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The attenuation coefficients of the individual components of the IEC-agar tissue mimicking material.

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Title: The attenuation coefficients of the individual components of the IEC-agar tissue mimicking material.

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Keywords: tissue mimicking material, TMM, ultrasound, high frequency, attenuation coefficient, agar, silicon carbide, aluminium oxide.

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Abstract: Tissue mimicking materials (TMMs) are widely used in quality assurance (QA) phantoms to assess the performance of ultrasound scanners. The International Electrotechnical Commission (IEC) define the acoustic parameters of TMMs (IEC, 2001) up to 10 MHz. To manufacture a TMM that closely mimics the acoustical properties of small animal soft tissue at high frequencies, the acoustic properties of each of the individual component ingredients used in the IEC agar-TMM recipe need to be quantified. This study aimed to evaluate whether the overall attenuation coefficient of the IEC agar-TMM is the linear sum of the attenuation coefficients of each of its ingredients. Eight batches of agar-based materials were manufactured with different combinations of ingredients from the IEC agar-TMM recipe. The percentage concentrations of each ingredient used in the individual mixes were identical to that specified in the IEC recipe. The attenuation of each of these batches was measured over the ultrasound frequency range of 12 - 50 MHz and the attenuation value of the agar component was subtracted from the attenuation values of the other batches. The batch attenuation values, representing the attenuation of individual components within the IEC agar-TMM, were then summated and yielded attenuation values which accurately reproduced the attenuation of the IEC-agar TMM.

This information forms a valuable resource for the future development of TMMs with acoustic properties similar to those of soft tissue at high frequencies.

Suggested Reviewers: Louise Cannon
Department of Life and Physical Sciences Gaillimh
louise.cannon@gmit.ie
She has performed acoustic measurements relevant for this paper.

Scott Inglis
Head of Electronic Engineering, Clinical Engineering, NHS Lothian
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He has performed acoustic measurements that are relevant for this paper.

Jacinta Browne
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She has undertaken relevant acoustic research in TMM

Opposed Reviewers:

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18th of May, 2018

Dr. Holland

Editor-in-Chief

Ultrasound in Medicine & Biology

University of Cincinnati, Cardiovascular Research Center

Cincinnati, OH, USA

Dear Dr. Holland

Please find attached my manuscript entitled “The attenuation coefficients of the individual components of the IEC-agar tissue mimicking material” for consideration for publication in Ultrasound in Medicine and Biology.

I declare that the “The attenuation coefficients of the individual components of the IEC-agar tissue mimicking material” have not been and will not be submitted elsewhere for publication.

I will advise the following potential reviewers for the manuscript:

Dr. Scott Inglis

Dr. Louise Cannon

Dr. Jacinta Browne

Sincerely,

RABELL-MONTIEL Adela

The authors are grateful to the Reviewers for their thorough review and constructive feedback on our manuscript.

The organisation of this response document will be as follows: answers to the questions raised by the Reviewers, will be indented. Proposed modifications to the manuscript will be underlined and in italic font. These locations and line numbers are based on the new manuscript.

Reviewers' comments:

Reviewer #1: Overall

The work presented in this manuscript is interesting with the primary aim to investigate what the individual contribution each of the components of the IEC TMM have on the its attenuation. However, the authors do not adequately discuss the results in particular, how their findings can be used to systematically modify the IEC TMM to match different types of tissue and how the data regarding the linear sum of each component contribute to the overall attenuation is useful. The discussion is lacking in terms of its critical assessment of the results obtained and what the meaning of the results are and so the discussion needs to include this.

The manuscript needs to be proof-read as the language used in places is more descriptive than scientific, in the discussion.

-We thank the reviewer for the helpful comments provided. We have extended the Discussion to include a more comprehensive critical assessment and discussion of the results, see specific comments on this below.

Specific Comments

Abstract - good representation of work.

Line 24 Change ".... over the ultrasound frequency of 12-50 MHz ..." to ".... over the ultrasound frequency range of 12-50 MHz ..."

-Agreed. Page 2 Line 24. ...over the ultrasound frequency range of 12 – 50 MHz and the attenuation value of the agar component

Introduction

Provides a good background and context for the investigation.

Methods

Were the Agar and glycerol kept at the same percentage or was it modified to account for the change in the SiC and the Al₂O₃ (0.3 and 3 micron) components.

-On Page 5 Line 78-80, we have added the following sentence "For example, when making the B_{Al2O3} batch we omitted the 0.53% of SiC from the IEC agar-TMM recipe given in Table 1 but did not adjust any of the other weights of the components to account for the ingredient omitted".

Results

Figure 2 should only include the data for the constituent ingredients and not the different types of agar.

-Thank you for your comment. The data from B_{Merck} has been deleted from Figure 2, leaving only B_{VWR} which is the agar used to make the samples used for the other batches.

Discussion

* The following results were presented but no discussion of these findings was presented. "The attenuation increased with increasing frequency for all of the different agar-based material batches (Figure 2). The attenuation from the agar-based material batches composed of $0.3\text{ }\mu\text{m} + 3\text{ }\mu\text{m}$ particles of Al_2O_3 (BAI_2O_3) overlapped with the IEC agar-TMM attenuation ($B_{control}$) between 12 - 25 MHz. The attenuation of the agar-based material batches composed of $SiC + 3\text{ }\mu\text{m} Al_2O_3$ ($BSiC+3\text{ }\mu\text{m } Al_2O_3$), $0.3\text{ }\mu\text{m } Al_2O_3$ ($B0.3\text{ }\mu\text{m } Al_2O_3$) and $3\text{ }\mu\text{m } Al_2O_3$ ($B3\text{ }\mu\text{m } Al_2O_3$) overlapped at low frequencies (12 - 18 MHz). The attenuation of the $BSiC+3\text{ }\mu\text{m } Al_2O_3$ and $B0.3\text{ }\mu\text{m } Al_2O_3$ showed a similar attenuation coefficient from 12 - 40 MHz, but at 50 MHz the difference increased by 2.6 dBcm-1142 . The attenuation from the SiC ($BSiC$) batch samples, and that from the two agar supplier ($BVWR$ and $BMerck$) overlapped in the frequency range of 12 - 23 MHz. The attenuation from $BSiC$ coincided with the attenuation from $B3\text{ }\mu\text{m } Al_2O_3$ at higher frequencies (43 - 50 MHz)."

Specifically, what do the authors think is the reason for the attenuation response to the lower and higher frequencies of the components such as SiC , 0.3 and 3 micron Al_2O_3 .

-Thank you for your comment. On Page 4 Line 64-65 we state that "Furthermore, it is known that the attenuation coefficient and the backscatter of the IEC agar-TMM depend on the percentage concentrations of the Al_2O_3 and the SiC (Cannon et al., 2011; Inglis et al., 2006)". Our attenuation studies at high frequency agree with these results.

-We have shown that attenuation of both sizes of Al_2O_3 particles and SiC do not vary as rapidly with frequency as agar (Figure 3). In future studies it would be interesting to measure the scattering coefficient from Al_2O_3 and SiC to determine the scattering contribution to the overall attenuation which is measured in this study,

What is the purpose of the different metal particles in the TMM based on the attenuation data for the different batches?

-The purpose of the metal particles within the IEC -TMM recipe is to adjust the acoustic properties (attenuation and scattering) of the TMM which was developed to mimic the acoustic properties of soft tissue (Teirlinck et al., 1997). In the case of this study, one ingredient at a time was removed from the TMM recipe to determine the effect of its removal on the acoustic properties of the remaining sample. These measurements were undertaken at high frequency. The attenuation results agreed with previous studies as stated above and presented on Page 4 Line 64-65.

* SiC is known to adjust the backscatter but how does this affect the attenuation and what is the meaning of the attenuation responses over the different frequency ranges?

-From Page 12 Line 213-214. "the attenuation shown for $B_{SiC-VWR}$, $B_{0.3\mu Al2O3-VWR}$ and $B_{3\mu Al2O3-VWR}$ (Figure 3) do not increase with increasing frequency as rapidly as their respective agar-based components in Figure 2". Furthermore, it can be seen in Figure 3 and Figure 4 that the attenuation of the TMM is mostly due to the attenuation of $B_{0.3\mu Al2O3-VWR}$ and $B_{3\mu Al2O3-VWR}$ particles.

