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

Article Type: Original Contribution

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 summed 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
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She has performed acoustic measurements relevant for this paper.

Scott Inglis
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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:
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<\mu>m + 3<\mu>m particles of Al2O3 (B_{Al2O3}) 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<\mu>m Al2O3 (BSiC+3<\mu>m Al2O3), 0.3<\mu>m Al2O3 (B0.3<\mu>m Al2O3) and 3<\mu>m Al2O3 (B3<\mu>m Al2O3) overlapped at low frequencies (12 - 18 MHz). The attenuation of the BSiC+3<\mu>m Al2O3 and B0.3<\mu>m Al2O3 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 B_{Merck}) overlapped in the frequency range of 12 - 23 MHz. The attenuation from BSiC coincided with the attenuation from B3<\mu>m Al2O3 at higher frequencies (43 - 50 MHz).”
Specifically, what to 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 Al2O3.

-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 Al2O3 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 Al2O3 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 Al2O3 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_{0.3µ Al2O3-VWR} and B_{3µ 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_{0.3µ Al2O3-VWR} and B_{3µ 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_{0.3µ Al2O3-VWR} and B_{3µ 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_{0.3µ Al2O3-VWR} and B_{3µ Al2O3-VWR} particles.

* 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.


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.

MATHERIALS 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, event 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 flaw. 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 film. 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 ±0.7mm. 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 ± 0.22 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 ± 0.1 °C, over the frequency range 1 – 60 MHz, were \( f \) 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+3µ Al2O3*, and B*3µ Al2O3*. 
The attenuation coefficients of the individual components of the IEC-agar

tissue mimicking material.

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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.

Key words: tissue mimicking material, TMM, ultrasound, high frequency, attenuation coefficient, agar, silicon carbide, aluminium oxide.
Tissue mimicking materials (TMMs) are widely used in quality assurance (QA) phantoms to assess the performance of ultrasound scanners. The International Electrotechnical Commission (IEC) provided a specification for the acoustic parameters for TMM (IEC, 2001). The specifications of the acoustic parameters are defined for frequencies between 2 and 10 MHz and are $1540 \pm 15 \text{ ms}^{-1}$ for 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-based TMM has become widely used and has been acoustically characterised up to 60 MHz (Brewin et al., 2008; Rabell-Montiel et al., 2016, 2017; Rajagopal et al., 2014; Sun et al., 2012).

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

Agar-based materials have been studied up to 14 MHz with changing agar concentrations up to 6.6% in weight (Gettings et al., 1977; Madsen et al., 2005; Manickam et al., 2014a, 2014b; Ross et al., 2006; Zell et al., 2007). The existing IEC agar-TMM recipe states that the agar component should be 3% of the total weight (Teirlinck et al., 1998). The acoustic properties of agar-based materials have been also measured by changing the concentration (0 – 250%) of the 3 composition ingredients, glycerol, silicon carbide (SiC) and aluminium oxide ($\text{Al}_2\text{O}_3$). These measurements were
performed using a Scanning Acoustic Macrooscope (SAM) system in the frequency range from 14.8 – 24.5 MHz at 20°C by Cannon et al., (2011). Furthermore, the attenuation of the agar-based material has also been assessed when increasing the percentage concentrations of both the 3µm and 0.3µm Al₂O₃ particles and SiC (400 grain) from 0 – 100% with the SAM system using a probe with a centre frequency of 7 MHz (Inglis et al., 2006). The results from these studies confirm that the attenuation of IEC agar-TMM increases with increasing frequency above 10 MHz (Brewin et al., 2008; Rabell-Montiel et al., 2016, 2017; Rajagopal et al., 2014; Sun et al., 2011, 2012). Furthermore, it is known that the attenuation coefficient and the backscatter of the IEC agar-TMM depends on the percentage concentrations of the Al₂O₃ and the SiC (Cannon et al., 2011; Inglis et al., 2006).

