Clinical Translation of Abdominal Histotripsy: A Review of Preclinical Studies in Large Animal Models

Katrina L Falk[1,2], **Paul Laeseke**[1,2], **Meridith A. Kisting**[2], **Annie Zlevor**[2], **Emily Knott**[3], **Amanda R Smolock**[4], **Charles Bradley**[5], **Eli Vlaisavljevich**[6], **Fred T. Lee Jr**[1,2,7], **Timothy Ziemlewicz**[2]

[1]Department of Biomedical Engineering, University of Wisconsin, 600 Highland Avenue, Madison, Wisconsin 53792 USA

[2]Department of Radiology, University of Wisconsin, 600 Highland Avenue, Madison, Wisconsin 53792 USA

[3]Cleveland Clinic Lerner College of Medicine

[4]Department of Radiology, Division of Interventional Radiology, Medical College of Wisconsin

[5]School of Veterinary Medicine, University of Pennsylvania

[6]Department of Biomedical Engineering and Mechanics, Virginia Polytechnic Institute and State University

[7]Department of Urology, University of Wisconsin, 600 Highland Avenue, Madison, Wisconsin 53792 USA

Abstract

Histotripsy is an emerging non-invasive, non-thermal, and non-ionizing focused ultrasound (US) therapy that can be used to destroy targeted tissue. Histotripsy has evolved from early laboratory prototypes to clinical systems which have been comprehensively evaluated in the preclinical environment to ensure safe translation to human use. This review summarizes the observations and results from preclinical histotripsy studies in the liver, kidney, and pancreas. Key findings from these studies include the ability to make a clinically relevant treatment zone in each organ with maintained collagenous architecture, potentially allowing treatments in areas not currently amenable to thermal ablation. Treatments across organ capsules have proven safe, including in anticoagulated models which may expand patients eligible for treatment or eliminate the risk associated with taking patients off anti-coagulation. Treatment zones are well-defined with imaging and rapidly resorb, which may allow improved evaluation of treatment zones for residual

Address correspondence to: Katrina Falk, MS, 600 Highland Avenue, Madison, WI 53792, Katrina.falk@wisc.edu.

or recurrent tumor. Understanding the effects of histotripsy in animal models will help inform physicians adopting histotripsy for human clinical use.

Keywords

histotripsy; ablation; abdomen; liver; kidney; renal; pancreas; tumor

Introduction

Histotripsy is an emerging non-thermal, non-invasive, and non-ionizing focused ultrasound (US) therapy that can be used to destroy targeted tissue. In recent years, histotripsy devices have rapidly progressed from early laboratory prototypes to clinical systems being evaluated for use in humans. There is now an extensive body of preclinical work which demonstrates that histotripsy has the potential to non-invasively destroy tissue with a high degree of accuracy and precision. This manuscript is a summary of observations and results from large animal preclinical histotripsy studies in the liver, kidney and pancreas with particular emphasis on destruction of targeted tissue, tissue selectivity, side effects, and imaging findings. Understanding the bioeffects of histotripsy in in vivo large animal models should help inform physicians pursuing the translation of the technology for human clinical use.

The term histotripsy was coined at the University of Michigan in 2004 and comes from the Greek root words 'histo', meaning soft tissue, and 'tripsy', meaning breakdown. [1] Focused, high amplitude, short duration, low duty cycle US pulses are applied to target tissue to create high peak negative pressures. When pressures exceed a tissue-specific threshold, cavitation is produced which results in the rapid expansion and collapse of nanometer-scale endogenous gas bubbles, causing high stress and strain and eventual rupture of cell membranes. [1-4] This type of histotripsy is called "cavitation" or "cavitation cloud" histotripsy (intrinsic/shock-scattering mechanism) and contrasts boiling histotripsy which uses longer (1-10 ms) pulses and has a larger bubble cloud. [5] Boiling histotripsy pulses are still much shorter than those used in thermal High Intensity Focused Ultrasound (HIFU) and also results in non-thermal mechanical histotripsy. [6] More information regarding specific parameters of various forms of histotripsy can be found in this special issue by Williams et al. To destroy a targeted tissue volume with cavitation histotripsy, the focal point is swept through a pre-planned pathway using mechanical (micropositioners), robotic, or electronic (beam steering) methods. The result is a treatment volume that corresponds to the prescribed plan with millimeter-level accuracy and is composed of an acellular liquid homogenate consisting of extracellular fluid, cellular debris, and newly aggregated red blood cells. [7,8] Most in vivo abdominal studies and all human clinical trials to date have used cavitation histotripsy, which will be termed 'histotripsy' in this work, unless specifically denoted.

There are technical differences between histotripsy and other minimally or non-invasive ablation techniques (Figure 1). Histotripsy uses high-amplitude, short duration ultrasound pulses at a low duty-cycle to induce rapid expansion and collapse of microbubbles to cause mechanical destruction of cell membranes. The mechanical nature of cavitation histotripsy starkly contrasts the clinically used thermal based modalities of HIFU, microwave (MW),