* What is the contribution of the different size Al₂O₃ particles to the attenuation response of the TMM? How does the combination of the two size particles behave over the different frequencies? A discussion of each of the above should be provided to provide more meaning to the data and for the results to be useful in terms of modifying the amount of the individual components to alter the TMMs acoustic properties over the frequency range investigated.

-In this study we do not measure the backscatter of the individual components but agree this would be an interesting area to pursue in future studies. We have shown on Page 12 Line 213-214 "the attenuation shown for $B_{SiC-VWR}$, $B_{0.3\mu Al2O3-VWR}$ and $B_{3\mu Al2O3-VWR}$ (Figure 3) do not increase with increasing frequency as rapidly as their respective agar-based components in Figure 2." Furthermore, it can be seen in Figure 3 and Figure 4 that the attenuation of the TMM is mostly due to the attenuation of $B_{0.3\mu Al2O3-VWR}$ and $B_{3\mu Al2O3-VWR}$.

* In the abstract it is proposed that the data presented in this manuscript forms a valuable resource for the future development of TMMs with acoustic properties similar to those of soft tissue at high frequencies, however, there is no discussion of how the TMM can be modified to match different tissue types. A discussion of which tissues could be matched using the TMM with different modifications should be included.

-New section added. Page 13 Line 239-270. "Matching the acoustic properties of the IEC agar-TMM to those of small animal soft tissue

The acoustic properties of mouse soft tissue (brain, liver, and kidney) have previously been measured over the frequency range of 12 – 32 MHz with the tissue immersed in PBS at

37°C (Rabell-Montiel et al., 2018). The SoS was found to be 1566.3 ± 9.9 ms-1 for brain, 1604.7 ± 16.8 ms-1 for liver and 1574.9 ± 10.8 ms-1 for kidney. The attenuation coefficients were found to be nonlinear as a function of frequency (f) and were modelled as second-degree polynomials: 0.7533f + 0.006477f² (R²=0.85) for brain, 0.7252f + 0.01414f² (R² =0.70) for liver, and 0.5771f + 0.006322f² (R²=0.83) for kidney.

The acoustic properties of an agar-based material have previously been studied by changing the percentage concentration of the ingredient components, based on the IEC agar-TMM recipe (Cannon et al., 2011; Inglis et al., 2006). In order to adjust the acoustic properties of the IEC agar-TMM to match those of small animal soft tissue, the results shown in this project have been compared with previously published work of the acoustic properties of small animal soft tissue (Rabell-Montiel et al., 2018).

Glycerol is the main component that modifies the SoS in the TMM. The IEC recommends a SoS value of 1540 ± 15 ms-1 for TMM, which is lower than the SoS measured from small animal soft tissue. Consequently, to achieve the SoS of mouse brain tissue, the concentration of glycerol must increase to approximately 130% compared to the original IEC agar-TMM recipe, whereas for liver the glycerol concentration will have to be increased above 150%. To match the SoS of the agar-TMM to the SoS of the kidney, the glycerol percentage concentration should be increased to 140%.

Figure 7 is adapted from Cannon et al., (2011) and Inglis et al., (2006), and shows the difference in the attenuation of the IEC agar-TMM when the percentage of the SiC and the Al₂O₃ sizes particles have been modified. The concentration of aluminium oxide was found to mainly contribute to the overall attenuation of the agar-TMM (Cannon et al., 2011; Inglis et al., 2006). The attenuation data from kidney, liver and brain tissues calculated in Rabell-Montiel et al., (2018) are included in the figure. From Figure 6, it can be seen that the attenuation from the IEC agar-TMM matched that from kidney within 1%. It is evident that in order to match the attenuation coefficient for liver tissue, the concentrations of Al₂O₃ sizes particles would need to be increased to concentrations great than 180% of the original IEC agar-TMM recipe. The attenuation coefficient from brain showed good agreement with the attenuation of the percentage of aluminium oxide (both particles sizes) at 250%. Therefore, to create a TMM which mimics the properties of small animal soft tissue, the largest modification to the IEC agar-TMM recipe should be the glycerol concentration (to match the SoS) and the Al₂O₃ particle concentrations (to match the attenuation).

* A discussion of how the data can be used to inform a systematic approach to matching different tissues needs to be discussed. Specifically, how can the information regarding the overall attenuation of the IEC agar-TMM being the linear sum of the individual components be used to simplify the process of formulating a TMM with the appropriate attenuation properties?

-Agreed. Added on New Section Page 13 Line 255-257. The aluminium oxide has been found to mainly contribute to the overall attenuation of the agar-TMM (Cannon et al., 2011; Inglis et al., 2006). Therefore, to create a TMM which mimics the properties of small animal soft tissue, the largest modification to the IEC agar-TMM recipe should be the glycerol concentration (to match the SoS) and the Al₂O₃ particle concentrations (to match the attenuation).

Reviewer #2: Manuscript Number: UMB-D-18-00008.

The attenuation coefficient of the individual components of the IEC-agar tissue mimicking material

General comments

The paper presents an interesting experimental research in which different amounts of scatters are combined in a standardized TMM recipient. The idea is to find out the influence of each individual substance in the overall ultrasonic parameters in the TMM. The results are useful to help development of TMM for different soft tissue, what is a great contribution to the scientific community. The major drawback of the paper, or better, the research is that no uncertainty estimation has been done. The use of standard deviation is much modest than presently is possible to be done, regarding the literature. An improvement in that aspect would grow up the overall quality of the paper, what is of interest.

Specific comments

ABSTRACT

1. The reference "IEC, 2001" is not well written in the reference section. The standard number (61685) should be informed therein.

-Thank you for your comment, this has now been modified in the reference section.

-Page 18 Line 314-315. International Electrotechnical Commission (IEC). IEC 61685: International Standard. Ultrasonics - Flow measurement systems - Flow test object. Geneva: Author; 2001.

INTRODUCTION

2. Line 39. The unit "dBcm" should be separated ("dB cm"). Please do the same for all units throughout the text.

-Agreed and modified throughout the document.

MATERIALS AND METHODS

3. Line 101. One issue should be better clarified. Table 3 reports a "peak negative pressure" that was measured many years before by another researcher? How reliable is the equipment to assure that there is no drift in this parameter much longer after? Please justify it consistently or, even better, report new measurements values.

-The peak negative pressures reported in Table 3 were indeed measured in 2012 (Sun et al., 2012). However, the authors have no reason to suppose that these values have changed significantly over the years as the same machine and probes are being used and the image quality has not shown any measurable degradations.

4. As the paper reports an experimental research, some concerns about uncertainty shall

be addressed. Recent papers published by UMB deals with this issue and could be used to deploy the bases for the uncertainty assessment (10.1016/j.ultrasmedbio.2014.04.018; 10.1016/j.ultrasmedbio.2016.09.007). Another fundamental paper on metrology and ultrasound that could be also cited is (10.1088/0026-1394/47/2/S13). Without at least a general explanation of uncertainty, experimental researches are flawed. The quality of this paper would be significantly improved if uncertainty is addressed.

-The first document (10.1016/j.ultrasmedbio.2014.04.018) relates to studies measured using TMM samples encapsulated in Mylar films. This document has been cited in our paper as Rajagopal et al., (2014). The acoustical characterisation study was based on a transmission substitution technique. The equipment used in this study corresponds to a broadband reflection substitution technique where a transducer was used as both the transmitter and receiver.

-The second document (10.1016/j.ultrasmedbio.2016.09.007) cited corresponds to studies of 10 mm and 20 mm thick samples of TMM. This document has been cited as Santos et al., 2017. The method used to collect the data was using two operators and an interval between subsequent measurements, in a similar manner to that employed by Rajagopal et al., (2014). This reference has been included in the paper as Santos et al., (2017).

-The third document (10.1088/0026-1394/47/2/S13) mentioned corresponded to a review of different measurement methods for the acoustic properties of materials. This document mentioned that most of the uncertainties in an experiment comes from the equipment (used for the measurement of the acoustic properties).

In Rajagopal's research the phase velocity was measured "using two pairs of acoustic pulses (transmitted and reflected) by calculating the time of flight (TOF) of the pulses prevented them to calculate the thickness of the sample". In our research the thickness of the samples was calculated by subtracting the return time interval of the ultrasound wave from the front and rear surfaces as explained in detail in Rabell-Montiel et al., 2017, based on the pulse-reflection substitution technique (AIUM, 2014). The thickness of the samples was previously compared to measurements obtain using a digital calliper and to measurements estimated using the scale-bar from the screen capture of the samples using high-resolution ultrasound equipment. The typical difference between the pulse-reflection substitution technique and the other two methods was approximately $\pm 0.7\text{mm}$. Therefore, the thickness of the sample measured using the pulse-reflection substitution technique was considered a sufficiently accurate method to determine the thickness of the sample.

