Original Article

Ultrasonic Attenuation of an Agar, Silicon Dioxide, and Evaporated Milk Gel Phantom

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Abstract

Background: It has been demonstrated that agar-based gel phantoms can emulate the acoustic parameters of real tissues and are the most commonly used tissue-mimicking materials for high-intensity focused ultrasound applications. The following study presents ultrasonic attenuation measurements of agar-based phantoms with different concentrations of additives (percent of agar, silicon dioxide and evaporated milk) in an effort of matching the material's acoustic property as close as possible to human tissues. **Methods:** Nine different agar-based phantoms with various amounts of agar, silicon dioxide, and evaporated milk were prepared. Attenuation measurements of the samples were conducted using the through-transmission immersion techniques. **Results:** The ultrasonic attenuation coefficient of the agar-based phantoms varied in the range of 0.30–1.49 dB/cm-MHz. The attenuation was found to increase in proportion to the concentration of agar and evaporated milk. Silicon dioxide was found to significantly contribute to the attenuation coefficient up to 4% weight to volume (w/v) concentration. **Conclusion:** The acoustic attenuation coefficient of agar-based phantoms can be adjusted according to the tissue of interest in the range of animal and human tissues by the proper selection of agar, silicon dioxide, and evaporated milk.

Keywords: Agar, attenuation, tissue-mimicking material, ultrasound

Introduction

The attenuation coefficient is recognized as being a foundational acoustic property of tissue since it essentially governs the wave-tissue interaction. This main ultrasonic feature of tissues is attracting the considerable interest of many researchers over the last decades. The research interest arises from the need to achieve an accurate measurement of the attenuation coefficient which has been questioned as the results of several studies deviate using various techniques. The correct quantification of human tissues and tissue-mimicking materials (TMMs) attenuation coefficient is of great importance since it represents a key factor in the establishment of optimal high-intensity focused ultrasound (HIFU) treatment strategies.[1]

An immersion technique to estimate the attenuation coefficient is the pulse-transmission method. This method was introduced by Nolle and Mowry[2] in order to estimate the attenuation coefficient in high polymers. Following the same path in the 1960s and 1970s, McSkimin[3] suggested a method to measure the attenuation coefficient which was similar to the pulse-transmission technique. The different approach is that the pulsed signal is transmitted through the sample and received by a receiving transducer placed on the opposite side from the transmitting transducer. Two signals are recorded and analyzed, one without the sample and the other with the sample. A technique that compares the signals as received with different sample thicknesses was presented by Umchid.[4] In another technique,[5] the attenuation coefficient was estimated using a transmitter and

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a receiver transducer with the main advantage that density estimation was not necessary.

The above techniques have been widely used to estimate the attenuation coefficient of numerous materials and tissues. Emphasis has been given in changing the attenuation coefficient concerning the percentages of different liquid or solid materials for the manufacture of TMMs with desired values close to those of humans. The main target of TMMs is the replacement of human tissues and their use for testing diagnostic ultrasound and HIFU applications. Although the attenuation measurement methods may have imperfections and inaccuracies, nevertheless, the estimation of the attenuation coefficient of human/animal tissues and TMMs with the same technique would give comparable results.

In recent years, there has been considerable interest in TMMs in the field of focused ultrasound. In the literature, there are a surprising number of developed TMMs for which their attenuation coefficient was estimated and identified with values close to many tissues. The attenuation coefficient of polyvinyl chloride (PVC)-plastisol gels doped with powder (PVC or graphite) was investigated using the through-transmission technique.[6] The acoustic attenuation values of this material were found to be similar to fat tissue. Polyacrylamide gels combined with bovine serum albumin (BSA) were also prepared and tested.[7] Different amounts of intralipid were added to modify the ultrasonic absorption properties. The increase of intralipid concentration caused an increase in the attenuation coefficient from 0.26 dB/cm-MHz without intralipid to 0.78 dB/cm-MHz with 60% volume to volume (v/v) intralipid concentration.

The pulse-echo method was used in another study to estimate the attenuation of agarose phantoms doped with alumina powder granulation (Al_2O_3) in three different manufacturing techniques (manual, mechanical, or magnetic stirring).[8] The acoustic attenuation of phantoms differed with the various stirring techniques. The developed phantoms with mechanical stirring presented a higher attenuation coefficient than the other two manufacturing techniques. Polyvinyl-alcohol cryogel phantoms with a different number of freeze-thaw cycles have been produced and their attenuation was investigated.[9] The attenuation coefficients were in the range of 0.085–0.124 dB/cm-MHz with the lowest value resulting from a single freeze-thaw cycle and the highest one from three cycles.