radiofrequency (RF), and cryoablation. HIFU is a focused ultrasound technique which uses long duration, relatively low-amplitude focused ultrasound pulses at a high duty-cycle to generate heat at the focal point, resulting in thermal destruction. [9-12] Microwave (MW) and radiofrequency (RF) ablation use percutaneous probes and different energy sources to heat tissue to temperatures >60 ° . [13-19] Cryoablation uses similar probes but instead destroys tissue through many freeze-thaw cycles (temperatures $< -40°$) which result in ice crystal formation, cell membrane rupture, and osmotic imbalances. [14,16,20] Histotripsy has potential advantages over these thermal ablation modalities. Although rare, severe side effects in thermal ablation can occur in adjacent anatomy due to heat diffusion, including bile duct strictures and chronic thrombosis of blood vessels during liver or pancreas treatments, as well as hydronephrosis and strictures in the collecting system and ureter during renal treatments. [21-29] Disadvantages of cryoablation are the lack of control of treatment zone, bleeding post procedure, and the potential for cryoshock, a life-threatening syndrome of multi organ failure and coagulopathy. [30,31] Finally, cavitation histotripsy differs from irreversible electroporation (IRE), which is a minimally invasive technique that uses low-energy, high-voltage electrical impulses to induce cellular apoptosis through electroporation. [32-34] The applied electric field alters the electrochemical gradient across cell membranes, forming instabilities and pores through which cell contents leak. [34,35] IRE also has disadvantages, including a frequent need to place multiple electrodes in precise patterns, synchronization with the cardiac cycle to avoid dysrhythmias, small irregular and unpredictable ablation zones, an inability to prescribe specific treatment sizes and shapes, and a higher disease recurrence with larger tumor volumes. [33,36,37]

In addition to technical differences, cavitation histotripsy also has histopathologic differences from other ablation modalities (Figure 1). As stated, the treated zone with histotripsy is comprised of a homogenous acellular eosinophilic slurry or liquid with fragments of pyknotic cell debris and erythrocytes due to foci of hemorrhage. [7,38] Heat-based thermal tissue destruction (HIFU, MW, RF) occurs via coagulative necrosis which is characterized by protein denaturation and irreversible damage to cellular structures. [11,12,15,18,39-41] Microscopic analysis of the thermally ablated tissue generally shows maintenance of cellular shape and an intact membrane with reduced cytoplasmic detail, cytoplasmic hypereosinophilia and nuclear changes that include pyknosis (shrinkage/condensation), karyorrhexis (fragmentation), and eventually karyolysis (dissolution). In the peracute to acute phase of thermal destruction, tissue architecture is maintained, and histopathologic changes may be quite subtle, necessitating immunohistochemical staining to demonstrate non-viability (eg. Negative NADH staining). [42] Histotripsy differs pathologically from cryoablation, where instantaneous cell necrosis leaves remnants of cellular outlines, foci of cell loss and loss of cytoplasmic and nuclear detail with karyhorhexis. [16,43] Calcium deposition (dystrophic mineralization) is often encountered adjacent to or within the cryoablated region. With time, the ablated foci across modalities are generally rimmed by a pseudocapsule of granulation tissue, collagen with admixed inflammatory cells (neutrophils, lymphocytes, plasma cells and histiocytes). IRE treatment has maintained tissue architecture in the acute phase and later showing pyknosis, which is a characteristic feature of cells having undergone apoptosis. [35,44,45] Other cellular injuries such as swelling, vacuolation, and cytoplasmic eosinophilia have been reported, but

in general, apoptosis occurs later which may make acute pathological confirmation of tissue destruction difficult in transitional zones at the edges of the ablated regions. [35] Similar to histotripsy, structures with abundant collagen, such as the ureter, bile ducts, and large blood vessels are relatively resistant to damage from IRE. [45-49]

Tissue selectivity is a potentially important feature of cavitation histotripsy. Different tissues have different intrinsic thresholds, which results in a dose-dependence of destruction. Anatomical structures with high collagen content such as large blood vessels, bile ducts and urothelial structures appear relatively resistant to damage from histotripsy due to high intrinsic mechanical strength (high Young's Modulus) compared to parenchymal organs and most tumors. [4,50,51] The differences in the vulnerability of various tissues to cavitation can be exploited to preserve critical normal structures in and around the histotripsy treatment zone. Damage to collagenous structures can be achieved eventually with a higher dose (increasing number of pulses) compared to liver, kidney, or pancreas parenchyma. [4,50-52]

Although not yet tested in large animals, histotripsy appears to provoke activation of an immunologic response in small animal tumor models. The suggested mechanism of action is the release of non-denatured intracellular proteins and neoantigens due to the mechanical fracture of cell membranes. Early work in murine models suggests that this response is more robust than after external beam radiation or thermal ablation. [53-55] Histotripsy has also been shown to induce an increased immune recognition of abscopal tumors and may have synergy with checkpoint inhibitors. [53,54,56] Of note, IRE has been noted to create a potential immune response due to the lack of protein denaturation. [35,47] Further discussion of the immunological effects of histotripsy is provided in a separate issue in this volume (Imran KM, Cho CS, Allen CA).

Preclinical Histotripsy Studies in the Liver, Kidney, and Pancreas

Liver

The possibility of using histotripsy for treatment of liver tumors was first described at the University of Michigan in 2013 using an early prototype system and an in vivo swine model. [7] In this initial study, histotripsy was used to destroy small volumes of normal hepatic tissue safely, accurately, and reproducibly. [7] Since then, human-scale swine models have been used to assess histotripsy's safety and efficacy [7,57-62], outcomes with and without anticoagulation [63,64], ability to treat across costal blockage [60,65], and tissue selectivity. [50] A list of the in vivo, large animal, transcutaneous liver histotripsy studies can be found in Table 1.

The safety and efficacy of cavitation histotripsy treatments in the liver has been explored in multiple preclinical animal studies. The initial feasibility study consisted of treating two 1 cm^3 cubes in the liver of a porcine model and acutely showed successful fractionation of liver while preserving major vessels and the gallbladder. [7] To expand the clinical relevance, a proof-of-concept study was later performed with 3 cm spherical diameters, acute and chronic arms, and evaluation of body wall damage. [57] MR Imaging and histopathology demonstrated successful treatments with complete destruction of cellular hepatic parenchyma within the treatment zone. [57,59] The additional findings of this study

(larger craniocaudal dimension of the treatment zone than planned, some local body wall damage, and vessel thrombi) laid the groundwork for the subsequent studies.