-Added to Page 11 Line 191-195. Additionally, the variation in SoS may be due to sample thickness as it is known that the sample thickness affects the accuracy of the measured SoS (Rabell-Montiel et al., 2017) when using the pulse-reflection substitution technique (AIUM, 2014). The temperature varied by a maximum of 0.5 °C during the measurements in our study, therefore temperature did not have a significant effect on the results obtained

-The non-linear effects considered non-significant in our study due to the high frequency and low pressures (Table 3) that were utilised for the measurements.

-The calculation of the ultrasonic attenuation depends on knowledge of the ultrasonic properties of the fluid in which the samples of interest are immersed. Therefore, corrections for attenuation through the medium must be included. In our study the medium fluid was Tissue Mimicking Preserving Fluid, which is composed by water, benzalkonium chloride and glycerol as mentioned in Page 5 Line 86-87. The acoustic properties of this fluid have been measured by the National Physical Laboratory with a SoS value of $1538.15 \pm 0.22 \text{ ms}^{-1}$ and an attenuation coefficient which behaved as a 2nd degree polynomial as $\alpha(f) = 0.00309 f^2 - 0.004996 f$ ($R^2 = 0.99$) at $19.2 \pm 0.1^\circ\text{C}$, over the frequency range 1 – 60 MHz, where f is the frequency. The details of including the ultrasonic properties of the TMM preserving fluid when measuring the acoustic properties of TMM samples has been discussed in Rabell-Montiel et al., 2017.

-Added to page 12 Line 203-208. The calculation of the ultrasonic attenuation depends on knowledge of the ultrasonic properties of the fluid in which the samples of interest are immersed. Therefore, corrections for attenuation of the medium are included in the calculation of the attenuation of the individual batches. In our study the fluid was TMM preserving fluid (Rabell-Montiel et al., 2017), which was previously characterised by the National Physical Laboratory (NPL, Teddington, UK). The uncertainties using this experimental technique and fluid have been addressed previously in Rabell-Montiel et al., (2017).

-Added to Page 13 Line 228. due to experimental.

-Added to Page 13 Line 230-231. experimental errors associated with this attenuation measurement would have increased the overall experimental error in the summation of the individual components

-It is known that the diffraction pattern will be altered with the presence of the sample with phase velocity different from the medium in which the sample is being measured. Since the TMM preserving fluid has been found to have a similar SoS as the IEC agar-TMM sample, we consider the diffraction effect between the edge of the medium and the sample are not significant.

-The experimental uncertainties have been addressed previously in Rabell-Montiel et al., (2017) as the experimental procedure was exactly the same in both that study and in this one. The temperature varied less than 0.5°C , therefore it is unlikely to have significant effect on the results obtained, as mentioned in Rabell-Montiel et al., (2017).

MATERIALS AND METHODS

5. Line 125. As no uncertainty model was informed, what " ± 5.1 " exactly means?. Is it the standard deviation, the standard deviation of the mean or another statistic? The same applies to Table 4. The SD is simply "standard deviation"? Despite it could be obvious, as standard deviation of the mean and standard deviation are significantly distinct, one could be confused when analysing the results.

-Based on the experiment results, SD stands for standard deviation of 30 values within the population.

-Added to Page 8 Line 127. *(SD) of the population*

DISCUSSION

6. Line 191. The expected difference mentioned by Rabell-Montiel et al. (2017) is based in experimental evidences, I guess. Without a proper statistical analysis or, even better, uncertainty assessment the basis for the difference expectation is weak. On the other hand, uncertainty much probably would explain the variation of the experimental results much robustly.

-Figure 2 shows the mean attenuation data as a function of frequency averaged over three measurements. The SD shown was calculated across 1 MHz interval frequency over from the 3dB bandwidth of the 4 transducers used.

7. Line 216 and somewhere else. No statistical method for comparing the results was proposed, what is also a weakness of the analysis of the results. The statement "difference falls within 1 SD" could be more concise if a t-test was applied, for instance. It is quite simple to implement and would sound much better for the readers with a background on statistics and metrology.

-The experimental uncertainties coming from the equipment used were tested in a previous study where the experimental method used was the same as in this study (Rabell-Montiel et al., 2017). In this study it has been assumed that the 1 SD presented in previous published studies provides an indication of the systematic uncertainties. Moreover, on each batch the standard deviations are very small for both the SoS and the attenuation.

-Added to Page 8 Line 127-128. Applying a student t-test, it was found that the SoS was statistically different ($p<0.05$) between the $B_{control}$ and B_{SiC} , B_{VWR} , $B_{SiC+3\mu Al2O3}$ and $B_{3\mu Al2O3}$.

1 The attenuation coefficients of the individual components of the IEC-agar

2 tissue mimicking material.

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6

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13 ABSTRACT

14 Tissue mimicking materials (TMMs) are widely used in quality assurance (QA) phantoms to
15 assess the performance of ultrasound scanners. The International Electrotechnical Commission (IEC)
16 define the acoustic parameters of TMMs (IEC, 2001) up to 10 MHz. To manufacture a TMM that
17 closely mimics the acoustical properties of small animal soft tissue at high frequencies, the acoustic
18 properties of each of the individual component ingredients used in the IEC agar-TMM recipe need to
19 be quantified. This study aimed to evaluate whether the overall attenuation coefficient of the IEC
20 agar-TMM is the linear sum of the attenuation coefficients of each of its ingredients. Eight batches of
21 agar-based materials were manufactured with different combinations of ingredients from the IEC
22 agar-TMM recipe. The percentage concentrations of each ingredient used in the individual mixes
23 were identical to that specified in the IEC recipe. The attenuation of each of these batches was
24 measured over the ultrasound frequency range of 12 – 50 MHz and the attenuation value of the agar
25 component was subtracted from the attenuation values of the other batches. The batch attenuation
26 values, representing the attenuation of individual components within the IEC agar-TMM, were then
27 summated and yielded attenuation values which accurately reproduced the attenuation of the IEC-
28 agar TMM.

29 This information forms a valuable resource for the future development of TMMs with
30 acoustic properties similar to those of soft tissue at high frequencies.

31

32 *Key words:* tissue mimicking material, TMM, ultrasound, high frequency, attenuation coefficient,
33 agar, silicon carbide, aluminium oxide.

34 INTRODUCTION

35 Tissue mimicking materials (TMMs) are widely used in quality assurance (QA) phantoms to
36 assess the performance of ultrasound scanners. The International Electrotechnical Commission (IEC)
37 provided a specification for the acoustic parameters for TMM (IEC, 2001). The specifications of the
38 acoustic parameters are defined for frequencies between 2 and 10 MHz and are $1540 \pm 15 \text{ ms}^{-1}$ for
39 the speed of sound (SoS) and $0.5 \pm 0.05 \text{ dB cm}^{-1} \text{ MHz}^{-1}$ for the attenuation coefficient. The IEC agar-
40 based TMM has become widely used and has been acoustically characterised up to 60 MHz (Brewin
41 et al., 2008; Rabell-Montiel et al., 2016, 2017; Rajagopal et al., 2014; Sun et al., 2012).

42 One approach to manufacture a TMM that closely mimics the acoustical properties of small
43 animal soft tissue at high frequencies (Rabell-Montiel et al., 2018), is to modify the properties of an
44 existing, well-established TMM such as the IEC agar-TMM phantom. The first step in this process is
45 to measure the acoustic properties of the individual component ingredients of the IEC agar-TMM
46 recipe at high frequencies routinely used for preclinical imaging. Quantifying the acoustic properties
47 of these individual components of the IEC agar-TMM will help to determine if it is possible to modify
48 the existing TMM recipe in order to match the acoustical properties of small animal soft tissue at
49 high ultrasonic frequencies. Additionally, the study of the individual components of IEC agar-TMM
50 will test whether the overall attenuation of the IEC agar-TMM is the linear sum of the individual
51 components and thus simplify the process of formulating a TMM with the appropriate attenuation
52 properties.