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

Manufacture of samples

Using a base of agar and glycerol in the same proportions as in the IEC agar-TMM recipe (Teirlinck et al., 1998), eight batches, each composed of 10 samples of agar-based TMM with varying constituent components, were manufactured (Table 2). The ingredients specified by the IEC for the TMM recipe are silicon carbide (SiC), and two particles sizes of aluminium oxide (0.3µm Al₂O₃ and 3µm Al₂O₃). The volumes of each of the ingredients used in the batches corresponded to the original recipe. For example, when making the B₃Al₂O₃ 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. These samples were manufactured using a technique developed in previous experiments (Rabell Montiel et al., 2017) and briefly described here. Once the individual batches of agar-based TMM cooled to 42°C, the mixture was poured into PVC rings (2 mm thick, 5.5cm inner diameter) which had been previously located on a warm surface. After the TMM mixture was poured a metal ruler was employed to wipe the excess of the TMM mixture using the upper surface of the PVC rings as a guide. The samples were then left to cool. The resultant thickness of the samples varied between 1.78 – 3.32 mm. Thin samples were needed due to the short focal length of the Vevo 770® transducers. The samples were then dislodged from the PVC ring and were placed in a sealed container filled with TMM preserving fluid (Brewin et al., 2008; Cannon et al., 2011; Inglis et al., 2006). The TMM preserving fluid was manufactured in-house and consisted of a mixture of water, glycerol and benzalkonium chloride.

Experimental set-up and data acquisition

For each batch, ten samples were manufactured giving a total of eighty samples which were acoustically assessed. The acoustic properties were measured in a reservoir filled with TMM preserving fluid at 20.7 ± 0.5 °C.
Figure 1 shows the schematic diagram of the experimental set-up used with the Vevo 770® scanner. Each sample to be assessed was placed on top of an immersed polymethylpentene reflector before scanning (TPX, Boedeker Plastics, Texas USA) (cylinder of 2.5 cm diameter and 5 mm thickness). The TPX reflector was fixed at the focal point of each transducer, using modelling clay (Plasticine, Flair, UK). To adjust the position of the transducer and the sample for scanning, a 3D positioning system (Visualsonics Inc., Canada) with a step size of 0.1 mm was used. Measurements were made using four transducers (Vevo 770®, Visualsonics, Inc. Toronto, Canada) with a combined frequency range from 12 – 50 MHz (Table 3). Measurements were undertaken at 10% output power. This power was sufficient to obtain data with a good signal-to-noise ratio without the generation of significant nonlinear effects (Sun et al., 2012). The data was analysed offline with a MatLab script (MatLab 2013a, MathWorks, Inc).

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

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

Acoustic difference in agar suppliers

The samples manufactured in this paper utilised agar manufactured by VWR (VWR International Ltd, Dublin, Ireland) as their base ingredient (Table 2). In order to measure the...
experimental error value in this study an extra batch of ten agar-based samples were manufactured from VWR agar and from a different agar supplier Merck Chemicals (Merck Chemicals Ltd, Nottingham, UK). The acoustic properties from these batches were measured in a similar manner to the other sample batches. The agar batches was named $B_{VWR}$ and $B_{VWR2}$ for VWR agar and $B_{Merck}$ for the Merck agar.
RESULTS

Table 4 shows the mean SoS of all agar-based material batches (Table 2). It can be seen that the largest difference in SoS was 13.7 ms\(^{-1}\) between \(B_{\text{SiC}}\) and \(B_{\text{VWR}}\). \(B_{\text{Merck}}\) had the largest standard deviation (SD) of the population of 12.5 ms\(^{-1}\). Applying a Student t-test, it was found that the SoS was statistically different \((p<0.05)\) between the \(B_{\text{control}}\) and \(B_{\text{SiC}}, B_{\text{VWR}}, B_{\text{SiC}+3\mu \text{Al}_2\text{O}_3}\) and \(B_{3\mu \text{Al}_2\text{O}_3}\).