The first of Smolock et al's additional findings was a significant difference in the diameter of the treatment zone in the craniocaudal dimension (elongation of up to 8 mm when creating a 3 cm sphere) from planned to actual in a porcine model, which was likely due to respiratory motion of the liver during treatment. [57] Respiratory motion has also posed difficulty when treating the liver with boiling histotripsy. [62] Avenues to overcome respiratory motion have been explored: use of robotic arm in ex vivo boiling histotripsy [66] and the change in the prescribed geometry [59] and use of high frequency jet ventilation in in vivo cavitation histotripsy. [67] Using a motion compensating robotic arm with skin tracking showed promising results to improve treatment size accuracy ex vivo. [66] In the in vivo study compensating for respiratory motion with geometry, a lens prescription with the short axis in the craniocaudal dimension (2.5 cm x 2.5 cm x 1.7 cm) resulted in a more spherical treatment than the sphere prescription, which created an ovoid shape with craniocaudal elongation as in prior studies. [59] Each prescription resulted in uniform tissue destruction within the treatment zone and there was no significant difference in transition zone from normal to treated tissue between the prescriptions. [59] More recently, high frequency jet ventilation (HFJV), which utilizes relatively rapid (~120 breaths/minute) low tidal volume breaths to minimize diaphragmatic movement and therefore decrease liver motion, has been trialed in swine with more spherical treatment zones achieved compared with conventional ventilation. [67]

The initial proof of concept study also reported local body wall damage (via MR imaging) immediately after treatment (3/3) and in 1/7 chronic animals. [57] Authors suggested the damage was due to a poor acoustic window and a nonoptimized prototype pulse sequence which resulted in pre focal energy deposition causing local heating. [57] To address this limitation, a subsequent study with an optimized pulse sequence to mitigate heat deposition and body wall injury was used to treat 3 cm treatment zones in the right liver. [58] With the optimized pulse sequence, there was no body wall damage (0/6) seen on MRI and necropsy. [58] Using the optimized pulse sequence, the same group explored the efficacy of a transcostal treatment, determining that clinical size treatments were possible with an increase in ultrasound power and increased time of energy delivery relative to subcostal treatments. [60] Although intercostal muscle edema was identified on post-treatment MRI, there was no histopathological evidence of injury to the chest wall. [60] Another transcostal study used subcutaneous thermocouples in a swine model and measured the temperature change when treating entirely transcostal with subsequent ex vivo imaging and pathology. Though there was no substantial increase in temperature nor rib damage with transcostal treatments, the prescribed treatment zone was smaller than a typical clinical treatment with any currently available modality by 5 mm^3. [65] Therefore, although safe and feasible, the accuracy of treating beneath the ribs remains to be determined.

Finally, vascular thrombosis, particularly portal venous thrombosis, has been noted with histotripsy. The original clinical proof of concept study demonstrated portal vein (9/10) and hepatic vein thrombi (2/10) acutely post treatment, with all thrombi resolving during a 4 week follow-up period. [57] Similar acute results were found in the study with the pulse

sequence optimized to mitigate body wall damage, where thrombi in the main portal vein (2/6, both nonocclusive), peripheral portal vein (1/6, distally occlusive), main hepatic vein (4/6, 1 occlusive), were seen. [58] However, since no chronic data was reported in this second study, the results currently suggest the majority of thrombi are nonocclusive and/or resolve. [57]

Although most in vivo histotripsy research focuses on destroying liver parenchyma, a porcine study was developed to determine the outcome of treating on or within a hepatic vein and if prophylactic heparin was necessary. [63] After 22 treatments involving hepatic veins of healthy swine, authors concluded that heparinization before treatment was not necessary to perform safe and effective treatments near hepatic vessels and also noted no significant bleeding events when heparinized. [63] Inversely, a porcine study was performed to determine if anticoagulation before a procedure would pose a challenge to abdominal histotripsy, as it does with other invasive ablation procedures. In this study, eight healthy swine were anticoagulated with warfarin and survived for 7 days post liver treatment. [64] No hemorrhage or hematoma was seen despite the treatment extending to or across the liver capsule in 5/7 cases and 6 of the swine having supratherapeutic warfarin levels on post-procedure day 7. [64] Portal vein thrombus was observed in 4/7 cases, where 3 cases resolved by day 7. [64] Notably, the liver and renal capsule were intact following treatment, presumably due to their collagenous architecture. [64] These reported anticoagulation results only refer to histotripsy treatment in abdominal organs and may not generalize to other areas of the body.

The lack of vascular damage or side effects with histotripsy demonstrate that large blood vessels, and other collagenous structures, such as intact bile ducts, are more resistant to histotripsy compared to other critical structures. [4,50,51] In a study of ten acute and chronic swine undergoing liver treatments, there was notable sparing of collagenous structures such as intact bile ducts traversing the treatment zone (2/2) on radiographic and histologic analysis. [57] Figure 2 shows a hepatic porcine treatment with post-treatment MRI and gross histology illustrating the tissue selective and tissue sparing nature of histotripsy. A varying degree of tissue selectivity may be leveraged depending upon the tissue properties of both the target tissue and the specific critical structure of interest. [4,50-52]

In the liver, the histotripsy treatment zone has been reported to involute. There was a 64% volume involution reported at post-treatment day 28 in a swine, which trends with other published work in a rodent model where near 100% involution was reported. [57,68,69] The rapid involution of treatment zones may be beneficial for assessing local treatment success and failures on follow-up imaging. In addition to cavitation histotripsy, wound healing from boiling histotripsy in rat livers was reported to follow normal wound healing of extracellular matrix remodeling, angiogenesis, and finally hepatocyte regeneration. [69] Immunologic effects of histotripsy for liver cancer have been recently explored in small-animal model experiments. Partial (goal of treating only 50-80% of the tumor) and complete histotripsy treatments have resulted in tumor regression and statistically significant improved survival outcomes in orthotopic rat hepatocellular carcinoma models. [70,71] Large-animal tumor models for histotripsy are being explored but have yet to become widely used. [72] For a

detailed description of current knowledge on histotripsy immune effects readers are referred to another article within this issue (Imran KM, Cho CS, Allen CA).

