

## Peer Review File

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### The first-round peer review

#### Reviewer A:

1. Lines 61-62: There are 2 forms of histotripsy. Earlier in the introduction, the authors state that histotripsy was invented at the University of Michigan; however, they discovered cavitation cloud histotripsy. Boiling histotripsy was invented/discovered at the University of Washington. The first paper on boiling histotripsy appears to be ref 21, by Canney et al., 2010. The authors should give credit for each type of histotripsy to the correct institution and authors.

**Thanks for your suggestion, we have made corrections in the article.**

2. Line 154: The author for ref 19 should be Maxwell, not Adam.

**We are sorry for our carelessness. We have made corrections in the article.**

3. Line 165: “heating effects are negligible”. The authors should specify that the heating does not extend beyond the focal zone. This could help readers who are confused by the fact that boiling histotripsy does indeed heat the tissue up, but it’s restricted to the focal region and does not diffuse into nearby tissues.

**We are sorry for our misnomer. We have made corrections in the article.**

4. Line 171: “pulse-delivery” should be “pulse delivery rate”.

**We are sorry for our misnomer. We have made corrections in the article.**

5. Line 177. The authors refer to a review article #24. They should instead cite the original papers that show the boiling bubble appears each and every pulse.

**Thanks for the correction, we have cited other references in the article.**

## Reviewer B

### Introduction:

Lines 34-48: Please provide references to support all the authors' statements in the introduction, up to line 48.

Thanks for your correction, we have added references in the corresponding parts.

Lines 38-39: While it is possible for HIFU to cause thermal trauma to tissues beyond the treatment focus, HIFU delivery is also designed to be precise for the specific reason of avoiding collateral thermal tissue trauma. Please expand on the statement to describe the limitation of HIFU in potentially causing collateral tissue trauma despite its precise nature, as it is not accurate to state that HIFU will definitely cause collateral thermal trauma.

Thanks for your correction, we have revised the statement accordingly.

### Mechanism of histotripsy:

General comment: It would be helpful to the readers if the authors could revise the language used to describe the different forms of histotripsy to align the descriptions of each type of histotripsy relative to one another. This can help readers better understand the differences between the various forms of histotripsy. For example, in lines 170-175, the authors list the technical differences between cavitation cloud histotripsy and millisecond boiling histotripsy, and it would be helpful to the reader for the authors to explain the pros and cons of both types of histotripsy, and how the technical differences translate into clinical application differences.

Thanks for your suggestion, we have made changes to clarify the specific type of histotripsy.

Lines 63-64: Please provide clarification regarding your statement that “the results produced after ablation are consistent” – for example, please state what is consistent about the ablation results, is it the degree of ablation, the precision of ablation, among other possible definitions of treatment consistency. Please also provide references for the statements.

Thanks for your correction, we have revised the statement accordingly and added references.

Lines 67-83: Please provide references for all the statements between lines 67-83. If the authors intended for reference #1 to serve as the reference for all of the statements, please provide more than one reference to support all the statements included.

Thanks for the correction, we have added the reference.

Line 84: Please consider using another verb besides “split”, since “to split the target tissue” can give an inaccurate impression, for example, that the tissue is split into sections by histotripsy instead of being disintegrated into its subcellular components.

Thanks for your suggestion, we have made changes in the article.

Lines 85-98: Please provide references for all the statements between lines 85-98.

Thanks for the correction, we have added references.

Line 97: Please explain what “duty cycle” is and how it influences the delivery of histotripsy.

Thanks for the correction, we have explained it on Line 42. If the duty cycle is too high, it will lead to the accumulation of thermal effects.

Lines 105-106: Please provide clarification on how cavitation memory “significantly affects the therapeutic effect of histotripsy”.

Thank you for your correction, we have made changes in the corresponding position of the article.

Line 107-108: Please provide a reference for the statement “Decreasing pulse repetition frequencies (PRF) of histotripsy allows sufficient time for residual microbubbles to dissolve between successive pulses, reducing the effects of cavitation memory.”

Thanks for the correction, we have added the reference.

Line 109: Please clarify the part of the statement “...the influence of cavitation effect can be ignored”. Did the authors mean to state instead that “the influence of cavitation memory can be ignored”?

We apologize for the carelessness and we have revised the statement accordingly.

Line 116: Please include the limitations of using a separate pulse sequence to induce bubble coalescence, so as to explain the statement in lines 118-120 implying that it is advantageous to avoid using a separate pulse sequence.

Thanks for your suggestion, it would be advantageous to make the histotripsy system as simple as possible.