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

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 0.3µm + 3µm particles of \(\text{Al}_2\text{O}_3\) \((B_{\text{Al}_2\text{O}_3})\) overlapped with the IEC agar-TMM attenuation \((B_{\text{control}})\) between 12 – 25 MHz. The attenuation of the agar-based material batches composed of SiC + 3µm \(\text{Al}_2\text{O}_3\) \((B_{\text{SiC}+3\mu \text{Al}_2\text{O}_3}), 0.3\mu \text{m } \text{Al}_2\text{O}_3\) \((B_{0.3\mu \text{Al}_2\text{O}_3})\) and 3µm \(\text{Al}_2\text{O}_3\) \((B_{3\mu \text{Al}_2\text{O}_3})\) overlapped at low frequencies (12 – 18 MHz). The attenuation of the \(B_{\text{SiC}+3\mu \text{Al}_2\text{O}_3}\) and \(B_{0.3\mu \text{Al}_2\text{O}_3}\) showed a similar attenuation coefficient from 12 – 40 MHz, but at 50 MHz the difference had increased to 2.6 dB cm\(^{-1}\). The attenuation from the SiC \((B_{\text{SiC}})\) batch samples, and that from the two agar supplier \((B_{\text{VWR}}\) and \(B_{\text{Merck}}\)\) overlapped in the frequency range of 12 – 23 MHz. The attenuation from \(B_{\text{SiC}}\) coincided with the attenuation from \(B_{3\mu \text{Al}_2\text{O}_3}\) at higher frequencies (43 – 50 MHz).
The largest difference in attenuation was 24.1 dB cm\(^{-1}\) between the attenuation of \(B_{\text{control}}\) and the attenuation of one of the agar samples \(B_{\text{Merck}}\) at 50 MHz. Also, at higher frequencies, the attenuation from \(B_{\text{SiC},0.3\ \text{Al}_2\text{O}_3}\) overlapped with the attenuation of \(B_{\text{Al}_2\text{O}_3}\) and \(B_{\text{SiC}}\) attenuation overlapped the attenuation of \(B_{\text{3µm Al}_2\text{O}_3}\).

The attenuation of the agar \(B_{\text{VWR}}\) samples was subtracted from the attenuation values of \(B_{\text{SiC}}, B_{0.3\ \text{Al}_2\text{O}_3}\) and \(B_{3\ \text{µm Al}_2\text{O}_3}\) (Figure 3) to yield the attenuation values of SiC, 0.3\(\mu\)m Al\(_2\)O\(_3\) and 3\(\mu\)m Al\(_2\)O\(_3\) respectively. These comprise the main ingredients of the IEC agar-TMM. The subtraction of the agar attenuation enabled the calculation of the attenuation value of each of the main IEC agar-TMM constituent components and enable direct comparison of their attenuation as a function of frequency.

The attenuation of \(B_{\text{SiC-VWR}}, B_{\text{VWR}},\) and \(B_{3\ \text{µm Al}_2\text{O}_3-VWR}\) overlapped at low frequencies (12 – 16 MHz) whereas the attenuation from \(B_{\text{SiC-VWR}}\) and \(B_{0.3\ \text{µm Al}_2\text{O}_3-VWR}\) overlapped at higher frequencies (44 – 50 MHz).

The attenuation values from each of the individual constituent components of the IEC agar-TMM (\(B_{\text{SiC-VWR}}, B_{\text{VWR}}, B_{0.3\ \text{µm Al}_2\text{O}_3-VWR}\) and \(B_{3\ \text{µm Al}_2\text{O}_3-VWR}\)) were summated together. This data is showed in Figure 4. The addition of the attenuation from these batches enabled a comparison between the attenuation of IEC agar-TMM (\(B_{\text{control}}\)) and its individual components.

The attenuation calculated from the addition of the individual components (\(B_{\text{SiC-VWR}}, B_{\text{VWR}}, B_{0.3\ \text{µm Al}_2\text{O}_3-VWR}\) and \(B_{3\ \text{µm Al}_2\text{O}_3-VWR}\)) was found to be higher by a maximum of +1.28 dB cm\(^{-1}\), across the frequency bandwidth of 12 – 50MHz when compared with the attenuation of the control samples (\(B_{\text{control}}\)).

