas (as in the case of the lung). As a result, certain parts of the body, like the central lung and possibly the pancreas, cannot receive histotripsy treatment using external ultrasound due to the presence of gas or bone obstructions. This may limit its usefulness in treating inaccessible or gas-filled hollow organs[11].

Histotripsy could also potentially cause blood vessel thrombosis in the treated area due to the activation and clustering of platelets caused by cavitation. This activation of the coagulation cascade may result in pathological ischaemia or the formation of an embolus, leading to damage to surrounding healthy tissue. The use of histotripsy in highly vascular organs is limited due to these potential complications[18].

Histotripsy has the potential to disrupt targeted tissue, but there is a theoretical concern that it could inadvertently release tumour cells, potentially increasing the risk of metastasis. This limitation is significant because seeding tumour cells can have disastrous consequences. Nonetheless, existing research has indicated that histotripsy does not result in an increased risk of metastasis; in fact, in some cases, it may have led to a decreased risk. This outcome is likely attributed to a concurrent immune response[38,41].

In consideration of the future, it is essential to evaluate different types of histotripsy treatments and doses to gain a deeper understanding of how ablation stimulates an immune response and its possible short- and long-term side effects. As there is a scarcity of human clinical trials on histotripsy, additional basic studies and pre-clinical human and animal trials are also necessary to develop missing mechanistic insights. In the near future, more translationally relevant studies will be needed to address this knowledge gap.

Histotripsy in action: clinical outcomes and case examples

The outlook for employing histotripsy for various medical conditions is outlined in Table 1 and also as follows.

Benign prostate hypertrophy (BPH)

In the initial phase of this clinical trial (NCT01896973), a prototype medical device was used to address benign prostate hypertrophy in 25 patients. There were no instances of severe intraoperative complications during the procedure. Among the subjects, 68% underwent general anaesthesia, while the remaining 32% received sedation. Notably, there was a substantial improvement in the International Prostate Symptom Score (IPSS) compared to the baseline before treatment, with a mean increase of 52.4% at 1 month, 50.8% at 3 months, and 44.0% at 6 months ($P < .001$)[57].

Liver tumours

The first human Phase I trial of hepatic histotripsy for non-curative patients with multiple liver malignancies was conducted in 2019 in Barcelona, Spain using a clinical prototype device by Histosonics, Inc. The study involved 8 patients with 11 tumours, and it showed no significant adverse events, met its primary endpoint of creating ablations as planned, and exhibited tumour regression and volume reduction in the treated areas,[58] and a

Table 1

Overview of histotripsy applications in-human trials.

Author	Objective	*n*	Outcome	Follow-up	Adverse effects
Vidal-Jove *et al.*[54]	Current hepatic locoregional therapies face efficacy limitations and toxicities, prompting a first in-human trial to assess the technical effectiveness and safety of histotripsy—a noninvasive, focused ultrasound therapy—in patients with primary and secondary liver tumours based on encouraging pre-clinical results.	8	The eight patients, with a median age of 60.4 years and an average targeted tumour diameter of 1.4 cm, all achieved the primary endpoint, and the secondary safety profile analysis revealed no device-related adverse events, with two patients showing a sustained decrease in tumour markers over the eight weeks post-procedure.	8 weeks	No adverse effects reported
Min Wah *et al.*[59]	The objective of this phase I/II trial conducted across multiple centres is to evaluate the preliminary safety and effectiveness of the prototype investigational 'System' in treating both primary and metastatic liver cancers.	45	Ongoing Trial, no result reported	5 years	NR
Messas *et al.*[56]	To evaluate the safety and efficacy of noninvasive ultrasound therapy (NIUT) in calcified aortic stenosis patient	10	The trial, assessed by an independent core laboratory, found no cognitive impairment or significant changes in aortic regurgitation, left ventricular function, or volumes at 1 month; overall, there was a nonsignificant increase in aortic valve area and a decrease in mean pressure gradient, but a subgroup of six responders showed a significant improvement, associated with a longer therapy duration and higher cumulative focal energy delivery, suggesting potential factors linked to treatment response.	1 month	During the procedure, adverse effects included atrial fibrillation, premature ventricular beats, and nonsustained ventricular tachycardia, managed with sedation; chest wall discomfort and arrhythmia were reported, and one patient experienced post-procedure right-sided heart failure, though no deaths or major cardiovascular events occurred at the 1-month follow-up.
Schuster *et al.*[53]	The primary objective of the inaugural human study is to evaluate the clinical safety and, as a secondary focus, the efficacy of histotripsy in treating symptomatic benign prostatic enlargement (BPE).	25	Histotripsy treatment in 25 men showed no serious intraoperative adverse events, and although debulking was not observed, there was significant improvement in IPSS scores at one, three, and 6 months postoperatively.	6 months	The adverse effects observed included three cases of transient urinary retention lasting less than three days, one case of serious urinary retention lasting 8 days, a minor anal abrasion, and microscopic haematuria.