The suggested attenuation coefficient slopes for use in phantom materials vary from 0.3 to 0.7 dB/cm-MHz.[10] Whole milk, evaporated milk, liquid, and solid agar-based TMMs were previously developed and tested.[11] Bovine milk has been reported to exhibit a proportionality between the attenuation coefficient and a frequency over a wide range of 1–40 MHz. The attenuation of homogenized whole milk was also measured, and a slope of 0.35 dB/cm-MHz was found.[11] Thereby, whole milk can be only considered as a valuable liquid material to replicate low soft-tissue attenuation coefficient. Evaporated milk contribution to attenuation was studied and an attenuation coefficient slope of about 0.8 dB/cm-MHz was calculated.[11] The dissolution of evaporated milk with water enables the selection of any lower slope attenuation coefficient in the range of 0.3–0.7 dB/cm-MHz. Estimating the attenuation of agar-based gels at frequencies >20 MHz makes them possible for potential use in high-frequency applications.[12] The attenuation of an agar-based phantom that mimics brain and muscle tissue was also reported in a study by Menikou and Damianou[13] The brain tissue-mimicking gel resulted in an attenuation value of 0.59 dB/cm-MHz and the muscle TMM in a value of 0.99 dB/cm-MHz. In this study, the effect of agar concentration on the attenuation coefficient has not been investigated while the attenuation of the agar-based phantoms was measured up to 4% w/v silicon dioxide concentration. All the agar-based phantoms contained evaporated milk; therefore, the recipe that includes only agar and silicon dioxide has not been further explored.

The slopes of the ultrasonic attenuation coefficient versus frequency for agar-based gels doped with glycerol have been shown to lay in the range of 0.35–0.46 dB/cm-MHz at 22°C, which serves as a good approximation for the attenuation of many soft tissues.[14] For weight percent concentrations of up to 10% of glycerol, the increase in attenuation coefficient slope was <0.02 dB/cm-MHz. Typical hydrogel values have been reported to be in-line with the attenuation values of nondoped agar-based gels and polyacrylamide (PAA) materials with values lower than 1 dB/cm.[15,16] Polydimethylsiloxane (PDMS) samples demonstrated significantly higher attenuation values compared with samples of agarose and PAA materials.[16] Although attenuation values increase with material concentration, there were no statistically significant variations between the different quantities of nondoped materials. For high nanoparticle concentrations, statistically important variations were observed approximately between doped and nondoped samples.[17] This demonstrates that the addition of barium titanate nanoparticles increases the attenuation coefficient of agarose, PAA, and PDMS gel phantoms.

Gelatin-based phantoms comprised of dry gelatin, n-propanol, and preservatives were produced and their attenuation was adjusted between the range of 0.2–1.5 dB/cm-MHz by varying the concentration of graphite.[18] It has been stated that the attenuation increases as the amount of gelatin powder increases.[19] In another study,[20] the attenuation of poly (vinyl alcohol) cryogel was found to be low in the range of 0.075–0.28 dB/cm-MHz. However, the addition of enamel paint increases the values close to the values of human tissues. Standard BSA polyacrylamide hydrogel was also found to have a low attenuation coefficient using the pulse-echo method.[21] Despite this, the addition of scattering glass beads and the rise of the concentration of acrylamide to 30% increased the attenuation coefficient of polyacrylamide gels close to the value of diagnostic ultrasound systems. Commercial phantoms that are used in HIFU applications are available with an attenuation coefficient of 0.6 dB/cm-MHz.[22] Furthermore, the attenuation

of polyacrylamide gels (with 9% BSA) was approximately 8 times lower than that of soft tissues.[23]

Agar has already been used as a fundamental material of TMMs due to its ability for having a high melting point (>90°C).[24] This particular property makes agar ideal material for HIFU applications and ablative procedures without losing its integrity. The agar was selected to provide intermediate stiffness and elasticity to the phantom. Agar-based phantoms can resist to HIFU forces without cracking when HIFU applications are evaluated. In addition, silicon dioxide powder was added to control scattering independently, whereas evaporated milk served as a low scatter material that enhanced absorption.[25]

This paper reports the observed changes in the attenuation coefficient of agar-based phantoms with different agar, silicon dioxide, and evaporated milk concentrations. The attenuation coefficient of each sample was estimated using the through-transmission techniques for varying agar, silicon dioxide, and evaporated milk concentrations. In comparison to other studies, this study includes the microscopic structure of the various phantom recipes. We have extracted significant results that could possibly justify the change of attenuation coefficient based on the concentration of the different materials. The attenuation coefficient of excised rabbit tissues using the same method has been measured. The attenuation of agar-based phantoms was matched to the measured corresponding values of the excised rabbit tissues.

Materials and Methods

Measurements of the attenuation coefficient were performed in tissue-mimicking agar-based materials and excised rabbit tissues. No patient data were included in this study. Therefore, no informed consent from patients or approval from an ethics committee was required.

Samples preparation

Agar-based gels with different concentrations of agar (Merck KGaA, EMD Millipore Corporation, Darmstadt, Germany), silicon dioxide powder (Sigma-Aldrich, St. Louis, Missouri, United States), and evaporated milk (Nounou, Friesland Campina, Marousi, Greece) were formed.