Lines 122-127: Please provide references for all the statements between lines 122-127.

Thanks for the correction, we have added some references.

Lines 128-131: Please clarify the relationship between the statement in lines 128-129, and the statement in line 130-131. For example, please clarify whether there is a disadvantage to “microbubbles randomly generated in an “all-or-none” pattern”, which could explain the start of the next statement using “But” as a conjunction.

Thank you for your suggestion. Due to misrepresentation, we have removed this part of the statement from the original text.

Line 154: Please revise the first author’s last name to “Maxwell” instead of “Adam”.

We apologize for this carelessness and we have made a correction.

Line 159: Please provide an explanation of what the authors mean by “nonlinear propagation effects”.

Thanks for your correction. We have changed the original content to “Boiling histotripsy uses shock waves based on nonlinear propagation effects to heat the target lesion, producing a millimeter-sized boiling bubble within milliseconds”, and added some references.

Lines 161-163: Please revise the statement in lines 161-163 that begins with “Before the thermal effect”. The statement is confusing, for example, how do the “boiling bubbles interact with the microbubble cloud”, since the previous statement explained that the boiling bubbles are what form the microbubble cloud. Please revise the use of the language and the image of a “fountain that can inhale the surrounding tissue into it and explodes” to improve a reader’s understanding of the process. For example, please clarify what the authors mean by “inhale”, what causes this “inhalation” effect?  
**We are sorry for our misrepresentation, we have removed and rewritten this paragraph from Line 165 to 175.**

Please also provide references for all the statements in lines 161-163.

**We are sorry for our misrepresentation, we have removed and rewritten this paragraph from Line 165 to 175.**

Lines 164-167: Please provide references for all the statements.

**Thanks for the correction, we have added the reference.**

Lines 170-175: Please provide references for all the statements.

**Thanks for the correction, we have revised the corresponding statement and added references.**

Lines 184-188: Please provide references for all the statements.

**Thanks for the correction, we have added the reference.**

Lines 191-192: Please provide references for the statement.

**Thanks for the correction, we have added the reference.**

Application of histotripsy in animal models:

Lines 199-200: Please provide the references to the “many studies” that the authors reference.

**Thanks for the correction, we have added the reference and revised the statement.**

Lines 206-207: Please revise “Robert et al” to “Roberts et al”.

**We apologize for this carelessness and we have made a correction.**

Line 227: Please clarify what the authors mean by “deep disease”.

**Thanks for your suggestion, we have removed this part of the statement due to adjustments to the content of the article.**

Lines 230-233: Please provide references for all the statements.

**Thanks for the correction, we have added references.**

Lines 233-235: Since the authors refer to “some research groups”, please provide references in addition to reference #28 to support the statement.

**Thanks for the correction, we have added another reference.**

Line 235: Please clarify the comparison treatments – i.e., “histotripsy is better tolerated by patients” compared to which treatments? Please provide references to support the authors’ claim that histotripsy is “better tolerated” by patients.

**Thanks for the correction, we have added references.**

Line 236: The use of the word “negligible” implies that there is a degree of thermal trauma to the surrounding tissue. Please clarify whether there is any degree of thermal trauma to the surrounding tissue, since the authors stated and implied in the previous section of the manuscript that histotripsy avoids thermal trauma to tissue adjacent to the ablation zone.

**Thanks for the correction, we have made the correction in the article.**

Line 237: Please clarify what the authors mean by “good effect”, similar uses of the phrase are found in other portions of the manuscript, please clarify accordingly.

**Thanks for the correction, we have made the correction in the article.**

Lines 237-238: Please provide references to support the authors’ claim that histotripsy is “expected to become an alternative treatment method for BPH patients in the future”.

**Thanks for the correction, we have rewritten this statement and added references.**

Line 240: Please clarify whether this canine model involved histotripsy ablation of prostatic hyperplasia or the normal prostate.

**Thanks for the correction, we've added "normal" to the sentence.**

Lines 241-245: Please clarify what animal model was used by Styn et al. Please also revise the language to describe the evaluation carried out by Styn et al. As the text stands, it could imply that Styn et al. evaluated and observed trauma to the urethral sphincter, neurovascular bundle (NVB), and rectum, followed by the statement that the NVB and urethral sphincter were not damaged. Please also clarify whether there was clinically significant trauma to the rectum after clinically applicable doses of histotripsy were applied.

**Thanks for the correction, we've added "normal" to the sentence.**

Lines 246-247: This statement is in contradiction to the statement in lines 237-238, where the authors claimed that histotripsy is “expected to become an alternative treatment method for BPH patients in the future”. Please revise.