**Acoustic properties between different agar suppliers**

As shown in Table 4, the SoS varied 12 ms\(^{-1}\) between the \(B_{\text{VWR}}\) and the \(B_{\text{Merck}}\). However, the SD for \(B_{\text{Merck}}\) was 10.7 ms\(^{-1}\) greater than the SD of \(B_{\text{VWR}}\).
The SoS of $B_{VWR2}$ was found to be $1543.9 \pm 6.0 \text{ ms}^{-1}$. The SoS difference between the $B_{VWR}$ and $B_{VWR2}$ was 1.4 ms$^{-1}$.

Figure 5 shows the attenuation versus frequency of $B_{VWR}$, $B_{Merck}$ and $B_{VWR2}$. It can be seen that the difference in attenuation coefficient between $B_{VWR}$ and $B_{Merck}$ was only 0.13 dB cm$^{-1}$ at 12 MHz but rose to 2.2 dB cm$^{-1}$ at 50 MHz. Also, at 50 MHz the attenuation difference of $B_{VWR2}$ was found to be lower by 0.64 dB cm$^{-1}$ when compared with the attenuation of $B_{VWR}$ and higher by 1.34 dB cm$^{-1}$ when compared with the attenuation of $B_{Merck}$. Moreover, the attenuation of the $B_{VWR2}$ lay between the attenuation of $B_{VWR}$ and $B_{Merck}$. 
The aim of this study was to investigate the acoustic properties of the individual components of the IEC agar-TMM. In addition, the acoustic properties of 2 different agars supplied by two manufacturers (VWR Chemicals and Merck) were also measured. Measurements were undertaken using the preclinical ultrasound scanner Vevo 770® (Table 3) over the frequency of 12 – 50 MHz.

**Speed of sound**

The SoS measured of B\(_{\text{control}}\) was found to be 7.5 ms\(^{-1}\) smaller than the IEC agar-TMM measured in previous studies (Rabell-Montiel et al., 2017) where a similar technique to measure the acoustical properties using TMM preservation fluid was employed. This difference lay within the measured batch-to-batch SoS variation reported in Rabell-Montiel et al., (2017). The significant difference in the SoS values between the different agar-based material samples (with varying constituent ingredients) and the IEC agar-TMM samples may indicate the SoS dependence on the composition ingredients included in the IEC agar-TMM recipe. 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.

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

**Subtraction of the agar attenuation**
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 used 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).

Comparing Figure 2 and Figure 3 the attenuation from $B_{\text{SiC}}$, $B_{0.3\mu \text{Al}_2\text{O}_3}$ and $B_{3\mu \text{Al}_2\text{O}_3}$ decreased after the subtraction of the attenuation values measured from $B_{\text{VWR}}$. Moreover, the attenuation shown for $B_{\text{SiC-VWR}}$, $B_{0.3\mu \text{Al}_2\text{O}_3-VWR}$ and $B_{3\mu \text{Al}_2\text{O}_3-VWR}$ (Figure 3) do not increase with increasing frequency as rapidly as their respective agar-based components in Figure 2. The difference in the attenuation of the different components after the subtraction of the agar attenuation suggest that the agar component does affect the attenuation in the overall attenuation of the IEC agar-TMM.

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

The summation of the attenuation of the individual IEC-agar TMM component ingredients ($B_{\text{SiC-VWR}}$, $B_{\text{VWR}}$, $B_{0.3\mu \text{Al}_2\text{O}_3-VWR}$ and $B_{3\mu \text{Al}_2\text{O}_3-VWR}$) was compared with the attenuation measured of $B_{\text{control}}$. Both attenuation curves were in good agreement across the full experimental spectral range as shown in Figure 4.