The following nine samples were prepared: three samples with different w/v agar concentrations for each sample (2%, 4%, and 6%); three samples with different concentrations (w/v) of silicon dioxide (2%, 4%, and 6%) for agar concentration of 6% w/v; and three samples with different v/v concentrations of evaporated milk (10%, 20%, and 30%) for 6% w/v agar and 4% w/v silicon dioxide.

The manufacturing process until the addition of agar was the same for all the samples. The procedure was slightly differentiated after the addition of agar and depended on the number of materials used. Initially, ultrapure degassed/deionized water was slowly heated and continuously stirred using a magnetic stirrer (SBS, A160, Steinberg Systems, Germany) for a period of 10 min until its temperature reached 50°C. During the procedure, the temperature increase was monitored using an electronic thermometer (Omega Thermometer, HH806AU, Omega Engineering, Norwalk, Connecticut, USA).

For every batch, the agar content was added slowly in degassed/deionized water to mitigate aggregation. It is worth mentioning that since agar was in granular form with a particle size of approximately 1400 μm, it was first ground into powder before mixing with water to promote homogeneous jellification in the absence of impurities. In case no other material was added, the mixture was let to heat until it reached 90°C and then to cool down to 50°C. High temperatures allowed agar's bonds to break and bind to the rest of the mixture (in case other materials were added). During the cooling period, the mixture was continuously stirred with the magnetic stirrer. The mixture started to solidify when the temperature dropped at around 50°C. The amount of water that evaporated during boiling was replaced. The evaporation of the water was estimated by following the same procedure without the insertion of the solid materials. The evaporated water was calculated by subtracting the remaining volume of water from the initial volume. Care was taken by stirring the solution gently to avoid the creation of air bubbles that are known to reflect ultrasound waves. When other materials were added to the mixture, the silicon dioxide was first added 2–3 min after the agar insertion. The mixture including both the agar and silicon dioxide was heated until it reached 90°C. Afterward, the mixture was left to natural cool down to 50°C. Following this, the evaporated milk (v/v) was heated to 30°C and added to the rest of the mixture. For a final step, the whole mixture was stirred well to allow full dissolvement of all ingredients.

The preparation procedure for each phantom was simple and did not last more than 20 min. The mixture was poured into a polylactic acid (PLA)-designed mold and was let to jellify overnight at room temperature. The dimensions of each mold were 40 mm in height, 26 mm in width, and 32 mm in length with 3 mm thickness. Two open circular holes of 22 mm diameter were left on both sides of the mold container to allow ultrasound waves to propagate through the sample. The designed molds could be easily attached and fitted to the experimental setup, making them ideal for accurate attenuation coefficient measurements.

Attenuation measurements for the nine samples were taken within 24 h postfabrication to avoid decomposition by microbial growth. Thus, preservatives were not added, but their use can be beneficial for prolonging shelf life needed when characterizing properties and their change over time. Figure 1a illustrates a computer-aided design drawing of the PLA mold and Figure 1b shows the samples with different agar, silicon dioxide, and evaporated milk concentrations.

Microstructure of agar-based gels

The morphology of the nine phantoms including different concentrations of agar, silicon dioxide, and evaporated milk was investigated using a scanning electron microscope (SEM) (FEI,

Quanta 200, Hillsboro, Oregon, United States). Before the SEM investigation, all samples were sputter coated with a thin (<10 nm) silver layer to reduce electron charging effects. SEM images were collected at 20 kV accelerating voltage in ×100 magnification.

Experimental setup to measure the attenuation coefficient of agar-based gels

Attenuation measurements were conducted using the through-transmission technique, previously reported by Madsen *et al.*[26] The attenuation coefficient is an important acoustic property of tissues and gel phantoms, especially designed for HIFU applications since it governs the efficiency of acoustic energy transformation into heat. Therefore, adequate matching with the respective replicated soft-tissue coefficient is a matter of great importance.

Two well-known methods are broadly used to estimate the attenuation coefficient; pulse-echo and through-transmission method. Both techniques require the submersion of the sample in a tank filled with degassed/deionized water and all measurements are performed with immersion planar ultrasonic transducers. The through-transmission technique that was used herein, involved two planar transducers; one for transmitting the continuous ultrasound signal and one for receiving.

For the experimental setup, a special acrylonitrile butadiene styrene (ABS) plastic holder was designed (Inventor Professional 2018, Autodesk, California, USA) and printed using a 3D printer (F270, Stratasys Ltd., Minnesota, USA) as shown in Figure 2. The ABS holder retained the planar transducers and the sample at fixed positions and provided stability for more accurate measurements. The holder included two cylindrical cavities for tightly fitting the transducers with their active elements facing each other. A 30 mm diameter planar immersion transducer (CeramTec, Plochingen, Germany) operating at 1.1 MHz transmitted an amplified sinusoidal acoustic wave signal that propagating through 26 mm of the sample. The same type of transducer located 65 mm from the transmitter was used to record the attenuated resulting signal, which was displayed on a digital oscilloscope (TDS 2012, Tektronix, Inc., Karl Braun Drive, United States). Each sample was positioned in the near field of the emitting transducer where the intensity of the sound wave is relatively constant with distance traveled. A sinusoidal signal (3 V peak-to-peak) was generated and converted to 300 mW acoustical power in the transmitting transducer. Figure 2 shows the experimental setup for the attenuation coefficient measurements.