IPSS, International Prostate Symptom Score; NR, not reported.

patient with colorectal cancer liver metastasis had sustained reduction of non-treated tumours in the liver following histotripsy suggesting both safety and potential efficacy, including a possible abscopal effect in humans[59].

Calcified aortic stenosis

In 2019, a Phase I human trial of cardiac histotripsy using Cardia wave's Valvosoft device was conducted in elderly patients with severe calcific aortic stenosis who were not suitable candidates for conventional treatments. One month after the procedure, six of ten patients exhibited significant improvements in aortic valve area and pressure gradients, with no major adverse effects observed. The study demonstrated the safety and feasibility of noninvasive therapy for calcific aortic stenosis in this patient population[60].

Histotripsy for liver tumours and cholangiocarcinoma

This study explored the potential of histotripsy as a treatment for cholangiocarcinoma (CC) tumours, given its dense fibrotic stromal components, and aimed to determine the specific treatment requirements. The present study included in vivo experiments in a patient-derived xenograft mouse model, indicating the effectiveness of histotripsy in ablating CC tumours, and ex vivo experiments comparing the histotripsy doses needed for CC, HCC, and CLM tumours, highlighting the higher doses required for CC tumours. These findings suggest histotripsy's promise for CC tumour ablation and emphasize the need for tailored treatment approaches[61].

Thrombolysis

A new histotripsy approach called microtripsy has been investigated as a noninvasive, drug-free method for breaking up retracted blood clots with improved precision and reduced risk of vessel damage compared to traditional approaches. The study hypothesized that microtripsy thrombolysis is effective on retracted clots and can be enhanced with electronic focal steering, and experimental results demonstrated successful clot recanalization, particularly with multi-focus and dual-pass treatments incorporating electronic focal steering, indicating the potential of microtripsy thrombolysis for retracted clot management[62].

Conclusion

In summary, histotripsy is a novel technique for medical care that offers a highly focused and minimally invasive method to treat a variety of illnesses, such as calcified aortic stenosis, benign prostatic hypertrophy, and liver tumours. The procedure is a promising option for patients and clinicians because it is non-surgical, disrupts tissue at the cellular level, and allows quick absorption. Even though there are some possible drawbacks and issues, such as the inapplicability of gas-filled locations, the risk of thrombosis, and concerns regarding tumour cell release, further research shows that these difficulties are frequently outweighed by their advantages. Histotripsy has great potential to transform the way many diseases are treated, as well as to enhance overall prognosis and quality of life as it develops and advances. Its usefulness and safety will improve with additional studies and clinical trials, guaranteeing that it becomes a crucial component of contemporary medical treatment plans.

Ethics approval and consent to participate

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Consent for publication

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Author contribution

The conceptualization was done by T.K.F.A. and M.A.R. The literature and drafting of the manuscript were conducted by A.M. A.M., M.A.S., and A.H. The editing and supervision were performed by A.H. and A.R. All authors have read and agreed to the final version of the manuscript.

Conflicts of interest disclosure

The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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References

[1] Kumar V, Kewlani P, Singh A, *et al*. Multifunctional Liposomes to Attain Targeting, Stimuli Sensitive Drug Release and Imaging Cancer. Am Jf Roentgenol. In: Santra, TS, Shinde, AUS, Eds. Advanced Drug Delivery. Studies in Mechanobiology, Tissue Engineering and Biomaterials, vol 26. Springer, Singapore.

[2] Dubinsky TJ, Cuevas C, Dighe MK, *et al*. High-intensity focused ultrasound: current potential and oncologic applications. Am J Roentgenol 2008;190:191–9.