Finally, the most recent safety study of histotripsy in a hepatic model compared it with the clinical standard of care, microwave ablation (MW). In this study histotripsy created more spherical treatment zones and demonstrated similar efficacy of tissue destruction compared to MW ablation. [38] There was less chronic damage to vessels and bile ducts created by histotripsy when compared to microwave ablation, particularly there was evidence for biliary damage in 0/8 histotripsy treatments and 5/6 microwave treatments. [38]

Boiling histotripsy has also been shown feasible in invasive and transcutaneous liver treatments. [61,62] Similar results to the above studies in cavitation histotripsy were reported: full liquefaction based on histologic analysis, some damage to the body wall, respiratory motion disruption of treatment zone, and US aberrations from the body wall. [62] Further work on boiling histotripsy to mitigate aberrations has also been done in vivo with success. [73]

Beyond liver tumors, histotripsy has been studied preclinically for disinfection of abdominal abscesses and decellularization of liver parenchyma for cell transplantation. [74-76] Even if drug resistant, bacteria are still susceptible to thermal and mechanical damage. Both boiling and cavitation histotripsy were used in a pilot study to treat abdominal abscesses in a porcine model. Authors found that histotripsy treatments were successful at decreasing the amount of bacteria colonies in these abscesses. [74] Boiling histotripsy has also been studied in vivo to decellularize a section of a porcine and rat livers to create a cavity for implantation of healthy hepatocytes. [75,76] Cells injected into this cavity proliferated and integrated into the recipient liver, highlighting this method for future cell therapy. [75,76]

Kidney

In addition to focal tumor treatments of the liver, renal applications are also promising. The first use of histotripsy in the kidney was reported by the University of Michigan in 2006 demonstrating the feasibility of using transcutaneous focused ultrasound to destroy renal tissue in a normal rabbit kidney. [77] Shortly thereafter, the same group studied the tissue pathological appearance after renal treatments in 29 rabbits at 0, 1, 2, 7, 21, 60 days. [78] The tissue transitioned from finely disrupted tissue with no cellular components (day 0) to a gradual inflammatory response with steadily decreasing area of cellular and architectural disruption and finally to a small fibrous scar by day 60. [78] To evaluate concerns of systemic spread of tumor from a mechanical treatment, the group at the University of Michigan performed a rabbit study of histotripsy in a VX-2 tumor model. This demonstrated that the number of metastatic lung lesions from a renally implanted tumor did not increase with histotripsy treatment. [79]

Although less in number compared with liver, human scale swine models have been used for renal histotripsy treatments. There are currently three preclinical studies evaluating the safety and efficacy of renal cavitation histotripsy in a human scale swine model. [64,80,81] The first was an evaluation of the efficacy and safety of renal histotripsy in a swine model [80], the second an evaluation of the safety of treatments in a swine model with

anticoagulation [64], and the third a comparison study performed between cryoablation and renal histotripsy. [81]

In the first feasibility study, renal histotripsy intentionally including the central collecting system was performed in acute and chronic porcine models. [80] There was complete histological destruction of the targeted renal parenchyma with predictable and spherical treatment zone sizes. [80] There was involution of the treatment zone over time (96% over 4 weeks), similar to that noted in the liver studies. Bilateral treatments were performed in a portion of the animals (n=4) demonstrating safety of this approach as there was no evidence of renal decompensation. [80] Temporary bladder and ureteral debris occasionally resulting in ureteral obstruction was noted in a small number of animals with this debris resolving by 1 week in each case. [80] Importantly, there was no urine leak, main vessel thrombosis, or mortality. [80] On CT imaging, histotripsy treatment zones in the cortical kidney were noted to be well demarcated and non-enhancing post contrast administration with small, peripheral wedge-shaped perfusion defects. [80] Similar results have been seen on MRI. Figure 3 illustrates a well demarcated wedge-shaped perfusion defect on a treated porcine kidney.

The second study evaluated the risk of hemorrhage from renal histotripsy in a warfarin anticoagulated swine model. Eight therapeutically anticoagulated swine underwent histotripsy treatments to the renal parenchyma and were survived 7 days post treatment while continuing anticoagulation. There were no cases of retroperitoneal hemorrhage, even with all eight treatments extending to the kidney capsule and 6/8 swine being supratherapeutic on warfarin at day 7. [64] The authors proposed that this finding may be explained by histologic mechanisms that involve endothelial injury of vessels at the periphery and within the treatment zone resulting in thrombosis. [64]

The third study is a comparison between renal histotripsy and a current clinical standard, renal cryoablation. [81] Similar efficacy, safety profile, and predictability of treatment zone size was achieved with histotripsy and cryoablation. Debris was noted in the collecting system of both treatment arms, 9/9 with histotripsy and 5/9 with cryoablation, without resultant obstruction. Additionally, more perirenal hematomas (8/9) were identified after cryoablation treatments than with histotripsy (1/9). [81]

Similar to the liver, histotripsy treatments in the kidney exhibited tissue selectivity. First described by Lake et al, there is a differential histotripsy treatment effect in the renal cortex versus collecting system when treating in-vitro renal tissue. [82] This aligns with histology from another paper, where there was patchy urothelium disruption in the ureter but no injury to underlying connective tissue. [80] Similar results were seen when treating the kidneys with boiling histotripsy. [62,83] The tissue selective properties of histotripsy may be useful for centrally located renal tumors and those adjacent to the ureter, locations challenging or not typically accessible with current ablation modalities. [84]

Pancreas

Recent studies have investigated the feasibility of histotripsy for the treatment of healthy pancreas and pancreatic tumors in both small and large animal models in vivo. [85]

Results from histotripsy studies in an immunocompetent subcutaneous mouse model of pancreatic cancer showed that histotripsy could effectively ablate Pan02 pancreatic tumors and stimulate immune pathways through the release of tumor-associated proteins and DNA. [86,87] This work matches previous studies of histotripsy that have demonstrated effective ablation and immune-stimulation in multiple tumor types [53,54], and suggests that histotripsy may have multiple roles in the treatment of pancreatic cancer including ablation, palliation, immune-modulation, and enhancing drug delivery.