Analytical method to calculate the attenuation coefficient of agar-based gels

The attenuation in dB was calculated from the difference between the peak-to-peak voltages of the recorded signal from each sample compared to a reference signal traveling for the same distance in degassed/deionized water under the same experimental conditions. The attenuation coefficient was calculated in units of dB/cm-MHz by dividing the measured attenuation in dB with the product of the transmitting frequency (1.1 MHz) and sample thickness. The calculated result included the attenuation induced by the propagation of the acoustic wave in water. One advantage of this technique is that there was no need to calculate the reflection or transmission coefficients at each interface.[27] The attenuation

coefficient in units of dB/cm-MHz was calculated using equation 1,[28]

$$\alpha = \frac{20}{ln(10)} * \left[\frac{ln(\frac{A_s}{A_r})}{\Delta_x * f} + a_w \right] \quad (1)$$

where A_s corresponds to the reference peak-to-peak voltage without the sample, A_r to the peak-to-peak voltage at the receiver side with the addition of each sample between the two planar transducers, α_w is the attenuation of water in Np/cm-MHz, $\Delta\chi$ is the sample's thickness in cm, and f is the transmitting frequency in MHz.

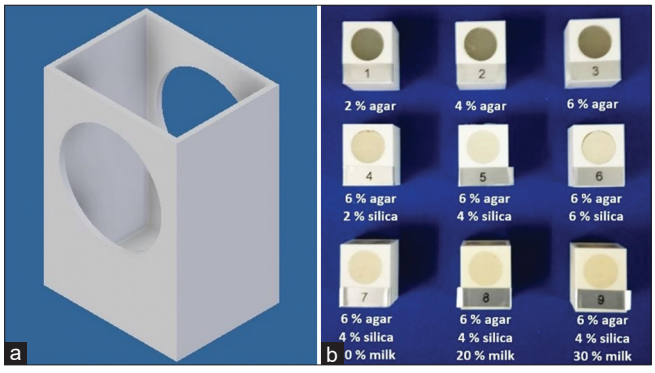

Figure 1: (a) Computer-aided design drawing of the polylactic acid mold, and (b) Front view of the developed samples with different amounts of agar, silicon dioxide, and evaporated milk

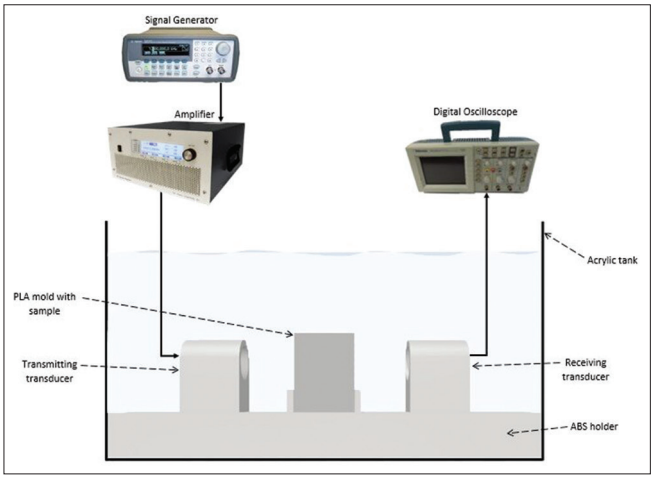

Figure 2: Experimental setup for the measurement of the ultrasonic attenuation coefficient of agar-based gels with different amounts of agar, silicon dioxide, and evaporated milk

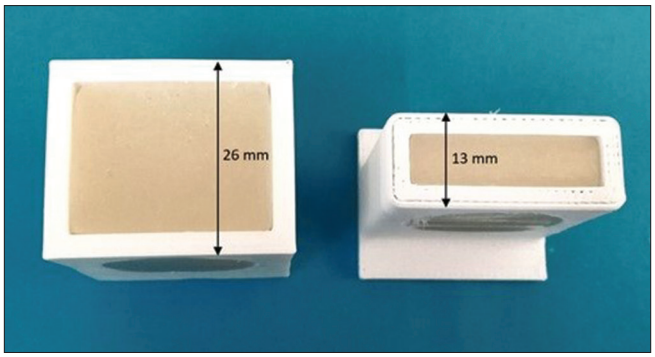

Figure 3: Top view of the thinner and thicker mold used in the variable-thickness technique

This procedure was repeated ten times, for each one of the phantom recipes under test. Ten different samples of each recipe were manufactured and attenuation calculation was performed for each one, thus evaluating the stability of the measurement system and estimating the uncertainty in measurements. Measurement uncertainties arose from measurement inaccuracy, influence of random noise, and possible variation among samples.