53 Agar-based materials have been studied up to 14 MHz with changing agar concentrations up
54 to 6.6% in weight (Gettings et al., 1977; Madsen et al., 2005; Manickam et al., 2014a, 2014b; Ross et
55 al., 2006; Zell et al., 2007). The existing IEC agar-TMM recipe states that the agar component should
56 be 3% of the total weight (Teirlinck et al., 1998). The acoustic properties of agar-based materials
57 have been also measured by changing the concentration (0 – 250%) of the 3 composition
58 ingredients, glycerol, silicon carbide (SiC) and aluminium oxide (Al_2O_3). These measurements were

59 performed using a Scanning Acoustic Macroscope (SAM) system in the frequency range from 14.8 –
60 24.5 MHz at 20°C by Cannon et al., (2011). Furthermore, the attenuation of the agar-based material
61 has also been assessed when increasing the percentage concentrations of both the 3µm and 0.3µm
62 Al₂O₃ particles and SiC (400 grain) from 0 – 100% with the SAM system using a probe with a centre
63 frequency of 7 MHz (Inglis et al., 2006). The results from these studies confirm that the attenuation
64 of IEC agar-TMM increases with increasing frequency above 10 MHz (Brewin et al., 2008; Rabell-
65 Montiel et al., 2016, 2017; Rajagopal et al., 2014; Sun et al., 2011, 2012). Furthermore, it is known
66 that the attenuation coefficient and the backscatter of the IEC agar-TMM depends on the
67 percentage concentrations of the Al₂O₃ and the SiC (Cannon et al., 2011; Inglis et al., 2006).

68 The aim of this study was to measure the attenuation of the individual components of the
69 IEC agar-TMM in order to determine whether the overall attenuation of the IEC agar-TMM is the
70 linear sum of the attenuation of its individual components. The acoustic properties of the
71 ingredients of the IEC agar-TMM have not been studied previously at high frequencies.

72 MATHERIALS AND METHODS

73 *Manufacture of samples*

74 Using a base of agar and glycerol in the same proportions as in the IEC agar-TMM recipe
75 (Teirlinck et al., 1998), eight batches, each composed of 10 samples of agar-based TMM with varying
76 constituent components, were manufactured (Table 2). The ingredients specified by the IEC for the
77 TMM recipe are silicon carbide (SiC), and two particles sizes of aluminium oxide ($0.3\mu\text{m}$ Al_2O_3 and
78 $3\mu\text{m}$ Al_2O_3). The volumes of each of the ingredients used in the batches corresponded to the original
79 recipe. For example, when making the $\text{B}_{\text{Al}_2\text{O}_3}$ batch we omitted the 0.53% of SiC from the IEC agar-
80 TMM recipe given in Table 1 but did not adjust any of the other weights of the components to
81 account for the ingredient omitted. These samples were manufactured using a technique developed
82 in previous experiments (Rabell Montiel et al., 2017) and briefly described here. Once the individual
83 batches of agar-based TMM cooled to 42°C, the mixture was poured into PVC rings (2 mm thick,
84 5.5cm inner diameter) which had been previously located on a warm surface. After the TMM
85 mixture was poured a metal ruler was employed to wipe the excess of the TMM mixture using the
86 upper surface of the PVC rings as a guide. The samples were then left to cool. The resultant thickness
87 of the samples varied between 1.78 – 3.32 mm. Thin samples were needed due to the short focal
88 length of the Vevo 770® transducers. The samples were then dislodged from the PVC ring and were
89 placed in a sealed container filled with TMM preserving fluid (Brewin et al., 2008; Cannon et al.,
90 2011; Inglis et al., 2006). The TMM preserving fluid was manufactured in-house and consisted of a
91 mixture of water, glycerol and benzalkonium chloride.

92 *Experimental set-up and data acquisition*

93 For each batch, ten samples were manufactured giving a total of eighty samples which were
94 acoustically assessed. The acoustic properties were measured in a reservoir filled with TMM
95 preserving fluid at 20.7 ± 0.5 °C.

96 Figure 1 shows the schematic diagram of the experimental set-up used with the Vevo 770®
97 scanner. Each sample to be assessed was placed on top of an immersed polymethylpentene reflector
98 before scanning (TPX, Boedeker Plastics, Texas USA) (cylinder of 2.5 cm diameter and 5 mm
99 thickness). The TPX reflector was fixed at the focal point of each transducer, using modelling clay
100 (Plasticine, Flair, UK). To adjust the position of the transducer and the sample for scanning, a 3D
101 positioning system (Visualsonics Inc., Canada) with a step size of 0.1 mm was used. Measurements
102 were made using four transducers (Vevo 770®, Visualsonics, Inc. Toronto, Canada) with a combined
103 frequency range from 12 – 50 MHz (Table 3). Measurements were undertaken at 10% output power.
104 This power was sufficient to obtain data with a good signal-to-noise ratio without the generation of
105 significant nonlinear effects (Sun et al., 2012). The data was analysed offline with a MatLab script
106 (MatLab 2013a, MathWorks, Inc).

107 The raw radio-frequency (RF) data was collected and analysed from four positions on each
108 sample. At each position, the RF data was obtained from previously selected regions-of-interest
109 (ROI). These ROIs were located at the upper surface of the TPX reflector with and without the
110 sample in place and from the front and rear surfaces of each sample. For each measurement, the RF
111 data was collected from 10 evenly spaced scan-lines within these preselected ROIs. The calculated
112 angular separation between the RF acquisition lines was 0.15°, therefore the lines were considered
113 effectively parallel and perpendicular to the TPX reflector.

114 A broadband reflection substitution technique (AIUM, 2014, Zequiri et al., 2010) was
115 employed to calculate the SoS, the thickness and the attenuation of the different material agar-
116 based material batches.

117 *Acoustic difference in agar suppliers*

118 The samples manufactured in this paper utilised agar manufactured by VWR (VWR
119 International Ltd, Dublin, Ireland) as their base ingredient (Table 2). In order to measure the

120 experimental error value in this study an extra batch of ten agar-based samples were manufactured
121 from VWR agar and from a different agar supplier Merck Chemicals (Merck Chemicals Ltd,
122 Nottingham, UK). The acoustic properties from these batches were measured in a similar manner to
123 the other sample batches. The agar batches was named B_{VWR} and B_{VWR2} for VWR agar and B_{Merck} for
124 the Merck agar.

125 RESULTS

126 Table 4 shows the mean SoS of all agar-based material batches (Table 2). It can be seen that
127 the largest difference in SoS was 13.7 ms^{-1} between B_{SiC} and B_{VWR} . B_{Merck} had the largest standard
128 deviation (SD) of the population of 12.5 ms^{-1} . Applying a Student t-test, it was found that the SoS was
129 statistically different ($p < 0.05$) between the B_{control} and B_{SiC} , B_{VWR} , $B_{\text{SiC+3}\mu\text{ Al2O3}}$ and $B_{\text{3}\mu\text{ Al2O3}}$.

130 Figure 2 shows the attenuation measured from each of the different batches with varying
131 constituents over the frequency range of 12 – 50 MHz. Since four transducers were used for this
132 study the attenuation values for each batch, shown in Figure 2, were averaged across 1 MHz
133 intervals based on the frequency bandwidth of the four transducers. From Table 3, it can be seen
134 that the 3dB bandwidth of the four frequency probes overlapped over the frequency range from 12
135 – 28 MHz. The attenuation values from 28 MHz to 40 MHz corresponded to the RMV704, RMV707B,
136 and RMV711 probes. The attenuation shown from 40 – 50 MHz correspond only to the RMV711
137 transducer. The SD shown in Figure 2 was calculated from the attenuation values measured across 1
138 MHz interval over the frequency range. For clarity of data visualization, the remainder of the figures
139 do not include SDs.

140 The attenuation increased with increasing frequency for all of the different agar-based
141 material batches (Figure 2). The attenuation from the agar-based material batches composed of
142 $0.3\mu\text{m} + 3\mu\text{m}$ particles of Al_2O_3 (B_{Al2O3}) overlapped with the IEC agar-TMM attenuation (B_{control})
143 between 12 – 25 MHz. The attenuation of the agar-based material batches composed of $\text{SiC} + 3\mu\text{m}$
144 Al_2O_3 ($B_{\text{SiC+3}\mu\text{ Al2O3}}$), $0.3\mu\text{m}$ Al_2O_3 ($B_{0.3\mu\text{ Al2O3}}$) and $3\mu\text{m}$ Al_2O_3 ($B_{3\mu\text{ Al2O3}}$) overlapped at low frequencies
145 (12 – 18 MHz). The attenuation of the $B_{\text{SiC+3}\mu\text{ Al2O3}}$ and $B_{0.3\mu\text{ Al2O3}}$ showed a similar attenuation
146 coefficient from 12 – 40 MHz, but at 50 MHz the difference had increased to 2.6 dB cm^{-1} . The
147 attenuation from the SiC (B_{SiC}) batch samples, and that from the two agar supplier (B_{VWR} and B_{Merck})
148 overlapped in the frequency range of 12 – 23 MHz. The attenuation from B_{SiC} coincided with the
149 attenuation from $B_{3\mu\text{ Al2O3}}$ at higher frequencies (43 – 50 MHz).