In addition to these feasibility studies in small animals, in vivo large animal swine models have been investigated for histotripsy ablation of the pancreas. [85,88] In one of these studies, the safety and feasibility of using histotripsy for the non-invasive ablation of the pancreas was tested in 11 healthy pigs using a custom 500kHz histotripsy transducer mounted on a prototype clinical histotripsy system. [88] Results from this study demonstrated that the pancreas could be successfully visualized and treated with histotripsy when the pigs were fed a specialty diet consisting of sweetened custard with simethicone and bisacodyl designed to minimize the intestinal contents and gas present on the day of treatment. To illustrate the importance of this diet to minimize intestinal gas, a separate group of pigs were fasted for 12 hours prior to histotripsy and were not fed the specialty diet. Ultrasound imaging of the pancreas was not feasible in any of these pigs.[88] Despite the lack of proper visualization of the pancreas on ultrasound prior to treatment, histotripsy was still performed and resulted in off-target injury to the intestines due to extensive pre-focal cavitation. In contrast, precise bubble clouds were safely generated in the pancreas of pigs that received the specialty diet without inducing any clinically relevant injuries to overlying tissue structures. [88] This finding matches prior clinical studies using thermal HIFU for the treatment of pancreatic cancer, which have shown a strong safety profile when applying HIFU through an acoustic window without gas blockage in the stomach and bowel. [89-92] It is also worth noting that four pigs in the initial histotripsy pig study were survived for 1-week post-treatment, with results showing that the treatments were well-tolerated with no signs of complications, behavioral changes, or changes in blood markers that might indicate other complications such as pancreatitis. [88] Additional studies are needed to confirm this finding in a larger number of animals and over longer time periods.

Ongoing work is underway to optimize histotripsy therapy methods for targeting the pancreas and to explore the differential thresholds for tissue selectivity to damage pancreatic tumors but preserve critical structures such as blood vessels, bile ducts, stomach, and bowel. Additional studies are needed to characterize these findings and develop improved computational models that can predict the level of tissue selectivity for specific clinical applications. [93]

Finally, the newest set of histotripsy studies on the pancreas has investigated the potential of histotripsy for treating pancreatic tumors in a novel SCID-like orthotopic pig tumor model. [94] This model, which grows human pancreatic tumors in the pancreas of immunocompromised pigs, has been used in initial pilot studies showing that histotripsy could effectively target and ablate pancreatic tumors in vivo, consistent with results from the healthy pig studies described above. [88,94]

Conclusion

Histotripsy is a non-invasive, non-thermal, non-ionizing focused ultrasound treatment that has benefits over previous minimally and non-invasive tissue destruction techniques by creating mechanical cell death versus thermal coagulative necrosis. preclinical work on the liver, kidney, and pancreas has spanned small and large animals through feasibility studies and tumor models. Although the liver has been most extensively studied both preclinically and clinically for tumor treatment with histotripsy, exploration of treatments in the kidney and pancreas are increasing. Preclinical studies have explored safety and efficacy of cavitation histotripsy treatments in abdominal organs through highly acoustic impeding structures, varying treatment sizes, with and without anticoagulation, and around critical structures. These important studies are instrumental to helping physicians understand the intricacies and nuances of histotripsy treatment in the abdomen which will lead to better clinical translatability and ultimately, patient care.

Acknowledgments

Data Availability Statement

Data sharing not applicable – no new data generated.