**Thanks for the correction, we have made changes in lines 251-255.**

Line 248: In the section on “Evaluation of histotripsy in the treatment of solid tumors”, the authors could consider adding bone tumors to the discussion, as there is a recent feasibility report on using histotripsy to ablate bone tumors.

**Thanks for your suggestion, we've added relevant research at the beginning of this section. However, because the focus of this part is on the application of liver tumors, it is not introduced in detail.**

Lines 250-253: Please provide references for all the statements, especially to support the authors’ claim that RF ablation “has become the first-line treatment for small hepatocellular carcinoma”. There are other standard-of-care first line treatments for HCC, including the small HCC lesions.

**Thanks for the correction, we have added the reference and revised the statement.**

Lines 262-264: Please provide references for all the statements.

Thanks for the correction, we have added a reference.

Lines 264-265: Please correct the reference provided to support this statement. Reference #8 does not report data to suggest that histotripsy has the potential to promote metastasis.

Sorry for our carelessness, we have replaced the reference with the correct one.

Lines 269-270: Please clarify whether the authors are referring to the risk of metastatic promotion after histotripsy ablation in general, or only after histotripsy ablation of liver tumors. Please also clarify why the authors imply that the potential tumor promoting effect of histotripsy does not apply to regional metastasis, and only to distant metastasis.

Thanks for your suggestion, we have added "solid tumor" to the sentence. In the section "Evaluation of tumor immune function in histotripsy", we present the study that histotripsy treatment is associated with a reduction in tumor intrahepatic metastasis.

Line 272: Please revise the phrase "Due to the specificity of its location and function", as the phrase may not provide an adequate explanation to the reader why surgical treatment of brain lesions requires "more damage control of surrounding normal structures".

Thanks for your suggestion, we have rewritten this section from Line 304-305.

Lines 273-274: Please provide references to support the statement, especially the authors' claim that non-invasive treatment can "achieve better therapeutic effect".

Thanks for your correction, we have removed this statement.

Line 276: Please correct the reference provided to support this statement. Reference #28 does not support the authors' statement.

Thanks for your correction, we have removed this statement.

Lines 290-292: Please remove this sentence, it is redundant as it has already been stated in lines 287-288.

Thanks for your correction, we have removed this sentence.

Lines 294-295: Please revise the statement, as not all patients with heart disease are geriatric, and not all of them have multiple complex co-morbidities.

Thanks for the correction, we have made the correction in the article on Line 346-347.

Lines 298-301: Please provide select details of the studies to help the reader better understand the clinical implications of the studies, for example, what anatomic structures did histotripsy treat?

Thanks for your suggestion, we've added more details.

Lines 304-309: Please provide references to support the statements.

Thanks for the correction, we have added references.

Lines 309-310: Please provide the references to support the authors' statement that "Numerous ex vivo and in vivo studies have demonstrated...."

Thanks for the correction, we have added the reference and revised the statement.

Lines 315-317: Please provide the references to support the authors' assertion that "Histotripsy is expected to overcome the limitations...and become a non-invasive method for the treatment of deep vein thrombosis". For example, are there phase 3 clinical trials reported that can support the assertion?

Thanks for the correction, we have changed the sentence to "This study preliminarily demonstrates the feasibility and safety of histotripsy for thrombus ablation."

Lines 317-318: Please provide references to support the statement.

Thanks for the correction, we have added references.

Lines 318-321: Please clarify the statement, for example, what effect of histotripsy is "worse" when treating mature versus newly developed thrombi, and why is it "worse".

Thanks for your suggestion, we have made changes on Line 386-388.

Lines 322-324: Please provide references to support the statement.

Thanks for the correction, we have added references.

Lines 325-326: Please clarify what the authors mean by "treatment effect", for example, does the phrase refer to the ablation quality of histotripsy, or other?

Thanks for the correction, we have made the correction in the article." The size of the fragments generated after ablation is an important factor affecting the safety of histotripsy in the treatment of thrombosis."

Lines 328-330: Please clarify why the result showing that "the largest diameter of the fragments produced by 3-cycle pulse histotripsy was smaller than the size of the anti-embolism filter" would demonstrate "the safety of histotripsy in the treatment of venous thrombosis". If the diameter of the fragment is smaller than the size of the filter, it seems that the fragment would be able to escape the filter, which in turn is detrimental to the patient.

Thanks for your question. If the diameter of the fragment is smaller than the size of the filter, the fragment is too small to form a dangerous embolism.