150 The largest difference in attenuation was 24.1 dB cm⁻¹ between the attenuation of $B_{control}$
151 and the attenuation of one of the agar samples B_{Merck} at 50 MHz. Also, at higher frequencies, the
152 attenuation from $B_{SiC+0.3 Al_2O_3}$ overlapped with the attenuation of $B_{Al_2O_3}$, and B_{SiC} attenuation
153 overlapped the attenuation of $B_{3\mu Al_2O_3}$.

154 The attenuation of the agar B_{VWR} samples was subtracted from the attenuation values of
155 B_{SiC} , $B_{0.3 Al_2O_3}$ and $B_{3\mu Al_2O_3}$ (Figure 3) to yield the attenuation values of SiC, 0.3μm Al₂O₃ and 3μm
156 Al₂O₃ respectively. These comprise the main ingredients of the IEC agar-TMM. The subtraction of the
157 agar attenuation enabled the calculation of the attenuation value of each of the main IEC agar-TMM
158 constituent components and enable direct comparison of their attenuation as a function of
159 frequency.

160 The attenuation of $B_{SiC-VWR}$, B_{VWR} , and $B_{3\mu Al_2O_3-VWR}$, overlapped at low frequencies (12 – 16
161 MHz) whereas the attenuation from $B_{SiC-VWR}$ and $B_{0.3\mu Al_2O_3-VWR}$ overlapped at higher frequencies
162 (44 – 50 MHz).

163 The attenuation values from each of the individual constituent components of the IEC agar-
164 TMM ($B_{SiC-VWR}$, B_{VWR} , $B_{0.3\mu Al_2O_3-VWR}$ and $B_{3\mu Al_2O_3-VWR}$) were summated together. This data is
165 showed in Figure 4. The addition of the attenuation from these batches enabled a comparison
166 between the attenuation of IEC agar-TMM ($B_{control}$) and its individual components.

167 The attenuation calculated from the addition of the individual components ($B_{SiC-VWR}$, B_{VWR} ,
168 $B_{0.3 Al_2O_3-VWR}$ and $B_{3 Al_2O_3-VWR}$) was found to be higher by a maximum of +1.28 dB cm⁻¹, across the
169 frequency bandwidth of 12 – 50MHz when compared with the attenuation of the control samples
170 ($B_{control}$).

171 *Acoustic properties between different agar suppliers*

172 As shown in Table 4, the SoS varied 12 ms⁻¹ between the B_{VWR} and the B_{Merck} . However, the
173 SD for B_{Merck} was 10.7 ms⁻¹ great than the SD of B_{VWR} .

174 The SoS of B_{VWR2} was found to be $1543.9 \pm 6.0 \text{ ms}^{-1}$. The SoS difference between the B_{VWR}
175 and B_{VWR2} was 1.4 ms^{-1} .

176 Figure 5 shows the attenuation versus frequency of B_{VWR} , B_{Merck} and B_{VWR2} . It can be seen
177 that the difference in attenuation coefficient between B_{VWR} and B_{Merck} was only 0.13 dB cm^{-1} at 12
178 MHz but rose to 2.2 dB cm^{-1} at 50 MHz. Also, at 50 MHz the attenuation difference of B_{VWR2} was
179 found to be lower by 0.64 dB cm^{-1} when compared with the attenuation of B_{VWR} and higher by 1.34
180 dB cm^{-1} when compared with the attenuation of B_{Merck} . Moreover, the attenuation of the B_{VWR2} lay
181 between the attenuation of B_{VWR} and B_{Merck} .

182 DISCUSSION

183 The aim of this study was to investigate the acoustic properties of the individual components
184 of the IEC agar-TMM. In addition the acoustic properties of 2 different agars supplied by two
185 manufacturers (VWR Chemicals and Merck) were also measured. Measurements were undertaken
186 using the preclinical ultrasound scanner Vevo 770® (Table 3) over the frequency of 12 – 50 MHz.

187 *Speed of sound*

188 The SoS measured of $B_{control}$ was found to be 7.5 ms^{-1} smaller than the IEC agar-TMM
189 measured in previous studies (Rabell-Montiel et al., 2017) where a similar technique to measure the
190 acoustical properties using TMM preservation fluid was employed. This difference lay within the
191 measured batch-to-batch SoS variation reported in Rabell-Montiel et al., (2017). The significant
192 difference in the SoS values between the different agar-based material samples (with varying
193 constituent ingredients) and the IEC agar-TMM samples may indicate the SoS dependence on the
194 composition ingredients included in the IEC agar-TMM recipe. Additionally, the variation in SoS may
195 be due to sample thickness as it is known that the sample thickness affects the accuracy of the
196 measured SoS (Rabell-Montiel et al., 2017) when using the pulse-reflection substitution technique
197 (AIUM, 2014). The temperature varied by a maximum of 0.5 °C during the measurements in our
198 study, therefore temperature did not have a significant effect on the results obtained.

199 It is known that the SoS in the IEC agar-TMM is largely controlled by the addition or
200 subtraction of the glycerol content in the manufacturing process (Brewin et al., 2008; Madsen et al.,
201 2005; Moran et al., 2009; Rajagopal et al., 2014). All the SoS values from each of the agar-based
202 material samples fall within the IEC SoS recommended values (IEC, 2001). This was expected as the
203 glycerol concentration was not modified in the manufacturing process of any of the agar-based
204 material batches.

205 *Subtraction of the agar attenuation*

206 The calculation of the ultrasonic attenuation depends on knowledge of the ultrasonic
207 properties of the fluid in which the samples of interest are immersed. Therefore, corrections for
208 attenuation of the medium are included in the calculation of the attenuation of the individual
209 batches. In our study the fluid used was TMM preserving fluid (Rabell-Montiel et al., 2017), which
210 was previously characterised by the National Physical Laboratory (NPL, Teddington, UK). The
211 uncertainties using this experimental technique and fluid have been addressed previously in Rabell-
212 Montiel et al., (2017).

213 Comparing Figure 2 and Figure 3 the attenuation from B_{SiC} , $B_{0.3\mu Al2O3}$ and $B_{3\mu Al2O3}$
214 decreased after the subtraction of the attenuation values measured from B_{VWR} . Moreover, the
215 attenuation shown for $B_{SiC-VWR}$, $B_{0.3\mu Al2O3-VWR}$ and $B_{3\mu Al2O3-VWR}$ (Figure 3) do not increase with
216 increasing frequency as rapidly as their respective agar-based components in Figure 2. The
217 difference in the attenuation of the different components after the subtraction of the agar
218 attenuation suggest that the agar component does affect the attenuation in the overall attenuation
219 of the IEC agar-TMM.

220 *Building up the IEC agar-TMM attenuation coefficient*

221 The summation of the attenuation of the individual IEC-agar TMM component ingredients
222 ($B_{SiC-VWR}$, B_{VWR} , $B_{0.3\mu Al2O3-VWR}$ and $B_{3\mu Al2O3-VWR}$) was compared with the attenuation measured of
223 $B_{control}$. Both attenuation curves were in good agreement across the full experimental spectral range
224 as shown in Figure 4.

225 Figure 4 shows the attenuation of $B_{control}$, the summation of the attenuation ($B_{SiC-VWR}$, B_{VWR} ,
226 $B_{0.3\mu Al2O3-VWR}$ and $B_{3\mu Al2O3-VWR}$) and the attenuation of the IEC agar-TMM previously reported in
227 Rabell-Montiel et al., (2017). As expected, the attenuation of $B_{control}$ was shown to be in good
228 agreement with the IEC agar-TMM attenuation (Rabell Montiel et al., 2017). The summation of the
229 attenuation values of $B_{SiC-VWR}$, B_{VWR} , $B_{0.3\mu Al2O3-VWR}$ and $B_{3\mu Al2O3-VWR}$ were a maximum of 1.8 dB cm^{-1}

230 higher when compared with the attenuation of the IEC agar-TMM over the frequency range of 12 –
231 50 MHz. This difference falls within one SD value of 2 dB cm^{-1} reported for attenuation
232 measurements of the IEC agar-TMM in Rabell-Montiel et al., (2017). Moreover, the difference in the
233 summated attenuation values compared to the B_{control} could also be due to experimental error in the
234 acoustic measurement of the agar (B_{VWR}). Since the agar is the base ingredient in all the agar-based
235 material batches, experimental errors associated with this attenuation measurement would have
236 increased the overall experimental error in the summation of the individual components ($B_{\text{SiC-VWR}}$,
237 B_{VWR} , $B_{0.3\mu\text{Al}_2\text{O}_3-\text{VWR}}$ and $B_{3\mu\text{Al}_2\text{O}_3-\text{VWR}}$).