Ultrasound imaging

A diagnostic ultrasound imaging system (UMT-150, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, P. R. China) was used to scan the sample with 6% w/v agar and 4% w/v silicon dioxide in order to check the echogenicity and the structural homogeneity of the sample.

Frequency dependence of attenuation

The frequency dependence of attenuation of the phantom recipe with 6% w/v agar and 4% w/v silicon dioxide was investigated in the frequency range of 1–2 MHz. For each ultrasonic frequency, the attenuation coefficient was calculated according to the previously described method, using equation 1.

Insertion loss

A slightly different method was then used, in order to estimate the insertion loss in the phantoms. The attenuation coefficient of the agar-based gels was estimated according to the variable-thickness method, using the same experimental set up described in the previous subsections (two identical transducers of 1.1 MHz central frequency fitted inside an acrylic tank at specific locations). Although, a different procedure was followed in the case of the variable-thickness technique, in which the signals through samples of different thicknesses are compared. For that purpose, specially designed thinner molds (with a total volume of 10.0 cm^3) were printed using PLA material. In this case, the acoustic waves propagated through a 13 mm sample, as shown in Figure 3.

First, the thinner sample (x_1 = 13 mm) was placed and the peak to peak voltage was measured on the oscilloscope. The procedure was repeated by replacing the specimen with the thicker one (x_2 = 26 mm). The attenuation coefficient of each recipe was estimated by including the measured voltage in the presence of the thinner Ax_1 and thicker Ax_{2s} samples, together with the thickness of the samples and the ultrasonic frequency , in the following equation:[13]

$$a = \frac{1}{f} * \frac{20}{x_2 - x_1} * log\left(\frac{A_{x_2}}{A_{x_1}}\right) \quad (2)$$

Ultrasonic attenuation in rabbit tissue

Ultrasonic attenuation in freshly excised rabbit tissues, including the liver, kidney, and muscle was estimated according to the through-transmission method of the two identical planar transducers of 1.1 MHz central frequency, previously described for the attenuation measurement of the agar-based gels. First, the soft tissues were carefully cut at a thickness of approximately 4 mm, placed in a vacuum chamber, and degassed such that microbubbles accidentally trapped in tissue were eliminated. The attenuation coefficient of each tissue was calculated using equation 1.

High-intensity focused ultrasound application

A HIFU transducer (MEDSONIC LTD, Limassol, Cyprus), with an operating frequency of 4.4 MHz, was used to sonicate an agar-based phantom. The phantom included 6% w/v agar and 4% w/v silicon dioxide. A 3D-printed (F270, Stratasys Ltd., Minnesota, USA) experimental setup was used to hold the transducer and the phantom stable at fixed positions. The whole setup was immersed in an acrylic water tank. A degassed/deionized water was included as a coupling material between the transducer and the phantom. The focal depth was set at 2 cm in the phantom. A magnetic resonance (MR) compatible thermocouple (5SC-TT-K-30-36, type K insulated beaded wire, 100 μm thick, Omega Engineering, Norwalk, Connecticut, USA) was accurately inserted at the focus in a plane perpendicular to the ultrasound beam.

The experimental setup was placed in an MRI scanner (1.5 T, Signa Excite, General Electric, Fairfield, CT, USA) to simultaneously measure the temperature change in the phantom using MR thermometry. An RF coil (GPFLEX, USA instruments, Cleveland, OH, USA) was used. An acoustic power of 25 W was applied for a sonication period of 36 s. A Fast-Spoiled Gradient echo sequence was used to acquire MR images for the MR thermometry image analysis. The following parameters were applied: repetition time (TR) = 40 ms, echo time (TE) = 20 ms, slice thickness = 5 mm, echo train length = 1, flip angle = 30°, and matrix = 256 × 256. During the sonication, MR images were taken every 12 s while an image was obtained when the transducer was deactivated. The temperature change was recorded using MR thermometry.

Results

Samples of different amounts of agar, silicon dioxide, and evaporated milk were prepared following a simple preparation procedure. The attenuation coefficient of each sample was calculated using the through-transmission immersion technique. In total, ten calculations of the attenuation coefficient were

made for each recipe, in ten different phantoms designed with the same concentration of agar, silicon dioxide, and evaporated milk, from which a mean value and a corresponding standard deviation were obtained. The estimated values are shown in Table 1. Figure 4 shows the estimated attenuation coefficient with respect to the measurement number, for a recipe with 6% and 4% w/v agar and silicon dioxide, respectively. The estimated values are normally distributed around a mean value of 1.10 ± 0.09 dB/cm-MHz. The optimum recipe was defined as the recipe that was found (6% w/v agar and 4% w/v silicon dioxide) to possess an attenuation coefficient close to that of human muscle.[29]

Initially, experiments were performed to estimate the attenuation coefficient by varying the agar concentration. Figure 5 shows the attenuation coefficient as a function of agar concentration (n = 10) without any amount of silicon dioxide and evaporated milk. This was done to assess the attenuation purely induced by agar in the absence of other materials that enhance scattering or absorption.