Figure 4 shows the attenuation of $B_{\text{control}}$, the summation of the attenuation ($B_{\text{SiC-VWR}}$, $B_{\text{VWR}}$, $B_{0.3\mu \text{Al}_2\text{O}_3-VWR}$ and $B_{3\mu \text{Al}_2\text{O}_3-VWR}$) and the attenuation of the IEC agar-TMM previously reported in Rabell-Montiel et al., (2017). As expected, the attenuation of $B_{\text{control}}$ was shown to be in good agreement with the IEC agar-TMM attenuation (Rabell Montiel et al., 2017). The summation of the attenuation values of $B_{\text{SiC-VWR}}$, $B_{\text{VWR}}$, $B_{0.3\mu \text{Al}_2\text{O}_3-VWR}$ and $B_{3\mu \text{Al}_2\text{O}_3-VWR}$ were a maximum of 1.8 dB cm$^{-1}$
higher when compared with the attenuation of the IEC agar-TMM over the frequency range of 12 –
50 MHz. This difference falls within one SD value of 2 dB cm\(^{-1}\) reported for attenuation
measurements of the IEC agar-TMM in Rabell-Montiel et al., (2017). Moreover, the difference in the
summated attenuation values compared to the B\(_{\text{control}}\) could also be due to experimental error in the
acoustic measurement of the agar (B\(_{\text{VWR}}\)). Since the agar is the base ingredient in all the agar-based
material batches, experimental errors associated with this attenuation measurement would have
increased the overall experimental error in the summation of the individual components (B\(_{\text{SiC-VWR}},\)
B\(_{\text{VWR}},\) B\(_{0.3\mu\text{Al}_2\text{O}_3-VWR}\) and B\(_{3\mu\text{Al}_2\text{O}_3-VWR}\)).

**Acoustic difference between two agar suppliers**

The difference between the summated attenuation of B\(_{\text{SiC-VWR}},\) B\(_{\text{VWR}},\) B\(_{0.3\mu\text{Al}_2\text{O}_3-VWR}\) and B\(_{3\mu\text{Al}_2\text{O}_3-VWR}\) and the attenuation of the IEC agar-TMM (1.84 dB cm\(^{-1}\), Figure 4) can be accounted for by
the variability in the attenuation between the two batches of agar (B\(_{\text{VWR}}\) and B\(_{\text{VWR2}}\)). This difference
in the attenuation is within one standard deviation attenuation expected for overall IEC agar-TMM
(Rabell-Montiel et al., 2016, 2017).

**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: 0.7533\(f\) + 0.006477\(f^2\) (\(R^2=0.85\)) for brain, 0.7252\(f\) + 0.01414\(f^2\) (\(R^2=0.70\)) for liver, and 0.5771\(f\) + 0.006322\(f^2\) (\(R^2=0.83\)) 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
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 all 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-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 Al\(_2\)O\(_3\) 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 Al\(_2\)O\(_3\) 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 Al\(_2\)O\(_3\) particle concentrations (to match the attenuation).
In this study, the acoustic properties of IEC agar-TMM ingredients were evaluated over the frequency range 12 – 50 MHz. The percentages of water, glycerol and benzalkonium chloride were not modified from the original recipe.

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

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

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

The authors will like to thank Kirsty McNeill from the NHS Medical Physics laboratory for her help during the production of this work. This study was funded by a CONACyT (Becas al Extranjero 2014) PhD studentship.
AIUM. *Methods for specifying acoustic properties of Tissue-Mimicking phantoms and objects*. 2014


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

Figure 2. Mean attenuation data as a function of frequency averaged over the three measurements. Each batch description can be found in Table 2. The SD shown has been calculated across 1 MHz interval frequency from the overlapped 3dB bandwidth of the 4 transducers used with the Vevo 770® ultrasound scanner. Data: $B_{\text{control}} =$ control. $B_{\text{SiC}} =$ silicon carbide, $B_{\text{VWR}} =$ VWR agar. $B_{\text{SiC} + 0.3 \mu \text{m} \text{Al}_2\text{O}_3} =$ silicon carbide and 0.3μm Al$_2$O$_3$. $B_{\text{SiC} + 3 \mu \text{m} \text{Al}_2\text{O}_3} =$ silicon carbide and 3μm Al$_2$O$_3$. $B_{\text{Al}_2\text{O}_3} =$ 3μm and 0.3μm Al$_2$O$_3$. $B_{0.3 \mu \text{m} \text{Al}_2\text{O}_3} =$ 0.3μm Al$_2$O$_3$. $B_{3 \mu \text{m} \text{Al}_2\text{O}_3} =$ 3μm Al$_2$O$_3$.