Lines 332-334: Please either provide references to strongly support the authors' prediction that histotripsy is "expected to become an effective method for the treatment of thrombosis", or revise the statement so that it is not so emphatically predictive.

Thanks for the correction, we have removed the relevant statement.

Clinical trial of histotripsy for benign prostatic hyperplasia: Please provide discussion on why improvements in histotripsy for BPH are needed, since the authors noted that the clinical trial subjects "experienced significant improvement in the International Prostate Symptom Score". Are there other endpoints that need to be achieved in the treatment of BPH?

Thanks for your question. In this trial, no debulking in prostate tissue was observed with transrectal ultrasound imaging or with endoscopic visualization and no clinically significant improvement in uroflow or postvoid residual urine (PVR). For the above

reasons, the parameters still need to be further adjusted. We've added instructions on Line 489-492.

Clinical trial of histotripsy for liver cancer: Please provide references that report the clinical trial and support the statements of the authors in this section.

Thanks for your correction, we did not find any literature related to this trial, but we have added a URL with information about the trial.

Line 369: Please clarify why these patients underwent surgery when histotripsy is supposed to be a non-invasive technique.

Sorry for our poor wording, we have made the relevant changes.

Line 372: Please clarify what the authors mean by “mislocalized”, for example, was the treatment of histotripsy for this 5 mm lesion off-target?

Thanks for the correction, we've made changes on Line 506-508 to better explain it.

Line 372-373: Please clarify what the clinical significance of the decrease in “tumor markers” is for these patients.

Thanks for your correction. Tumor markers were an endpoint of the trial, and a reduction in tumor markers was observed in one patient with distant effects after treatment.

Lines 373-374: Please clarify exactly how many patients with colorectal cancer “experienced distant tumor shrinkage”, and define what these “distant” tumors are – were they tumors that were not treated by histotripsy?

Thanks for the correction. Only one patient had untreated distant tumor shrinkage, which we have corrected.

Line 376: Please clarify and define what the authors mean by “distant effects”.

Thanks for your question, we have explained it on Line 511-513.

Clinical trial of histotripsy for calcified aortic stenosis: Please clarify why these patients underwent surgery when histotripsy is supposed to be a non-invasive technique.

Thanks for the correction, we have made the correction in the article.

Lines 387-389: Please explain why “stronger ultrasound energy and longer duration of treatment” was used for some patients only, since the total number of 10 patients implies that 6 patients received this “stronger” and “longer” treatment, compared to 4 patients. Please also clarify what the authors mean by “stronger ultrasound energy” by explaining exactly what histotripsy parameter was different to create this “stronger energy”.

Thanks for your question. The energy received for each patient's treatment is calculated and counted after treatment. We have made corrections in the manuscript to reduce misunderstandings.

Advantages:

General comment: The authors are encouraged to consider additional discussion to explain the various advantages of histotripsy they list to help the reader understand



exactly why these advantages are unique to histotripsy, and to what other treatment methods are the authors making comparison when listing these advantages.

Additionally, the authors are encouraged to clarify that the translation of these advantages to demonstrable clinical benefits is still a work in progress for the field.

Line 393: Please provide strong support and clarification for the authors' assertion that surgical incisions result in "tumor spread", as this statement is not applicable to all surgical procedures.

Thanks for the correction, we have added references.

Lines 394-395: Please provide strong support for this statement, for example, have there been reports detailing the ability of histotripsy to reduce tumor recurrence, and specifically, to what kind of recurrence are the authors referring – local or distant recurrence?

Thanks for the correction, we have revised the statement and added references.

Lines 396-398: Please provide references to support the statements.

Thanks for the correction, we have added references.

Lines 399-400: Please provide references to support the statement.

Thanks for the correction, we have added references.

Lines 401-403: Please provide references to support the statement.

Thanks for the correction, we have added references.

Limitations:

Lines 405-414: Please provide references to support the statements.

Thanks for the correction, we have added references.

Lines 405-406: Please provide additional information to explain the extent of the depth limitation of histotripsy, for example, has the maximum depth of treatment been established?

Thank you for your suggestion, but we have not collected information about it, what is known is that the deeper the ultrasound penetrates the tissue and the more layers of tissue it penetrates, the greater the aberration.

Lines 407-409: The authors are encouraged to consider additional discussion to explain why "image-guided restrictions" is a clinical limitation.

Thanks for your suggestion, we have removed this section in the revised manuscript.

Lines 410-411: Please revise this statement, as it is inaccurate. For example, the authors described the use of histotripsy to treat metastatic hepatic tumors in an earlier section of the manuscript.