The next step was to quantify the attenuation coefficient of agar-based gels by varying the amount of silicon dioxide and evaluate its effect on attenuation. Figure 6 shows the attenuation coefficient dependence of silicon dioxide concentration (n = 10) using 6% agar and 0% evaporated milk. At low doses of silicon dioxide (<4%), the attenuation coefficient increased with an increase in silicon dioxide percentage. Although the increase of silicon dioxide concentration above 4% increased scattering, that scattering increment further lowered the ultrasonic absorption at a significant level as found in a previous study.[25] Although the scattering is expected to increase, the absorption which is the main factor of the attenuation significantly decreased and the total result led to an attenuation decrease. The attenuation decreases with increasing silicon dioxide concentration (over 4 %) possible due to the domination of the absorption decrease over the increase of scattering. According to the linear regression analysis (R^2 = 0.995), for silicon dioxide concentration up to 4%, the attenuation coefficient increased by 0.101 dB/cm-MHz for every 1% increase of silicon dioxide concentration.

The effect of the variation of the evaporated milk amount on the attenuation coefficient of agar-based gels was also assessed.

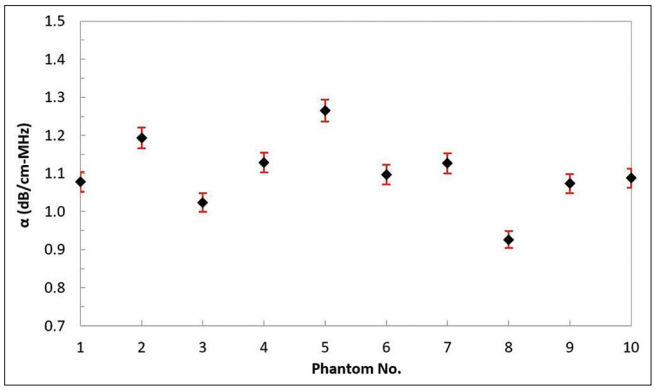

Figure 4: Attenuation coefficient versus measurement number of the phantom with the optimum recipe (6% agar, 4% silicon dioxide). Uncertainty bars represent standard deviations

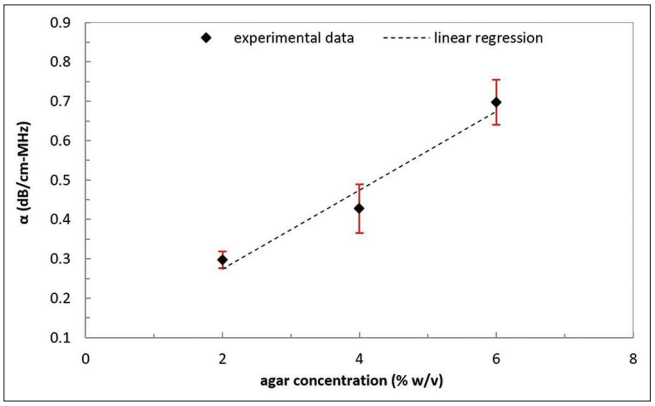

Figure 5: Attenuation coefficient versus percentage of agar (n=10) for 0% silicon dioxide and 0% evaporated milk. Uncertainty bars represent standard deviations and the dashed line represents linear regression fitting (R^2 = 0.961)

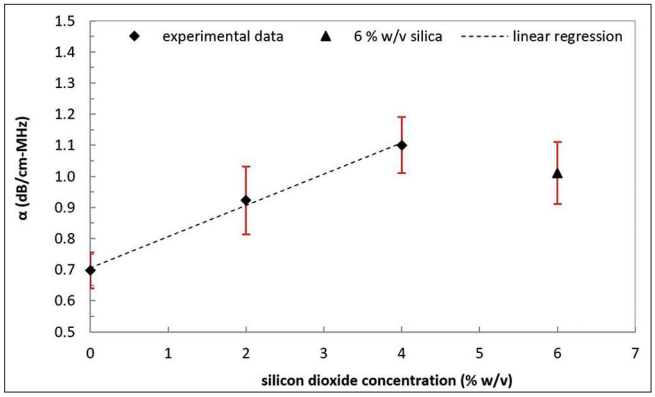

Figure 6: Attenuation coefficient versus silicon dioxide (n = 10) with 6% agar and 0% evaporated milk. Uncertainty bars represent standard deviations and the dashed line represents linear regression fitting (R^2 = 0.995)