Figure 3. Attenuation data as a function of frequency after the subtraction of the agar (BVWR) attenuation value from the main ingredients of the IEC agar-TMM ($B_{\text{SiC-VWR}}, B_{0.3 \mu \text{m} \text{Al}_2\text{O}_3-\text{VWR}}$, and $B_{3 \mu \text{m} \text{Al}_2\text{O}_3-\text{VWR}}$). Data averaged over three measurements.

Figure 4. Attenuation versus frequency of $B_{\text{control}}$ (IEC agar-TMM), build-up attenuation from the IEC-agar TMM component ingredients ($B_{\text{SiC-VWR}}, B_{\text{VWR}}, B_{0.3 \mu \text{m} \text{Al}_2\text{O}_3-\text{VWR}}$, and $B_{3 \mu \text{m} \text{Al}_2\text{O}_3-\text{VWR}}$) in comparison with the IEC agar-TMM attenuation (Brewin et al., 2006; Inglis et al., 2006; Rajagopal et al., 2014; Rabell Montiel et al., 2017; Sun et al., 2012).

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

Figure 6. Attenuation versus frequency graph comparing the polynomial fit found in this study and the attenuation data from the UTMMs and the IEC agar-TMM (IEC, 2001; Rabell-Montiel et al., 2017).

Figure 7. Effects on the attenuation when increasing the concentrations of aluminium oxide and silicon carbide, aluminium oxide only and silicon carbide only (Cannon et al., 2011, Inglis et al., 2006). The power-law fits from the biological tissues measured in Rabell-Montiel et al. (2018) have been added for reference purposes. The red double bracket indicates the IEC (IEC, 2001) guideline.
Table 1. Ingredients of agar-based tissue mimicking material (TMM).

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% Weight Concentration</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>78.83%</td>
<td>Sigma-Aldrich company Ltd</td>
</tr>
<tr>
<td>Glycerol 99% (pure)</td>
<td>11.21%</td>
<td>Sigma-Aldrich company Ltd</td>
</tr>
<tr>
<td>Agar</td>
<td>3%</td>
<td>VWR International Ltd.</td>
</tr>
<tr>
<td>3µm Al₂O₃ powder</td>
<td>0.95%</td>
<td>Logitech Ltd.</td>
</tr>
<tr>
<td>0.3µm Al₂O₃ powder</td>
<td>0.88%</td>
<td>Logitech Ltd.</td>
</tr>
<tr>
<td>400 grain SiC power</td>
<td>0.53%</td>
<td>Logitech Ltd.</td>
</tr>
<tr>
<td>10% solution of Benzalkonium chloride (C₆H₅CH₂N(CH₃)₂RCl)</td>
<td>4.6%</td>
<td>(50% solution, diluted in-house to 10%) Sigma-Aldrich Company Ltd.</td>
</tr>
</tbody>
</table>
Table 2. The components included in each of the TMM batches manufactured. SiC = silicon carbide and Al₂O₃ = aluminium oxide.