Thank you for the correction. We have revised and explained in the manuscript on Line 550-552.

Lines 410-415: Please revise the section to clarify what the authors mean by "application limitation", for example, do they mean that histotripsy's clinical application is limited due to its current state of technology? There seems to be an overlap between this section and other sections such as "image-guided restrictions".

Please explain the list of items in lines 412-414, for example, what is “energy transfer technology” and how does that help improve the application of histotripsy to clinical use?

Thanks for your correction, we have removed this section in the revised manuscript.

Optimization of histotripsy systems:

Line 416: Please clarify why histotripsy “treatment accuracy” needs improvement when the authors had indicated in lines 399-400 the precision of histotripsy treatment. This comment applies to the discussion in lines 419-440.

Thanks for your question. Although histotripsy has advantages in treatment accuracy, if the histotripsy can be made more accurate on this basis, it will be helpful for its future clinical application.

Line 417: Please clarify what the differences are between “tissue depth and tissue that ultrasound passes through” to help the reader better understand the authors’ intended meaning.

Thanks for your suggestion, we have revised the manuscript on Line 554-556.

Lines 433-435: Please explain how the system discussed can allow histotripsy to be delivered through bone, for example in “transcranial brain histotripsy”, as the delivery of ultrasound waves through bone is a challenge.

Thanks for your correction, we have removed this section in the revised manuscript.

## Reviewer C

Line 37-39: Saying 'During the treatment, when the target lesion heats up, it will also cause thermal damage to normal tissues'

that seems to be redundant- omit the sentence

Thanks for your correction, we have removed this section in the revised manuscript.

68: could introduce more biological effects or rephrase sentence to just state that acoustic cavitation occurs at higher intensities (an increase of pressure)

Thanks for the correction, we have made the correction on Line 67-69.

70-73: Could replace liquid with a more generic term since cavitation can occur in soft tissues too

Thanks for your suggestion, we have made the correction on Line 69-73. We've replaced "liquid" with "tissue"

106: provide examples of how the therapeutic effect is increased or decreased by the cavitation memory effect

Thanks for your suggestion, we have made the correction on Line 108-112.

161 -163: The ending on line 163 could use some rephrasing.

We are sorry for our misrepresentation, we have removed and rewritten this part on Line 169-175.

200: Grammar- should be "... the use of phantoms..."

Thanks for the correction, we have made the correction on manuscript.

204-227: Could introduce some potential diseases to create a better transition into the following sections and paint a better picture

Thanks for your suggestion. In this section we would like to present some in vivo experimental studies validating that histotripsy can produce mechanical ablation.

234: Why has it not met the expectations?

Thanks for your question, we have replaced some references in this section.

253: Histotripsy is capitalized?

We apologize for the carelessness and We have made corrections.

441-446: Seems a little vague, could elaborate on how the increase of cavitation cloud generation can improve efficiency

Thank you for your suggestion. We have removed this section in the manuscript because we have presented the relevant research earlier on Line 112-125.

## Reviewer D

This review paper described the mechanisms involved in cavitation cloud histotripsy and in boiling histotripsy, their potential clinical applications, and their current development status. Though the authors provided a broad aspect of histotripsy (both cavitation cloud histotripsy and boiling histotripsy), there are several areas in the manuscript that need to be improved.

### 1. Mechanisms of boiling histotripsy – Lines 161-164, Pg 8

In boiling histotripsy, cavitation clouds can form due to the shock scattering (interaction of incident shockwaves with scattered fields by the boiling bubble) after the formation of a boiling bubble at the HIFU focus, resulting in the formation of a tadpole shaped lesion. More detailed mechanisms on boiling histotripsy can be found in Pahk KJ et al. 2021. The interaction of shockwaves with a vapour bubble in boiling histotripsy: The shock scattering effect. Ultrasonics Sonochemistry.

Thanks for the correction, we have rewritten this part on Lines 163-175

### 2. Lines 199-201. More details on types of phantom used should be provided as there are a number of tissue phantoms which have been employed in histotripsy study (e.g., liver tissue phantom, agar phantom, RBC-embedded phantom, etc).

Thanks for your suggestion. In this section, we are dedicated to presenting the progress of animal experiments. It is for this reason that we omit the introduction of phantoms. However, we have cited some references on phantoms, which we hope will be helpful to the reader's understanding.

### 3. Line 220. There a number of in vivo experiments performed with boiling histotripsy. Please refer to more recent boiling histotripsy publications.

Thanks for your correction. We have added studies of boiling histotripsy in both chapters 4.1 and 4.8.