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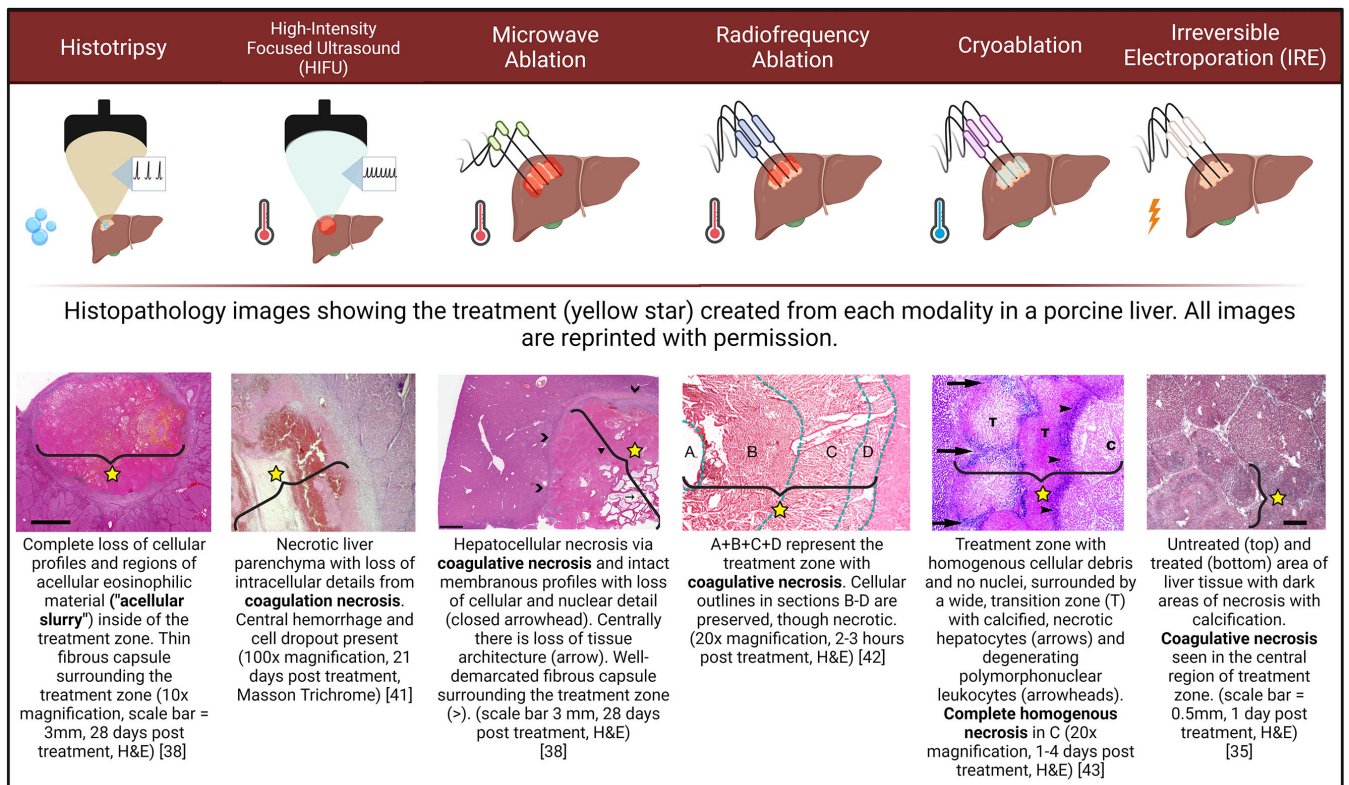
Histotripsy	High-Intensity Focused Ultrasound (HIFU)	Microwave Ablation	Radiofrequency Ablation	Cryoablation	Irreversible Electroporation (IRE)

Histopathology images showing the treatment (yellow star) created from each modality in a porcine liver. All images are reprinted with permission.

Complete loss of cellular profiles and regions of acellular eosinophilic material ("**acellular slurry**") inside of the treatment zone. Thin fibrous capsule surrounding the treatment zone (10x magnification, scale bar = 3mm, 28 days post treatment, H&E) [38]	Necrotic liver parenchyma with loss of intracellular details from **coagulation necrosis**. Central hemorrhage and cell dropout present (100x magnification, 21 days post treatment, Masson Trichrome) [41]	Hepatocellular necrosis via **coagulative necrosis** and intact membranous profiles with loss of cellular and nuclear detail (closed arrowhead). Centrally there is loss of tissue architecture (arrow). Well-demarcated fibrous capsule surrounding the treatment zone (>). (scale bar 3 mm, 28 days post treatment, H&E) [38]	A+B+C+D represent the treatment zone with **coagulative necrosis**. Cellular outlines in sections B-D are preserved, though necrotic. (20x magnification, 2-3 hours post treatment, H&E) [42]	Treatment zone with homogenous cellular debris and no nuclei, surrounded by a wide, transition zone (T) with calcified, necrotic hepatocytes (arrows) and degenerating polymorphonuclear leukocytes (arrowheads). **Complete homogenous necrosis** in C (20x magnification, 1-4 days post treatment, H&E) [43]	Untreated (top) and treated (bottom) area of liver tissue with dark areas of necrosis with calcification. **Coagulative necrosis** seen in the central region of treatment zone. (scale bar = 0.5mm, 1 day post treatment, H&E) [35]

Figure 1:

Histopathologic comparison of tissue destruction modalities. Differences in procedural application and mechanism of action between histotripsy, high intensity focused ultrasound (HIFU), microwave ablation (MW), radiofrequency ablation (RF), cryoablation, and irreversible electroporation (IRE) are illustrated in the pictograms under each modality. Histotripsy uses cavitation to mechanically destroy tissue. HIFU, MW, RF and cryoablation use thermal mechanisms to create coagulative necrosis and IRE uses electricity to disrupt the cell membrane potential. Pathologic differences are illustrated below each modality. Images have been reprinted with permission and license numbers can be found in the Acknowledgements section.
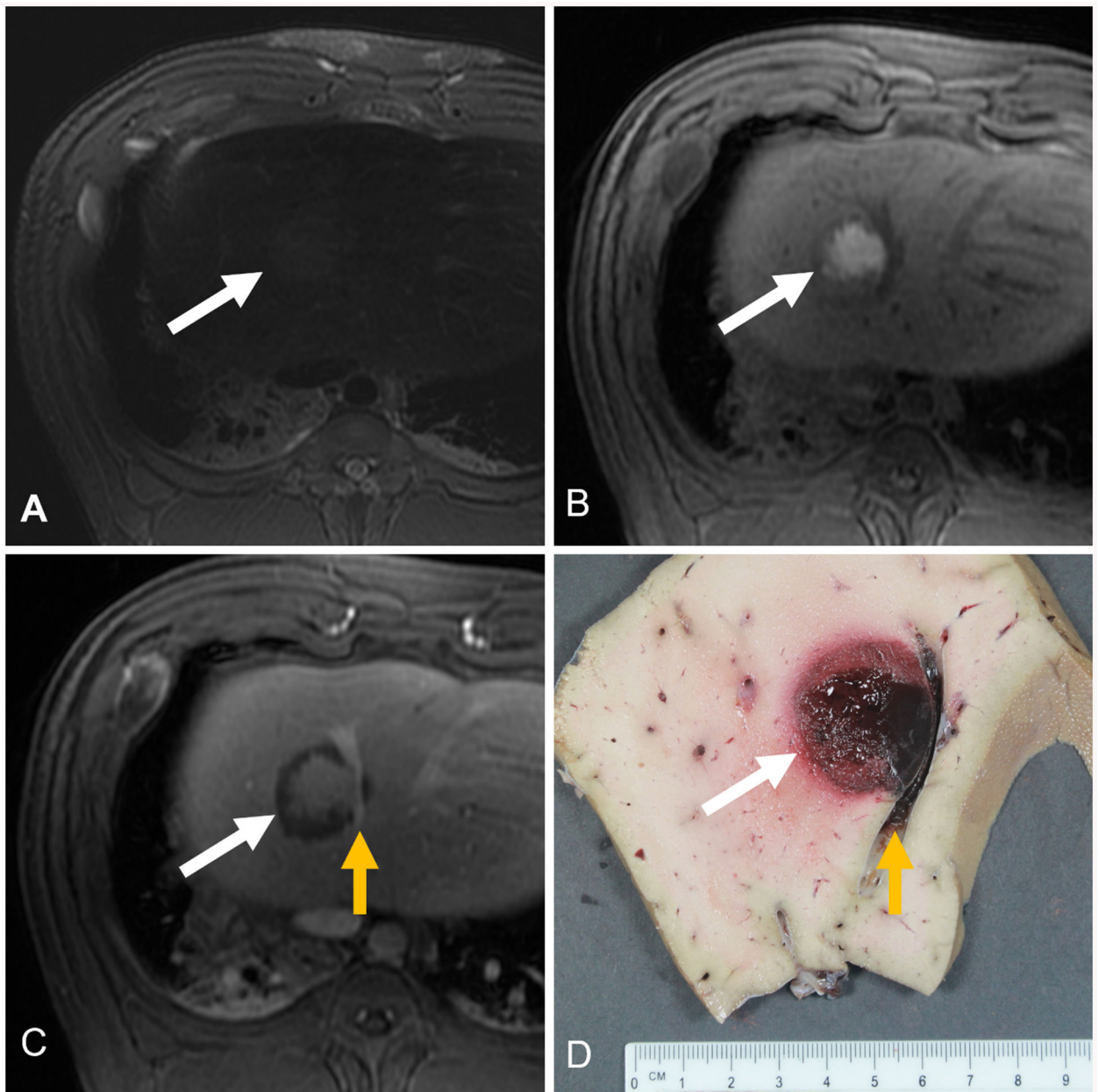
Figure 2:
MRI and gross pathology images of a histotripsy treatment (white arrows) in a porcine liver.
A) Axial T2-weighted fat-saturated MRI following histotripsy of a healthy swine liver. B)
Axial T1 weighted MRI before the administration of IV contrast. C) Axial T1 weighted MRI
after the administration of a gadolinium-based contrast, shown in the portal venous phase.
A patent vessel runs through the treatment zone (yellow arrow). D) Corresponding gross
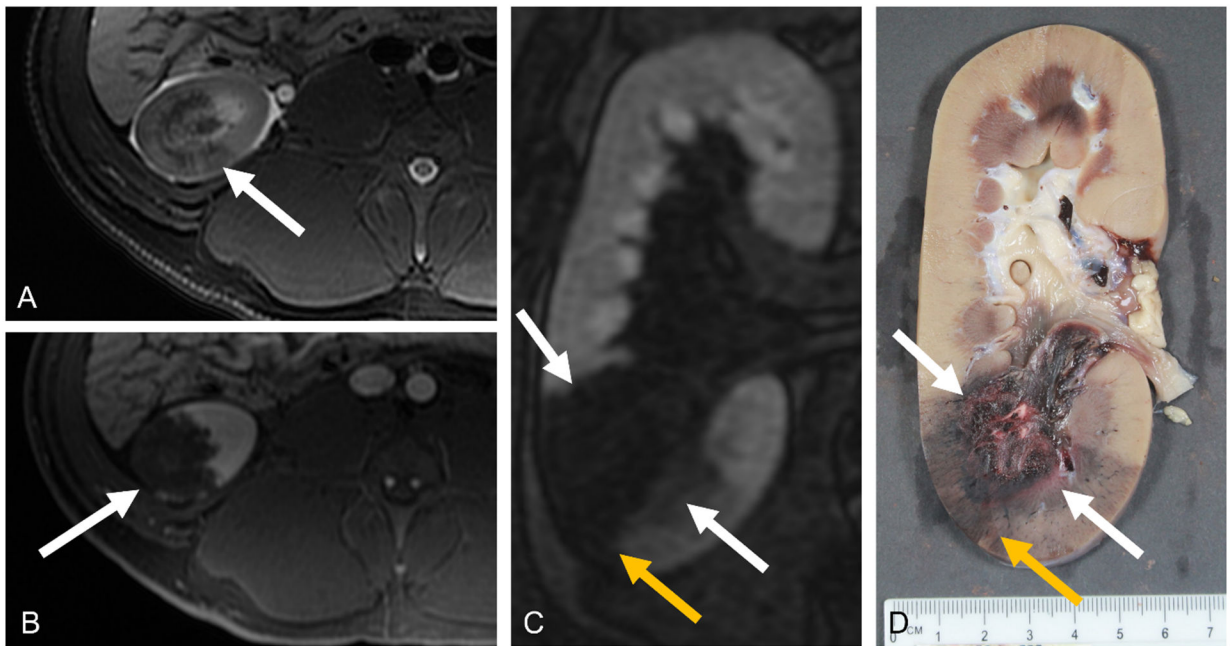pathology of histotripsy treatment with patent vessel.

Figure 3:
MRI and gross pathology images of a histotripsy treatment (white arrow) in a porcine kidney. A) Axial T2-weighted fat-saturated MRI following histotripsy of a healthy swine kidney. B) Axial T1 weighted MRI with IV contrast (gadobenate dimeglumine) in the corticomedullary phase after treatment (white arrow). C) Coronal MRI with contrast 13 minutes after administering IV contrast. Note the wedge-shaped perfusion defect (yellow arrow) extending peripheral to the treatment zone (white arrows), causing the treatment zone to appear larger. D) Corresponding gross image demonstrating the central area of treatment (white arrows) with adjacent perfusion deficit (yellow arrow).