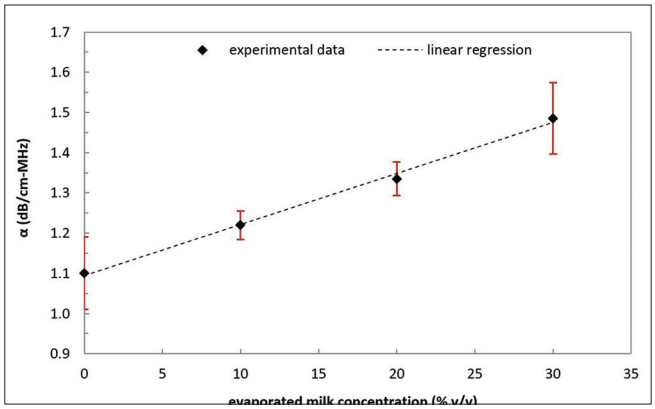

Figure 7: Attenuation coefficient versus percentage of evaporated milk (n = 10) for 4% silicon dioxide and 6% agar. Uncertainty bars represent standard deviations and the dashed line represents linear regression fitting (R^2 = 0.996)

Figure 7 shows the attenuation coefficient versus the percentage of evaporated milk (n = 10) for a preliminary agar and silicon dioxide concentration of 6% and 4%, respectively. The sample lost its stiffness with an evaporated milk percentage of over 30% as suggested in a previous study[25] and became too difficult to handle. Therefore, further investigation for higher evaporated milk concentrations was excluded. An attenuation coefficient increment of 0.013 dB/cm-MHz was observed for every 1% increase of evaporated milk. The contribution of evaporated milk to the increment of ultrasonic attenuation was found to be significantly smaller than the one found for silicon dioxide.

Further to the through-transmission method in which the signal through the reference (water) path is essential, the variable-thickness method was also used, in order to assess the insertion loss through the phantoms. As previously mentioned, using the specific technique, the signals through samples of different thicknesses were compared. Thereby, the ultrasonic attenuation due to the reflection phenomenon on the water/phantom interface was eliminated, and thus, the results represented only the insertion losses (mostly absorption). The estimated attenuation coefficients were found to be smaller or very similar to the ones obtained by the through-transmission method previously used. This is mostly attributed to the fact that agar-based phantoms possess acoustic impedance similar to that of water,[13] and thus, the reflection on a water/agar-based gel interface is significantly small. The results obtained by both methods were compared as shown in Table 1, indicating a good agreement with the underlying theory.

The sample with the optimum recipe (6% w/v agar and 4% w/v silicon dioxide) was scanned with a diagnostic ultrasound imaging system (UMT-150). The sample appeared with increased echogenicity due to the ability of silicon dioxide to scatter ultrasound waves as travel through the sample [Figure 8].

For the optimum recipe (6% w/v agar and 4% w/v silicon dioxide) further investigation was conducted for estimating the frequency dependence of attenuation. Figure 9 shows the estimated attenuation coefficient plotted against the probe frequency, in the frequency range of 1–2 MHz. The attenuation coefficient was found to vary from 0.97 (at 1.1 MHz) to 2.93 dB/cm-MHz (at 1.9 MHz). The least-squares method that was used to determine the line of best fit to data indicated a linear behavior (R^2 = 0.978), as shown in Figure 9.

The attenuation coefficient of freshly excised rabbit tissues was also estimated using the same experimental setup and formula used for the ultrasonic investigation of the agar-based phantoms. Overall, ten measurements of the reference and attenuated (in the presence of the tissue) signal were made, approximately 1 h after excision. Nonuniformities in the rabbit excised tissues existed due to the physical diversity of the rabbits. This led to higher measurement uncertainty. The mean value of attenuation coefficient for samples of about 4 mm thickness, at 1.1 MHz, was found to be 0.86 ± 0.20, 1.18 ± 0.46, and 1.46 ± 0.44 dB/MHz-cm for the liver, muscle, and kidney, respectively. The estimated values for the soft tissues were compared to the ones obtained for the agar-based phantoms as shown in Table 2. The recipes with no evaporated milk were preferred due to their increased durability. An optimum amount of 6% w/v agar, along with a silicon dioxide concentration of 0%–4% can be used to mimic the rabbit tissues. In general, the phantom with 6% and 4% w/v agar and silicon dioxide concentration, respectively, was considered optimum for mimicking the rabbit tissue.

SEM images of the nine agar-based phantoms were retrieved to observe potential changes in the internal microstructure composition dependent on the amount of agar [Figure 10], silicon dioxide [Figure 11], and evaporated milk [Figure 12].