<table>
<thead>
<tr>
<th>Batch name</th>
<th>Composition ingredients</th>
<th>Agar</th>
<th>SiC</th>
<th>0.3µm Al₂O₃</th>
<th>3µm Al₂O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>B&lt;sub&gt;control&lt;/sub&gt;</td>
<td>Control (IEC agar-TMM)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>B&lt;sub&gt;SiC&lt;/sub&gt;</td>
<td>SiC</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B&lt;sub&gt;VWR&lt;/sub&gt;</td>
<td>Agar (VWR International Ltd.)</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B&lt;sub&gt;Merck&lt;/sub&gt;</td>
<td>Agar (Merck Chemicals Ltd.)</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B&lt;sub&gt;SiC+0.3µ Al₂O₃&lt;/sub&gt;</td>
<td>SiC + 0.3µm Al₂O₃</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>B&lt;sub&gt;SiC+3µ Al₂O₃&lt;/sub&gt;</td>
<td>SiC + 3 µm Al₂O₃</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>B&lt;sub&gt;Al₂O₃&lt;/sub&gt;</td>
<td>0.3µm Al₂O₃ + 3µm Al₂O₃</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>B&lt;sub&gt;0.3µ Al₂O₃&lt;/sub&gt;</td>
<td>0.3µm Al₂O₃</td>
<td>✓</td>
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<td></td>
<td>✓</td>
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<tr>
<td>B&lt;sub&gt;3µ Al₂O₃&lt;/sub&gt;</td>
<td>3µm Al₂O₃</td>
<td>✓</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Model</th>
<th>Central Frequency (MHz)</th>
<th>Focal Length (mm)</th>
<th>Measured 3dB bandwidth (MHz)</th>
<th>Peak negative pressure (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>704</td>
<td>40</td>
<td>6</td>
<td>18 – 40</td>
<td>0.52</td>
</tr>
<tr>
<td>707B</td>
<td>30</td>
<td>12.7</td>
<td>12 – 32</td>
<td>1.05</td>
</tr>
<tr>
<td>710B</td>
<td>25</td>
<td>15</td>
<td>12 – 28</td>
<td>1.06</td>
</tr>
<tr>
<td>711</td>
<td>55</td>
<td>6</td>
<td>25 – 50</td>
<td>0.23</td>
</tr>
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</table>
Table 4. The mean and the SD of the SoS (ms⁻¹) measured with the Vevo 770® across all the agar-TMM component batches.

<table>
<thead>
<tr>
<th>Batch</th>
<th>$B_{\text{control}}$</th>
<th>$B_{\text{SiC}}$</th>
<th>$B_{\text{VWR}}$</th>
<th>$B_{\text{Merck}}$</th>
<th>$B_{\text{SiC+0.3µ Al}2\text{O}3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SoS (ms⁻¹ ± SD)</td>
<td>1536.6 ± 2.0</td>
<td>1531.6 ± 6.7</td>
<td>1545.3 ± 1.8</td>
<td>1533.3 ± 12.5</td>
<td>1539.2 ± 8.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Batch</th>
<th>$B_{\text{SiC+3µ Al}2\text{O}3}$</th>
<th>$B_{\text{Al}2\text{O}3}$</th>
<th>$B_{0.3µ \text{ Al}2\text{O}3}$</th>
<th>$B_{3µ \text{ Al}2\text{O}3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SoS (ms⁻¹ ± SD)</td>
<td>1542.0 ± 3.8</td>
<td>1536.7 ± 8.7</td>
<td>1537.2 ± 6.0</td>
<td>1546.8 ± 4.5</td>
</tr>
</tbody>
</table>
Figure 2
Click here to download Figure: figure2.docx

![Graph showing the relationship between Frequency (MHz) and Attenuation (dB cm⁻¹) for different materials: B_control, B_SiC, B_VWR, B_SiC+0.3μ Al₂O₃, B_SiC+3μ Al₂O₃, B_A1₂O₃, B_0.3μ Al₂O₃, and B_3μ Al₂O₃. The graph illustrates attenuation levels across a range of frequencies, with each material having a distinct trend.](figure2.png)
Figure 3
Click here to download Figure: fig 3.docx
Figure 4
Click here to download Figure: fig 4.docx
Figure 5
Click here to download Figure: fig 5.docx
\[ \alpha = 0.7533f + 0.006477f^2 \] brain

\[ \alpha = 0.7252f + 0.01414f^2 \] liver

\[ \alpha = 0.5771f + 0.006322f^2 \] kidney
Figure 7

Click here to download Figure: figure7.docx

![Graph showing attenuation coefficient vs. percentage concentrations for different materials.

- Aluminium Oxide and Silicon Carbide (Inglis et al., 2006)
- Aluminium Oxide and Silicon Carbide (Cannon et al., 2011)
- Aluminium Oxide (Cannon et al., 2011)
- Silicon Carbide (Cannon et al., 2011)
- Brain tissue (Rabell-Montiel et al., 2018)
- Liver tissue (Rabell-Montiel et al., 2018)
- Kidney tissue (Rabell-Montiel et al., 2018)