### 4. Optimization of histotripsy systems – line 415

A new histotripsy method termed pressure-modulated shockwave histotripsy in addition to boiling histotripsy has recently been proposed and demonstrated – Control of the dynamics of a boiling vapour bubble using pressure-modulated high intensity focused ultrasound without the shock scattering effect: A first proof-of-concept study, Ultrasonics Sonochem.(2021).

Thanks to your suggestion, we have added an introduction of this study on Line 583-594.

### 5. Specific comments

- Please remove millisecond from millisecond-boiling histotripsy throughout the manuscript. It should be boiling histotripsy, not millisecond-boiling histotripsy.

Thanks for your correction, we have made the correction in the manuscript.

- Line 154: should be Maxwell et al. (19).

We apologize for the carelessness and we have revised the statement.

-Line 192. There is a positive relationship between the number of pulses used and the degree of mechanical damage produced (i.e., The number of pulses has a great impact on lesion formation).

Thanks for your correction. We have removed this misstatement.

## Reviewer E

In this manuscript, the authors examine some of the fundamental mechanisms of histotripsy and review evidence for clinical applications. I do not recommend this manuscript for publication.

1. The authors have missed some very important reviews and papers in the field of histotripsy. For example, how does this review add to what has been written by DOI:10.3109/02656736.2015.1007538 and DOI:10.1080/02656736.2021.1905189? At first sight, I believe that the current review is, in fact, less thorough and precise concerning the physics and applications of the process.

Thanks for your review and questions. We hope to focus this review on presenting research advances on clinical evaluation of histotripsy. As surgeons, we try to describe the physics of histotripsy as accurately as possible by thoroughly searching and reading the relevant literature. There are some errors and ambiguities in the manuscript we have submitted, which we have revised based on the reviewers' comments and further knowledge of our own. We would be very grateful if you could point out errors and deficiencies in our revised manuscript. This will help us to understand histotripsy more deeply.

2. Unfortunately, the review suffers from a lack of precision and clarity in terminology. For example:

- It would be helpful to define what is meant by "Traditional HIFU".
- What are the temperature ranges in question?
- How can one quantify thermal damage as "large"?
- HIFU has more than one mechanical effect (acoustic radiation forces, cavitation, etc).

Thank you for your question. In the revised manuscript, we have revised and minimized ambiguity in the description.

3. It is not a good idea to mention the UoM team as the developers of histotripsy when the team at the University of Washington has also had an important role in its development. To be completely accurate, sonication protocols resembling to histotripsy have been known in the literature for more than 30 years.

Thanks for your correction. We have revised on Line 40.

4. Method: I do not think that "mechanical high-intensity focused ultrasound" is a good term for a literature search. Anything like ultrasound + cavitation, bubble activity, bubble damage to soft tissue, tissue emulsification, or tissue fractionation would have returned better results.

Thanks for your suggestion. We followed your suggestion and performed a new search, and selected important papers from recent years to add to the manuscript for introduction.

5. Although I can see that a lot of effort has been put into this manuscript, it is not very well written and it is sometimes difficult to read.

Once again, thank you very much for your comments and suggestions. We tried our best to improve the manuscript and made some changes in the manuscript.

Subsequently, we will accept the journal's extensive language editing to improve the English presentation of the manuscript.

## Reviewer F

This paper is a narrative review on histotripsy, an ultrasound mechanical ablation technique. This review has several major issues. First, this review has made dozens of incorrect statements, which conflict with the references cited. Second, this review paper is missing several recent key papers in the histotripsy field. Third, the English writing is unsatisfactory. Because of these major shortcomings, I cannot recommend this paper for publication.

1. This review has made multiple incorrect statements. Examples are given below. Please note that this is not a comprehensive list, as this review contains too many incorrect statements, suggesting authors' lack of in-depth understanding of the literature.

Page 5, line 92 – “But unlike ESWL, cavitation cloud histotripsy uses pulses of 5-20 cycles (4-20 $\mu$ s) to create a rarefactional phase of shorter duration (<1 $\mu$ s).” – This statement is wrong. Cavitation cloud histotripsy uses 1-20 cycle pulses, and every cycle contains a rarefactional phase.

Thanks for the correction. We are sorry for our misrepresentation. We have made changes in the manuscript.