[3] Bailey M, Khokhlova V, Sapozhnikov O, *et al*. Physical mechanisms of the therapeutic effect of ultrasound (a review). Acoustl Phys 2003;49: 369–88.

[4] Williams RP, Simon JC, Khokhlova VA, *et al*. The histotripsy spectrum: differences and similarities in techniques and instrumentation. Int J Hyperthermia 2023;40:2233720.

[5] Xu Z, Hall TL, Vlaisavljevich E, *et al*. Histotripsy: the first noninvasive, non-ionizing, non-thermal ablation technique based on ultrasound. Int J Hyperthermia 2021;38:561–75.
[6] Marrero JA, Pelletier S. Hepatocellular carcinoma. Clin Liver Dis 2006; 10:339–51.
[7] Xu Z, Owens G, Gordon D, *et al*. Noninvasive creation of an atrial septal defect by histotripsy in a canine model. Circulation 2010;121: 742–9.
[8] Li S, Wei Y, Zhang B, *et al*. Research progress and clinical evaluation of histotripsy: a narrative review. Ann Transl Med 2023;11:263.
[9] Vlaisavljevich E, Greve J, Cheng X, *et al*. Non-invasive ultrasound liver ablation using histotripsy: chronic study in an in vivo rodent model. Ultrasound Med Biol 2016;42:1890–902.
[10] Khokhlova VA, Fowlkes JB, Roberts WW, *et al*. Histotripsy methods in mechanical disintegration of tissue: towards clinical applications. Int J Hyperthermia 2015;31:145–62.
[11] Xu Z, Hall TL, Vlaisavljevich E, *et al*. Histotripsy: the first noninvasive, non-ionizing, non-thermal ablation technique based on ultrasound. Int J Hyperthermia 2021;38:561–75.
[12] Bader KB, Vlaisavljevich E, Maxwell AD. For whom the bubble grows: Physical principles of bubble nucleation and dynamics in histotripsy ultrasound therapy. Ultrasound Med Biol 2019;45:1056–80.
[13] Pahk KJ, Gélat P, Sinden D, *et al*. Numerical and experimental study of mechanisms involved in boiling histotripsy. Ultrasound Med Biol 2017; 43:2848–61.
[14] Khokhlova VA, Bawiec CR, Cunitz BW, *et al*. Real-time imaging and control of boiling histotripsy lesion formation using Doppler ultrasound. J Acoust Soc Am 2019;146(4_Supplement):2992.
[15] Pahk KJ, De Andrade MO, Kim H, *et al*, The effects of the size of a boiling bubble on lesion production in boiling histotripsy. J Phys Conf Ser; 2019;1184:012007.
[16] Khokhlova TD, Monsky WL, Haider YA, *et al*. Histotripsy liquefaction of large hematomas. Ultrasound Med Biol 2016;42:1491–8.
[17] Maxwell AD, Park S, Vaughan BL, *et al*. Trapping of embolic particles in a vessel phantom by cavitation-enhanced acoustic streaming. Phys Med Biol 2014;59:4927–43.
[18] Knott EA, Swietlik JF, Longo KC, *et al*. Robotically-assisted sonic therapy for renal ablation in a live porcine model: initial preclinical results. J Vasc Interv Radiol 2019;30:1293–302.
[19] Zhang X, Macoskey JJ, Ives K, *et al*. Non-invasive thrombolysis using microtripsy in a porcine deep vein thrombosis model. Ultrasound Med Biol 2017;43:1378–90.
[20] Hendricks-Wenger A, Sereno J, Gannon J, *et al*. Histotripsy ablation alters the tumor microenvironment and promotes immune system activation in a subcutaneous model of pancreatic cancer. IEEE Trans Ultrason Ferroelectr Freq Control 2021;68:2987–3000.
[21] Arnold L, Hendricks-Wenger A, Coutermarsh-Ott S, *et al*. Histotripsy ablation of bone tumors: Feasibility study in excised canine osteosarcoma tumors. Ultrasound Med Biol 2021;47:3435–46.
[22] Vlaisavljevich E, Kim Y, Allen S, *et al*. Image-guided non-invasive ultrasound liver ablation using histotripsy: feasibility study in an in vivo porcine model. Ultrasound Med Biol 2013;39:1398–409.
[23] Smolock AR, Cristescu MM, Vlaisavljevich E, *et al*. Robotically assisted sonic therapy as a noninvasive nonthermal ablation modality: proof of concept in a porcine liver model. Radiology 2018;287:485–93.
[24] Hall TL, Hempel CR, Wojno K, *et al*. Histotripsy of the prostate: dose effects in a chronic canine model. Urology 2009;74:932–7.
[25] Sukovich JR, Cain CA, Pandey AS, *et al*. In vivo histotripsy brain treatment. J Neurosurg 2018;131:1331–8.
[26] Swietlik JF, Mauch SC, Knott EA, *et al*. Noninvasive thyroid histotripsy treatment: proof of concept study in a porcine model. Int J Hyperthermia 2021;38:798–804.
[27] Ter Haar GR. High intensity focused ultrasound for the treatment of tumors. Echocardiography 2001;18:317–22.
[28] Maxwell AD, Cain CA, Hall TL, *et al*. Probability of cavitation for single ultrasound pulses applied to tissues and tissue-mimicking materials. Ultrasound Med Biol 2013;39:449–65.
[29] Vlaisavljevich E, Lin K-W, Maxwell A, *et al*. Effects of ultrasound frequency and tissue stiffness on the histotripsy intrinsic threshold for cavitation. Ultrasound Med Biol 2015;41:1651–67.