238 *Acoustic difference between two agar suppliers*

239 The difference between the summated attenuation of $B_{\text{SiC-VWR}}$, B_{VWR} , $B_{0.3\mu\text{Al}_2\text{O}_3-\text{VWR}}$ and $B_{3\mu\text{Al}_2\text{O}_3-\text{VWR}}$ and the attenuation of the IEC agar-TMM (1.84 dB cm^{-1} , Figure 4) can be accounted for by
240 the variability in the attenuation between the two batches of agar (B_{VWR} and $B_{\text{VWR}2}$). This difference
241 in the attenuation is within one standard deviation attenuation expected for overall IEC agar-TMM
242 (Rabell-Montiel et al., 2016, 2017).

244 Matching the acoustic properties of the IEC agar-TMM to those of small animal soft tissue

245 The acoustic properties of mouse soft tissue (brain, liver, and kidney) have previously been
246 measured over the frequency range of 12 – 32 MHz with the tissue immersed in PBS at 37°C (Rabell-
247 Montiel et al., 2018). The SoS was found to be $1566.3 \pm 9.9 \text{ ms}^{-1}$ for brain, $1604.7 \pm 16.8 \text{ ms}^{-1}$ for liver
248 and $1574.9 \pm 10.8 \text{ ms}^{-1}$ for kidney. The attenuation coefficients were found to be nonlinear as a
249 function of frequency (f) and were modelled as second-degree polynomials: $0.7533f + 0.006477f^2$
250 ($R_2=0.85$) for brain, $0.7252f + 0.01414f^2$ ($R_2=0.70$) for liver, and $0.5771f + 0.006322f^2$ ($R_2=0.83$) for
251 kidney.

252 The acoustic properties of an agar-based material have previously been studied by changing
253 the percentage concentration of the ingredient components, based on the IEC agar-TMM recipe

254 (Cannon et al., 2011; Inglis et al., 2006). In order to adjust the acoustic properties of the IEC agar-
255 TMM to match those of small animal soft tissue, the results shown in this project have been
256 compared with previously published work of the acoustic properties of small animal soft tissue
257 (Rabell-Montiel et al., 2018).

258 Glycerol is the main component that modifies the SoS in the TMM. The IEC recommends a
259 SoS value of $1540 \pm 15 \text{ ms}^{-1}$ for TMM, which is lower than all the SoS measured from small animal
260 soft tissue. Consequently, to achieve the SoS of mouse brain tissue, the concentration of glycerol
261 must increase to approximately 130% compared to the original IEC-TMM recipe, whereas for liver
262 the glycerol concentration will have to be increased above 150%. To match the SoS of the agar-TMM
263 to the SoS of the kidney, the glycerol percentage concentration should be increased to 140%.

264 Figure 7 is adapted from Cannon et al., (2011) and Inglis et al., (2006), and shows the
265 difference in the attenuation of the IEC agar-TMM when the percentage of the SiC and the Al_2O_3
266 sizes particles have been modified. The concentration of aluminium oxide was found to mainly
267 contribute to the overall attenuation of the agar-TMM (Cannon et al., 2011; Inglis et al., 2006). The
268 attenuation data from kidney, liver and brain tissues calculated in Rabell-Montiel et al., (2018) are
269 included in the figure. From Figure 6, it can be seen that the attenuation from the IEC agar-TMM
270 matched that from kidney within 1%. It is evident that in order to match the attenuation coefficient
271 for liver tissue, the concentrations of Al_2O_3 sizes particles would need to be increased to
272 concentrations great than 180% of the original IEC agar-TMM recipe. The attenuation coefficient
273 from brain showed good agreement with the attenuation of the percentage of aluminium oxide
274 (both particles sizes) at 250%. Therefore, to create a TMM which mimics the properties of small
275 animal soft tissue, the largest modification to the IEC agar-TMM recipe should be the glycerol
276 concentration (to match the SoS) and the Al_2O_3 particle concentrations (to match the attenuation).

277 CONCLUSIONS

278 In this study, the acoustic properties of IEC agar-TMM ingredients were evaluated over the
279 frequency range 12 – 50 MHz. The percentages of water, glycerol and benzalkonium chloride were
280 not modified from the original recipe.

281 The mean SoS of the $B_{control}$ was found to be $1536.6 \pm 2.0 \text{ ms}^{-1}$. The SoS was found to be in
282 good agreement with those studies published of the acoustic properties of IEC agar-TMM acoustics
283 (Brewin et al., 2008; Browne et al., 2003; Rabell Montiel et al., 2017b; Rajagopal et al., 2014; Santos
284 et al., 2017, Sun et al., 2012) and falls within the IEC recommended guideline (IEC, 2001).

285 The attenuation coefficients of the IEC agar-TMM component ingredients were found to
286 increase with increasing frequency. By summing together, the attenuation values from each of the
287 individual constituent ingredients, the attenuation of the IEC-agar TMM was reproduced. The SD
288 between the addition of the attenuation values and the IEC agar-TMM, and the difference in the
289 agar attenuation from two different manufacturers was within the expected SD value when using
290 the same experimental measurement technique as reported in previous studies (Rabell-Montiel et
291 al., 2017).

292 Finally, this information forms a valuable source for the future development of TMMs with
293 acoustic properties similar to that of soft tissue at high frequencies by the modification of the
294 existing IEC agar-TMM recipe.

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365 LIST OF FIGURES

366 Figure 1. Experimental set-up using the preclinical ultrasound scanner Vevo 770®.

367 Figure 2. Mean attenuation data as a function of frequency averaged over the three measurements.

368 Each batch description can be found in Table 2. The SD shown has been calculated across 1

369 MHz interval frequency from the overlapped 3dB bandwidth of the 4 transducers used with the

370 Vevo 770® ultrasound scanner. Data: $B_{control}$ = control. B_{SiC} = silicon carbide, B_{VWR} = VWR agar.

371 $B_{SiC + 0.3\mu Al_2O_3}$ = silicon carbide and $0.3\mu m Al_2O_3$. $B_{SiC + 3\mu Al_2O_3}$ = silicon carbide and $3\mu m Al_2O_3$.

372 $B_{Al_2O_3} = 3\mu m$ and $0.3\mu m Al_2O_3$. $B_{0.3\mu Al_2O_3} = 0.3\mu m Al_2O_3$. $B_{3\mu Al_2O_3} = 3\mu m Al_2O_3$.

373 Figure 3. Attenuation data as a function of frequency after the subtraction of the agar (BVWR)

374 attenuation value from the main ingredients of the IEC agar-TMM ($B_{SiC-VWR}$, $B_{0.3\mu Al_2O_3-VWR}$ and $B_{3\mu$

375 Al_2O_3-VWR). Data averaged over three measurements.

376 Figure 4. Attenuation versus frequency of $B_{control}$ (IEC agar-TMM), build-up attenuation from the IEC-

377 agar TMM component ingredients ($B_{SiC-VWR}$, B_{VWR} , $B_{0.3\mu Al_2O_3-VWR}$ and $B_{3\mu Al_2O_3-VWR}$) in comparison

378 with the IEC agar-TMM attenuation (Brewin et al., 2006; Inglis et al., 2006; Rajagopal et al.,

379 2014; Rabell Montiel et al., 2017; Sun et al., 2012).

380 Figure 5. Mean attenuation versus frequency of the different agar suppliers (B_{VWR} , B_{MERCK} and B_{VWR2}).

381 Figure 6. Attenuation versus frequency graph comparing the polynomial fit found in this study and

382 the attenuation data from the UTMMs and the IEC agar-TMM (IEC, 2001; Rabell-Montiel et al.,

383 2017).