Page 5, line 94 – “The high-pressure wave peak positive pressure is about 80 MPa, and the peak negative pressure is about 20 MPa. In general, cavitation cloud histotripsy has a relatively low frequency (usually from 0.75 MHz to 1 MHz), short pulses (1-20 cycles), and frequent pulse-delivery (10 Hz – 1 kHz).” – Cavitation cloud histotripsy has been demonstrated using frequency ranges of 250kHz – 5MHz, at peak negative pressure above 15MPa. It does not have a requirement for the peak positive pressure.

Thanks for the correction. We are sorry for our misrepresentation. We have made changes in the manuscript.

Page 6, line 106 – “.....but the treatment efficiency of histotripsy under this condition is too low to meet clinical needs.” – This statement is incorrect. The treatment efficiency of histotripsy (the effectiveness per pulse) at a low PRF is high, but the treatment speed is slow.

Thanks for the correction. We are sorry for our misrepresentation. We have made changes in the manuscript.

Page 7, line 123 – “Under high PRF conditions, pulses interval is shorter than the time for bubble dissolution. Previously generated microbubbles remain intact, and new pulses generated new microbubbles,..” Previously generated microbubbles do not remain intact. They collapse to form bubble fragments, which last for several seconds after each pulse.



Thanks for the correction. We are sorry for our misrepresentation. We have made changes in the manuscript.

Page 7, line 130 – “But in general, high PRF (500-2000 Hz) and large pulse number (>2000 pulses) are considered to be the best parameters for creating bubble clouds (2,11-13). Histotripsy at this parameter can result in a homogeneous liquefied lesion with smooth and sharp borders.” The parameters listed are not the best parameter set as stated. This is also an example of the unsatisfactory English – “Histotripsy at this parameter...”.

Thanks for the correction. We are sorry for our misrepresentation. We have removed the relevant erroneous description in the manuscript.

Page 7, last paragraph and Page 8 first paragraph – The authors spent almost a page to describe that some references (15, 17) indicate that the cavitation threshold decreased with stiffer tissue (or tissue with a higher Young’s modulus), while other references (18, 19) indicate that the cavitation threshold does not change with tissue stiffness. Therefore, the authors conclude that “In conclusion, further research is needed to determine the tissue properties that influence the cavitation threshold.” In fact, these references do not indicate conflicting results. References 15 and 17 show that the shockscattering cavitation threshold is reduced with increasing Young’s modulus, because the bubble expansion is reduced in tissue with increasing Young’s modulus, and smaller bubbles are less effective reflectors for shockwaves. Therefore, it requires shockwaves with a higher positive pressure in the stiffer tissue to achieve the same cavitation intrinsic threshold. References 18 and 19 show that the cavitation intrinsic threshold does not change with tissue mechanical properties. The results from these papers actually match with each other well.

Thanks for the correction. We are sorry for our misrepresentation. We have made changes in the manuscript.

Page 8, line 161 – “Before the thermal effect becomes significant, the boiling bubbles interact with the microbubble cloud to form a "fountain" that can inhale the surrounding tissue into it and explodes.” Incorrect statement about how the “fountain” is formed.

Thanks for the correction. We are sorry for our misrepresentation. We rewrote the part of mechanism of boiling histotripsy on Line 163-173.

Page 9, line 173 – “The peak positive pressure of millisecond-boiling histotripsy is about 40 MPa, and the peak negative pressure is about 10 MPa, which is about half of the sound pressure of cavitation cloud histotripsy.” - Wrong statement about the pressure levels used in boiling histotripsy.

Thanks for the correction. We are sorry for our misrepresentation. We have made changes in the manuscript.

Page 9, line 175 – “It is worth noting that, unlike randomly generated cavitation

clouds, each pulse in millisecond-boiling histotripsy can produce a boiling bubble, which ensures the stability of the treatment (24).” When the peak negative pressure is sufficiently high, cavitation bubble cloud is consistently generated by each pulse, not randomly generated.

**Thanks for the correction. We are sorry for our misrepresentation. We have removed the relevant erroneous description in the manuscript.**

Page 13, line 255 – “Vlaisavljevich et al. (34) conducted experiments on percutaneous porcine liver histotripsy.” Then in this paragraph, the authors repeatedly mentioned percutaneous ablation from this study. But Vlaisavljevich et al. (34) in fact conducted transcutaneous treatment, not percutaneous treatment.

**Thanks for the correction. We are sorry for our misrepresentation. We have made changes in the manuscript.**

2. This review paper is missing several recent key papers in the histotripsy field. A quick search in Pubmed on histotripsy generated 268 papers, yet this review only covered about 50 related to the histotripsy field. Many important papers are missing. Page 11, line 220 – “Up to now, there are few animal experiments on millisecond-boiling histotripsy, and more studies is needed to evaluate.” This review only cited two ex vivo studies on boiling histotripsy, entirely ignoring the in vivo studies on boiling histotripsy. Here are a couple of references.