[30] Vlaisavljevich E, Maxwell A, Mancia L, *et al*. Visualizing the histotripsy process: Bubble cloud–cancer cell interactions in a tissue-mimicking environment. Ultrasound Med Biol 2016;42:2466–77.
[31] Mancia L, Vlaisavljevich E, Xu Z, *et al*. Predicting tissue susceptibility to mechanical cavitation damage in therapeutic ultrasound. Ultrasound Med Biol 2017;43:1421–40.
[32] Mancia L, Vlaisavljevich E, Yousefi N, *et al*. Modeling tissue-selective cavitation damage. Phys Med Biol 2019;64:225001.
[33] Hu Z, Yang XY, Liu Y, *et al*. Release of endogenous danger signals from HIFU-treated tumor cells and their stimulatory effects on APCs. Biochem Biophys Res Commun 2005;335:124–31.
[34] Eranki A, Srinivasan P, Ries M, *et al*. High-intensity focused ultrasound (HIFU) triggers immune sensitization of refractory murine neuroblastoma to checkpoint inhibitor therapy. Clin Cancer Res 2020;26: 1152–61.
[35] Longo KC, Knott EA, Watson RF, *et al*. Robotically assisted sonic therapy (RAST) for noninvasive hepatic ablation in a porcine model: mitigation of body wall damage with a modified pulse sequence. Cardiovasc Intervent Radiol 2019;42:1016–23.
[36] Hall TL, Kieran K, Ives K, *et al*. Histotripsy of rabbit renal tissue in vivo: temporal histologic trends. J Endourol 2007;21:1159–66.
[37] Wheat JC, Hall TL, Hempel CR, *et al*. Prostate histotripsy in an anticoagulated model. Urology 2010;75:207–11.
[38] Qu S, Worlikar T, Felsted AE, *et al*. Non-thermal histotripsy tumor ablation promotes abscopal immune responses that enhance cancer immunotherapy. J Immunother Cancer 2020;8:e000200.
[39] Khokhlova TD, Schade GR, Wang Y-N, *et al*. Pilot in vivo studies on transcutaneous boiling histotripsy in porcine liver and kidney. Sci Rep 2019;9:20176.
[40] Worlikar T, Mendiratta-Lala M, Vlaisavljevich E, *et al*. Effects of histotripsy on local tumor progression in an in vivo orthotopic rodent liver tumor model. BME Front 2020;2020:20–4.
[41] Styn NR, Hall TL, Fowlkes JB, *et al*. Histotripsy of renal implanted VX-2 tumor in a rabbit model: investigation of metastases. Urology 2012;80: 724–9.
[42] Schade GR, Wang Y-N, D'Andrea S, *et al*. Boiling histotripsy ablation of renal cell carcinoma in the eker rat promotes a systemic inflammatory response. Ultrasound Med Biol 2019;45:137–47.
[43] Schade GR, Keller J, Ives K, *et al*. Histotripsy focal ablation of implanted prostate tumor in an ACE-1 canine cancer model. J Urol 2012;188: 1957–64.
[44] Hempel CR, Hall TL, Cain CA, *et al*. Histotripsy fractionation of prostate tissue: local effects and systemic response in a canine model. J Urol 2011; 185:1484–9.
[45] Gerhardson T, Sukovich JR, Chaudhary N, *et al*. Histotripsy clot liquefaction in a porcine intracerebral hemorrhage model. Neurosurgery 2020;86:429.
[46] Khokhlova TD, Kucewicz JC, Ponomarchuk EM, *et al*. Effect of stiffness of large extravascular hematomas on their susceptibility to boiling histotripsy liquefaction in vitro. Ultrasound Med Biol 2020; 46:2007–16.
[47] Li Y, Liu Y, Li R, *et al*. Histotripsy liquefaction of large hematoma for intracerebral hemorrhage using millisecond-length ultrasound pulse groups combined with fundamental and second harmonic superposition: a preliminary study. Ultrasound Med Biol 2020;46:1244–57.
[48] Owens GE, Miller RM, Owens ST, *et al*. Intermediate-term effects of intracardiac communications created noninvasively by therapeutic ultrasound (histotripsy) in a porcine model. Pediatr Cardiol 2012;33: 83–9.
[49] Messas E, Rémond MC, Goudot G, *et al*. Feasibility and safety of noninvasive ultrasound therapy (NIUT) on an porcine aortic valve. Phys Med Biol 2020;65:215004.
[50] Villemain O, Robin J, Bel A, *et al*. Pulsed cavitational ultrasound softening: a new noninvasive therapeutic approach for calcified bioprosthetic valve stenosis. Basic Transl Sci 2017;2:372–83.
[51] Duryea AP, Hall TL, Maxwell AD, *et al*. Histotripsy erosion of model urinary calculi. J Endourol 2011;25:341–4.
[52] Matula TJ, Wang Y-N, Khokhlova T, *et al*. Treating porcine abscesses with histotripsy: a pilot study. Ultrasound Med Biol 2021;47:603–19.
[53] Smallcomb M, Simon JC. Investigation into tendon histotripsy. J Acoust Soc Am 2019;145:1862.
[54] Bigelow TA, Northagen T, Hill TM, *et al*. The destruction of escherichia coli biofilms using high-intensity focused ultrasound. Ultrasound Med Biol 2009;35:1026–31.
[55] Bigelow TA, Thomas CL, Wu H, *et al*. Histotripsy treatment of S. Aureus biofilms on surgical mesh samples under varying pulse