384 Figure 7. Effects on the attenuation when increasing the concentrations of aluminium oxide and

385 silicon carbide, aluminium oxide only and silicon carbide only (Cannon et al., 2011, Inglis et al.,

386 2006). The power-law fits from the biological tissues measured in Rabell-Montiel et al., (2018)

387 have been added for reference purposes. The red double bracket indicates the IEC (IEC, 2001)

388 guideline.

Table 1. Ingredients of agar-based tissue mimicking material (TMM).

Ingredients	% Weight Concentration	Manufacturer
Water	78.83%	
Glycerol 99% (pure)	11.21%	Sigma-Aldrich company Ltd
Agar	3%	VWR International Ltd.
3µm Al₂O₃ powder	0.95%	Logitech Ltd.
0.3µm Al₂O₃ powder	0.88%	Logitech Ltd.
400 grain SiC power	0.53%	Logitech Ltd.
10% solution of Benzalkonium chloride (C₆H₅CH₂N(CH₃)₂RCI)	4.6%	(50% solution, diluted in-house to 10%) Sigma-Aldrich Company Ltd.

Table 2

Table 2. The components included in each of the TMM batches manufactured. SiC = silicon carbide and Al₂O₃ = aluminium oxide.

Batch name	Composition ingredients	Agar	SiC	0.3µm Al₂O₃	3µm Al₂O₃
B_{control}	Control (IEC agar-TMM)	✓	✓	✓	✓
B_{SiC}	SiC	✓	✓		
B_{VWR}	Agar (VWR International Ltd.)	✓			
B_{Merck}	Agar (Merck Chemicals Ltd.)	✓			
B_{SiC+0.3µ Al₂O₃}	SiC + 0.3µm Al ₂ O ₃	✓	✓	✓	
B_{SiC+3µ Al₂O₃}	SiC + 3 µm Al ₂ O ₃	✓	✓		✓
B_{Al₂O₃}	0.3µm Al ₂ O ₃ + 3µm Al ₂ O ₃	✓		✓	✓
B_{0.3µ Al₂O₃}	0.3µm Al ₂ O ₃	✓		✓	
B_{3µ Al₂O₃}	3µm Al ₂ O ₃	✓			✓

Table 3. Parameters of the four transducers used in this work provided by the manufacturer (VisualSonics, 2006). The acoustic peak negative pressure was taken from Sun et al., 2012.

Model RMV	Central Frequency (MHz)	Focal (mm)	Length	Measured 3dB bandwidth (MHz)	Peak negative pressure (MPa)
704	40	6		18 – 40	0.52
707B	30	12.7		12 – 32	1.05
710B	25	15		12 – 28	1.06
711	55	6		25 – 50	0.23

Table 4. The mean and the SD of the SoS (ms⁻¹) measured with the Vevo 770® across all the agar-TMM component batches.

Batch	B_{control}	B_{SiC}	B_{VWR}	B_{Merck}	B_{SiC+0.3μ Al2O3}
SoS (ms⁻¹ ± SD)	1536.6 ± 2.0	1531.6 ± 6.7	1545.3 ± 1.8	1533.3 ± 12.5	1539.2 ± 8.4
Batch					
	B_{SiC+3μ Al2O3}	B_{Al2O3}	B_{0.3μ Al2O3}	B_{3μ Al2O3}	
SoS (ms⁻¹ ± SD)	1542.0 ± 3.8	1536.7 ± 8.7	1537.2 ± 6.0	1546.8 ± 4.5	

Figure 1

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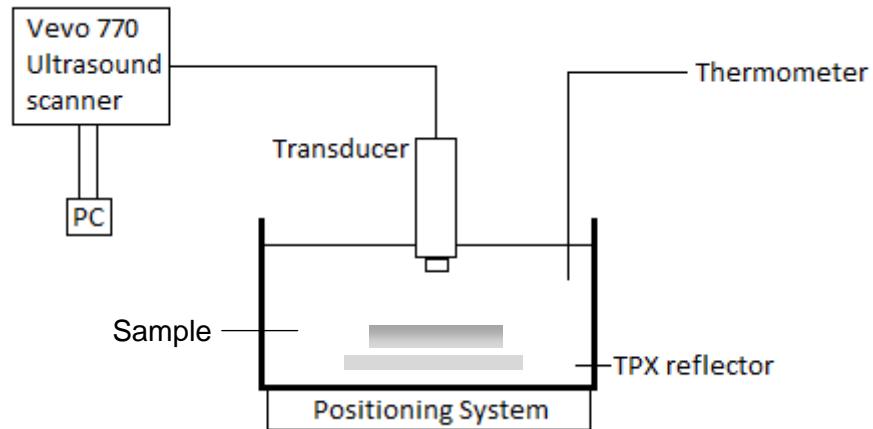


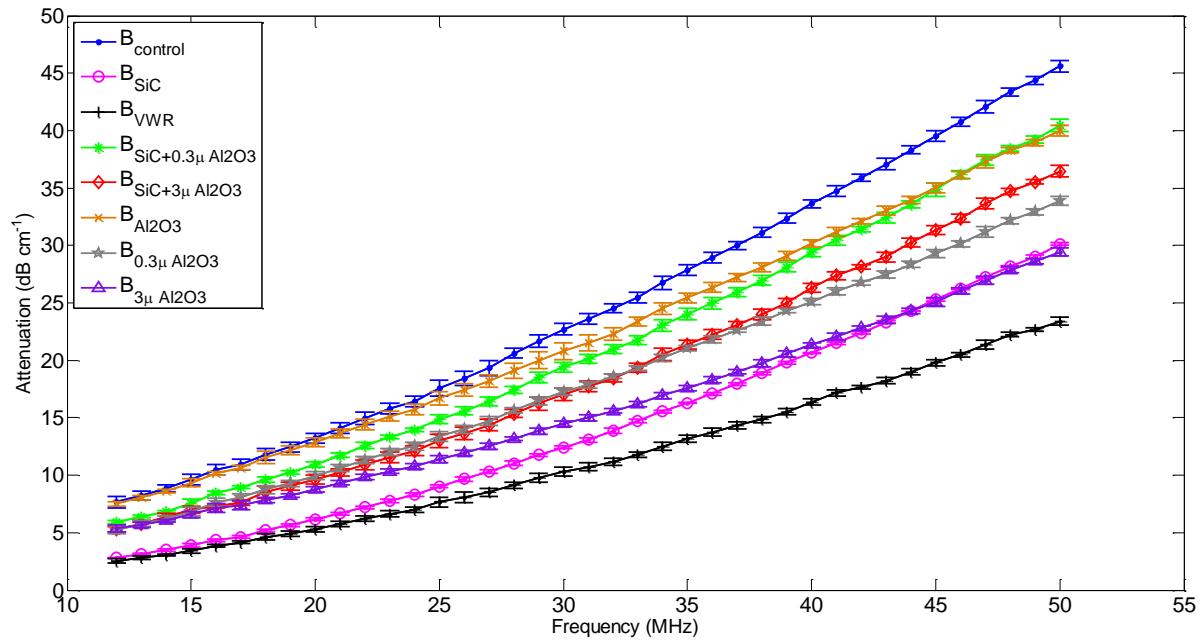
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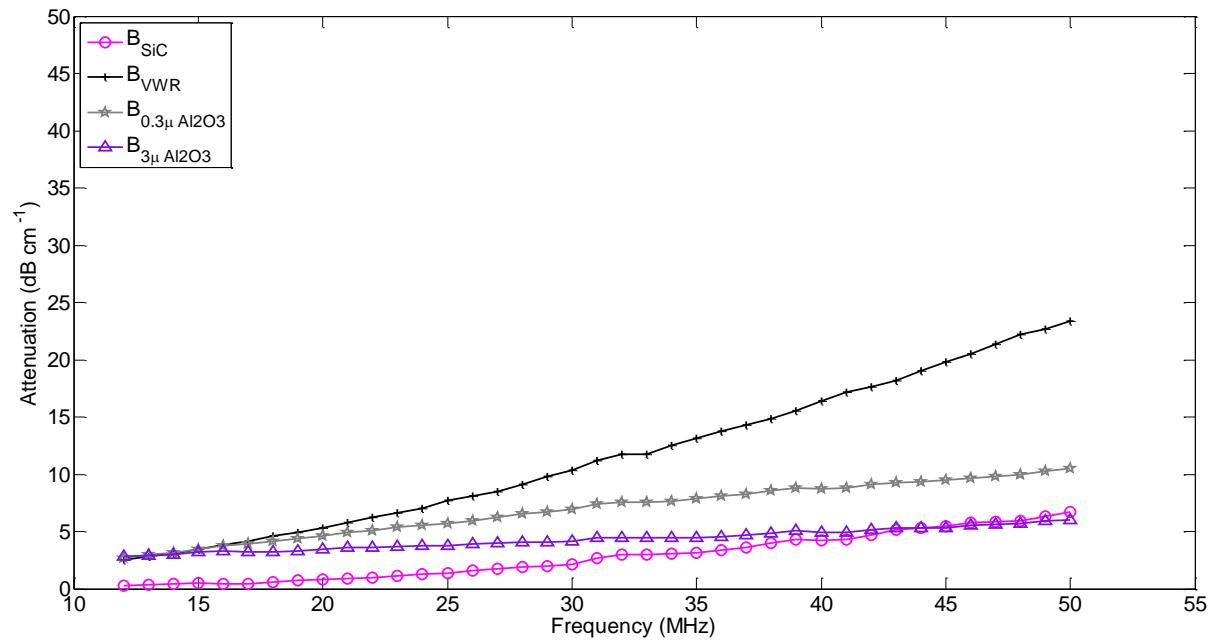
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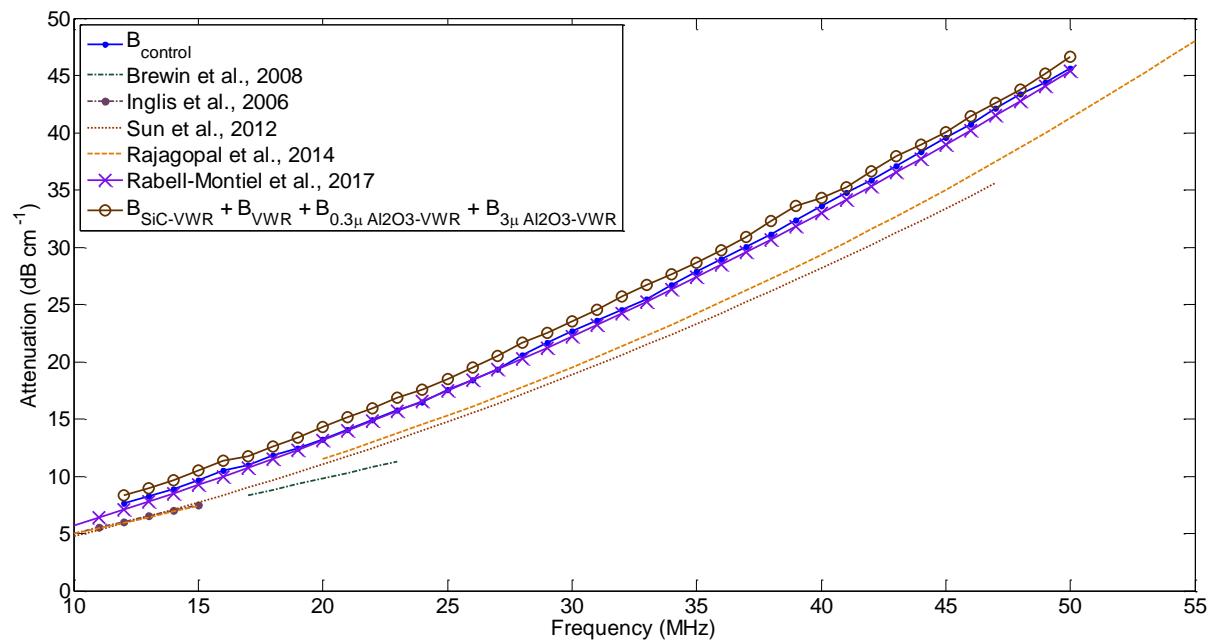
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Figure 5