Khokhlova, T. D., Schade, G. R., Wang, Y. N., Buravkov, S. V., Chernikov, V. P., Simon, J. C., Starr, F., Maxwell, A. D., Bailey, M. R., Kreider, W. & Khokhlova, V. A. Pilot in vivo studies on transcutaneous boiling histotripsy in porcine liver and kidney. *Sci Rep* 9, 20176, (2019). PMC6934604

Schade, G. R., Wang, Y. N., D'Andrea, S., Hwang, J. H., Liles, W. C. & Khokhlova, T. D. Boiling Histotripsy Ablation of Renal Cell Carcinoma in the Eker Rat Promotes a Systemic Inflammatory Response. *Ultrasound Med Biol* 45, 137-147, (2019). PMC6546431

Page 12, 4.3 Evaluation of histotripsy in the treatment of solid tumors - This section missed a few key recent references.

Worlikar, T., Mendiratta-Lala, M., Vlaisavljevich, E., Hubbard, R., Shi, J., Hall, T. L., Cho, C. S., Lee, F. T., Greve, J. & Xu, Z. Effects of Histotripsy on Local Tumor Progression in an in vivo Orthotopic Rodent Liver Tumor Model. *BME Frontiers*, (2020).

Worlikar, T., Zhang, M., Ganguly, A., Hall, T. L., Shi, J., Zhao, L., Lee, F. T., Mendiratta-Lala, M., Cho, C. S. & Xu, Z. Impact of Histotripsy on Development of Intrahepatic Metastases in a Rodent Liver Tumor Model. *Cancers (Basel)* 14, (2022). PMC8996987

Page 13, 4.4 Evaluation of histotripsy in the treatment of brain diseases – This section missed the most relevant references as listed below.

Lu, N., Hall, T. L., Choi, D., Gupta, D., Daou, B. J., Sukovich, J. R., Fox, A.,

Gerhardson, T. I., Pandey, A. S., Noll, D. C. & Xu, Z. Transcranial MR-Guided Histotripsy System. *IEEE Trans Ultrason Ferroelectr Freq Control* 68, 2917-2929, (2021). PMC8428576

Lu, N., Gupta, D., Daou, B. J., Fox, A., Choi, D., Sukovich, J. R., Hall, T. L., Camelo-Piragua, S., Chaudhary, N., Snell, J., Pandey, A. S., Noll, D. C. & Xu, Z. Transcranial Magnetic Resonance-Guided Histotripsy for Brain Surgery: Pre-clinical Investigation. *Ultrasound Med Biol* 48, 98-110, (2022).

Page 14, 4.5 Evaluation of histotripsy in the treatment of heart diseases. There are over ten references on this topic, but only three were mentioned, and none covered the calcified aortic stenosis.

Messas, E., Remond, M. C., Goudot, G., Zarka, S., Penot, R., Mateo, P., Kwiecinski, W., Escudero, D. S., Bel, A., Ialy-Radio, N., Bertrand, B., Bruneval, P., Marijon, E., Spaargaren, R., Tanter, M. & Pernot, M. Feasibility and safety of non-invasive ultrasound therapy (NIUT) on an porcine aortic valve. *Phys. Med. Biol.* 65, 215004, (2020).

Thanks for the additional references. Following your suggestion, we have added and introduced many relevant studies in the revised manuscript.

3. Third, the English writing is unsatisfactory. There are numerous grammar mistakes and awkward English expressions throughout the paper. For example, there are four sentences in the Abstract, two of which have issues (see below).

“Traditional HIFU has high local working temperature and large thermal damage, which significantly affects the therapeutic effect.”

“This review introduces the mechanism of histotripsy, summarizes the research progress of animal models for clinical evaluation and clinical application, and analyzes the advantages and limitations of histotripsy, in order to provide reference for future research directions, product optimization and clinical applications.”

Thanks for your correction. Subsequently, we will accept the journal's extensive language editing to improve the English presentation of the manuscript.

## The second-round peer review

Line 23 – ‘Traditional HIFU based on thermal effect has high local working temperature, which may cause...’ Reword sentence structure -> ‘Traditional HIFU, based on thermal effects, has high local working temperature, which may cause...’

Thanks for your suggestion, we have modified the structure of the abstract and made changes based on your suggestions.

Line 25 - change sentence structure

Thanks for your suggestion, we have modified the structure of the abstract and made changes based on your suggestions.