Table 1:

Summary of Large Animal Preclinical Transcutaneous Liver Histotripsy Studies in Swine

Author	Year	Organ Treated	Objective	Number of Swine	Survival Period	Size of Treatment	Imaging Modality	Imaging Findings	Conclusion
Vlaisavljevich [7]	2013	Liver	Proof of concept	6	Acute (6 swine)	1 cm cube (12 treatments)	MRI of ex vivo liver	Hyperintense lesion on T2 weighted MRI	Creation of non-invasive lesions with tissue selective properties was possible.
Vlaisavljevich [50]	2014	Liver	Tissue selectivity	2	Acute (2 swine)	3 x 2 x 2 cm³ (1 treatment) and 3 x 3 x 2 cm³ (1 treatment)	MRI of ex vivo liver	T1 and T2 weighted images showed major hepatic vessels structurally intact inside of the treatment zone	Large vessels with increased mechanical strength are more resistant to treatment.
Kim [65]	2014	Liver	Transcostal treatment	8	Acute (8 swine)	5 cm cube (8 treatments)	MRI of ex vivo liver and ribs	Hyperintense lesions on T2 weighted MRI. No damage to ribs	Transcostal hepatic treatment without aberration correction is possible.
Vlaisavljevich [63]	2017	Liver	Safety profile with and without heparinization	22: 11 heparinized, 11-non heparinized	Acute (22)	Parenchyma: 5 x 3 mm rectangle (22 treatments) xVessel: single bubble cloud	Parenchyma: Ultrasound Vessel: Doppler Ultrasound	Parenchyma: Hypo-echoic lesions were observed in the treated regions for both groups Vessel: continuous blood flow, some with an altered flow pattern	Treatment near and inside of large hepatic veins can be safely and effectively accomplished without systemic heparinization.
Smolock [57]	2018	Liver	Feasibility of creating a clinically relevant zone and 1 month survival study	10	Acute (3 swine), 28 Days (7 swine)	3 cm sphere (10 treatments)	MRI	Acute: -3/3 body wall injury -3/3 portal vein thrombus -1/3 hepatic vein thrombus -no bile duct injuries 28 Days: -1/7 body wall injury -6/7 portal vein thrombus -1/7 hepatic vein thrombus -no bile duct injuries -treatment volume decreased by 64%	Destruction of liver tissue with clinically relevant treatment zone sizes are possible with sparing of vessels and bile ducts
Longo [58]	2019	Liver	Body wall damage mitigation	6	Acute (6 swine)	3 cm sphere (6 treatments)	MRI	-3/6 portal vein thrombus (2 were nonocclusive) -4/6 hepatic vein thrombus (3 were nonocclusive)	Thermal injury to body wall can be mitigated with an altered pulse sequence.
Khokhlova * [62]	2019	Liver and Kidney	Feasibility and safety of boiling histotripsy for subcostal liver lesions	6	Acute (6 swine)	~1 cm treatment zone	Ultrasound	-4/6 livers were treated -treatments showed homogenized voids and full liquefaction on histology -difficult to view treatments due to respiratory motion and US aberrations -3/4 livers had mechanical and	It was feasible to destroy liver and renal tissue with boiling histotripsy but some side effects were identified.

Author	Year	Organ Treated	Objective	Number of Swine	Survival Period	Size of Treatment	Imaging Modality	Imaging Findings	Conclusion
Longo [59]	2020	Liver	Altered treatment shape	6	Acute (6 swine)	2.5 cm sphere (6 treatments), 2.5 cm ellipsoid (6 treatments)	MRI	thermal damage noted on the fatty layer of the body wall Treatment zones were slightly larger than prescribed in all dimensions. No adverse events reported	Decreasing prescribed cranial-caudal diameter helps correct for effects of respiratory motion.
Knott [60]	2021	Liver	Transcostal treatment	6	Acute (6 swine)	3 cm sphere (6 treatments)	MRI	-5/5 intercostal muscle edema -4/5 portal vein thrombus (nonocclusive) -no bile duct injuries (one animal did not receive MRI)	Transcostal hepatic treatments are safe and possible.
Knott [38]	2022	Liver	Comparison to microwave ablation	14 (8 histotripsy, 6 MW)	28 Days (14 swine)	2-3 cm sphere (10 histotripsy treatments, 6 MW ablations)	CT	Histotripsy: 7/8 portal vein thrombus (4 resolved by day 28), 0/8 hematoma, 0/8 gallbladder injury. MW: 6/6 portal vein thrombus (none resolved), 2/6 hematoma, 1/6 gallbladder injury	Histotripsy has a similar safety and efficacy profile to microwave ablation.
Thomas * [73]	2022	Liver and Kidney	Aberration Correction in Subcostal Treatment	4	Acute (4 swine)	Single boiling bubble (5 areas in liver, 3 in kidney)	Ultrasound	The acoustic power to create a boiling bubble was reduced by at least 45% with aberration correction compared to without aberration correction	Aberration correction effectively lowers acoustic power needed to achieve a boiling histotripsy bubble in vivo
Mauch [64]	2023	Liver and Kidney	Treatment with anticoagulation	8 (all anticoagulated with warfarin)	7 Day (8 swine)	2.5 cm sphere (7 liver), 2 cm sphere (8 kidney)	CT	Liver: No hemorrhage. 4/8 portal vein thrombus (3 resolved by day 7). Kidney: No hemorrhage. 3/8 transient hematuria, 2/8 urine leak	No increase in bleeding risk in an anticoagulated swine

*
denotes study done with boiling histotripsy