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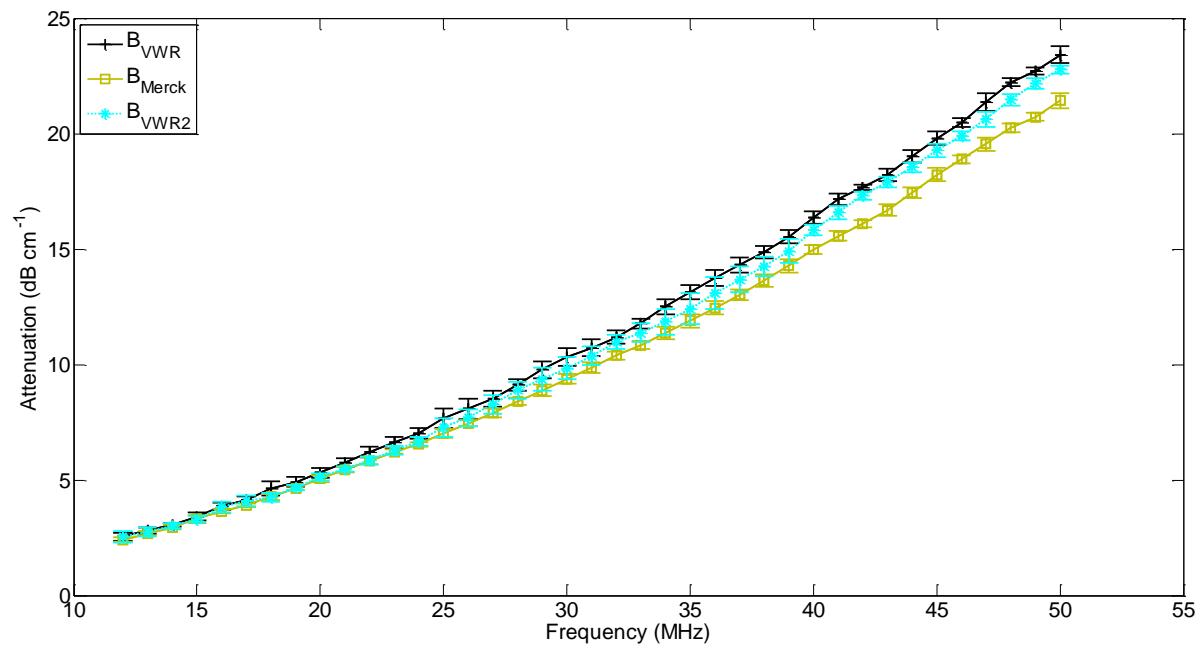


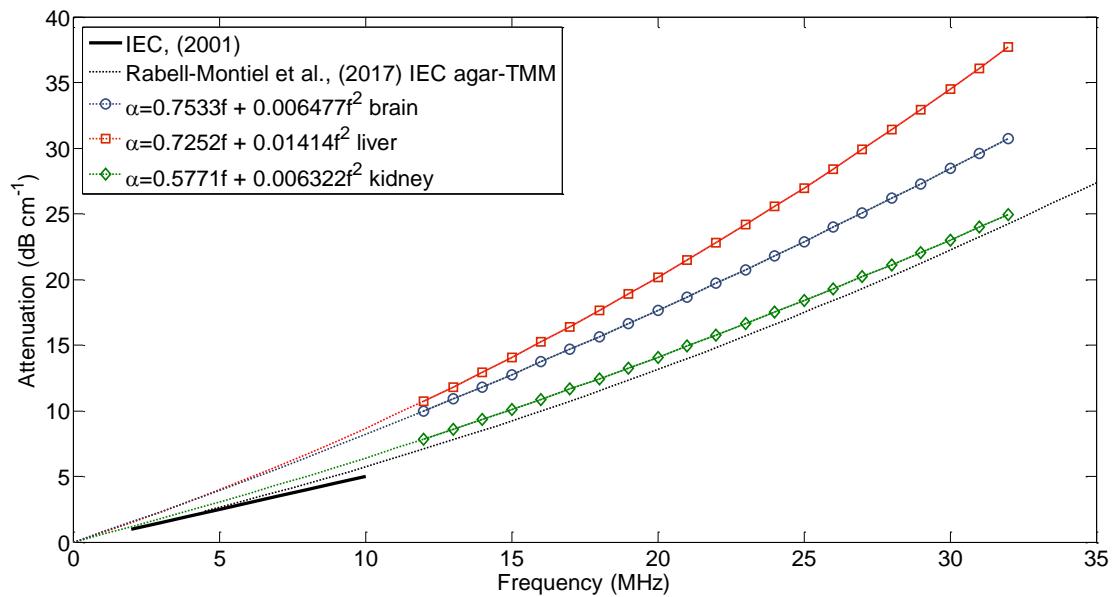
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