durations. IEEE Trans Ultrason Ferroelectr Freq Control 2017;64: 1420–8.
[56] Bigelow TA, Thomas CL, Wu H, *et al*. Impact of high-intensity ultrasound on strength of surgical mesh when treating biofilm infections. IEEE Trans Ultrason Ferroelectr Freq Control 2018;66:38–44.
[57] Schuster TG, Wei JT, Hendlin K, *et al*. Histotripsy treatment of benign prostatic enlargement using the Vortx Rx system: initial human safety and efficacy outcomes. Urology 2018;114:184–7.
[58] Vidal-Jove J, Serres X, Vlaisavljevich E, *et al*. First-in-man histotripsy of hepatic tumors: the THERESA trial, a feasibility study. Int J Hyperthermia 2022;39:1115–23.
[59] Vidal-Jové J, Serres-Créixams X, Ziemlewicz TJ, *et al*. Liver histotripsy mediated abscopal effect—Case report. IEEE Trans Ultrason Ferroelectr Freq Control 2021;68:3001–5.
[60] Messas E, IJsselmuiden A, Goudot G, *et al*. Feasibility and performance of noninvasive ultrasound therapy in patients with severe symptomatic aortic valve stenosis: a first-in-human study. Circulation 2021;143:968–70.
[61] Hendricks-Wenger A, Weber P, Simon A, *et al*. Histotripsy for the treatment of cholangiocarcinoma liver tumors: In vivo feasibility and ex vivo dosimetry study. IEEE Trans Ultrason Ferroelectr Freq Control 2021;68:2953–64.
[62] Zhang X, Owens GE, Cain CA, *et al*. Histotripsy thrombolysis on retracted clots. Ultrasound Med Biol 2016;42:1903–18.