A HIFU sonication was performed using a single element focused transducer and the temperature measurement was recorded in the phantom using a thermocouple at the focal point as shown in Figure 13. The temperature change at the

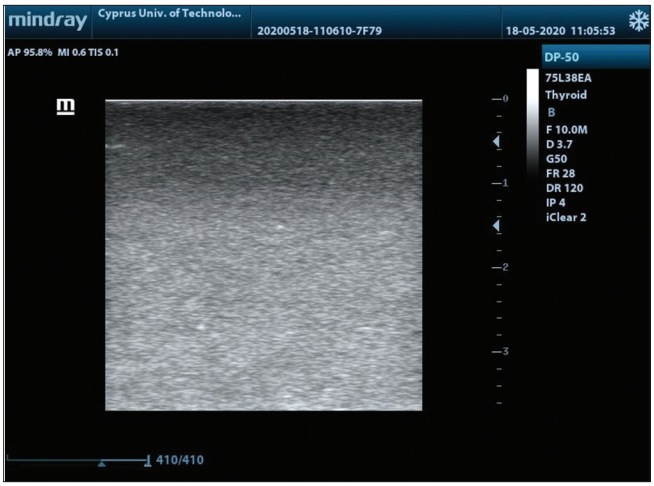

Figure 8: Ultrasound image of the sample with the optimum recipe (6% agar and 4% silicon dioxide)

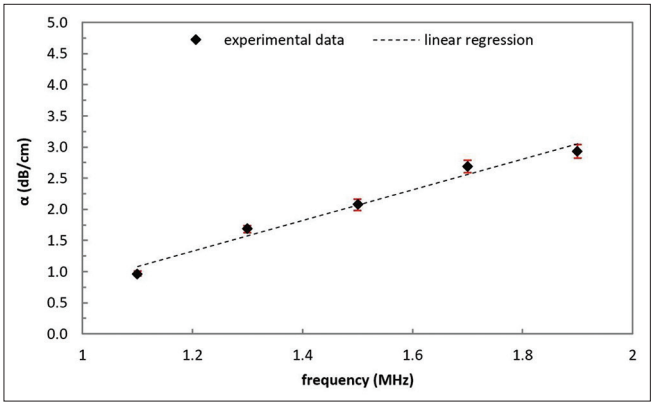

Figure 9: The attenuation coefficient versus frequency of the phantom with the optimum recipe (6% agar and 4% silicon dioxide). Uncertainty bars represent standard deviations and the dashed line represents linear regression fitting (R^2 = 0.9776)

Table 1: The mean value of the attenuation coefficient and the corresponding standard deviation of each phantom, compared to the insertion loss obtained by the variable-thickness method

Phantom recipe			Mean value of the attenuation coefficient±SD (dB/cm-MHz)	Insertion loss (dB/cm-MHz)	SD (dB/cm-MHz)
Agar (% w/v)	Silicon dioxide (% w/v)	Evaporated milk (% v/v)			
2	0	0	0.30±0.02	0.22	0.02
4	0	0	0.43±0.06	0.39	0.03
6	0	0	0.70±0.06	0.67	0.04
6	2	0	0.92±0.11	0.94	0.06
6	4	0	1.10±0.09	1.03	0.06
6	6	0	1.01±0.10	0.97	0.06
6	4	10	1.22±0.04	1.25	0.08
6	4	20	1.34±0.04	1.30	0.08
6	4	30	1.49±0.09	1.41	0.09

SD: Standard deviation

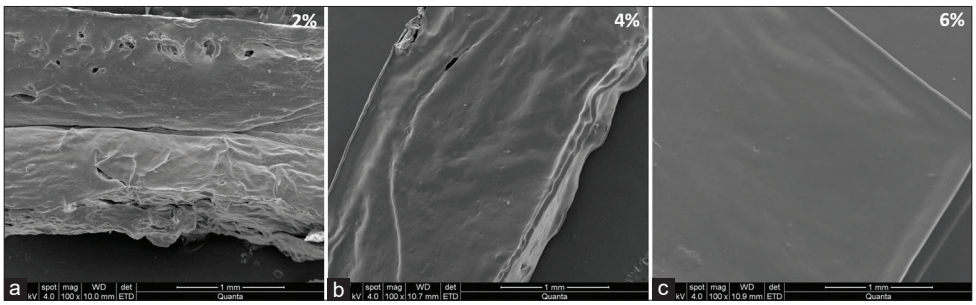

Figure 10: Scanning electron microscope images of the internal structure of phantoms with different w/v agar concentrations, (a) 2%, (b) 4%, and (c) 6%

maximum sonication time was 34.5°C. The temperature change was also recorded using MR thermometry. MR thermal maps in plane perpendicular and parallel to the ultrasound beam were obtained and the temperature change at 36 s sonication was 35.2°C [Figure 14].

Discussion

In this article, the variation of ultrasonic attenuation coefficient with different concentrations of evaporated milk, agar, and silicon dioxide was evaluated. A repeatability test ($n = 10$) was performed for each one of the tested recipes and attenuation coefficients were acquired using the through-transmission method. Small day-to-day variability was observed for most of the agar-based gels, indicating the stability of the measurement system. The variable-thickness technique was also used and resulted in slightly lower values of attenuation coefficients, as expected, proving extra evidence of the accuracy of the estimated values.

Depending on the agar, silicon dioxide, and evaporated milk concentration, the attenuation coefficient varied in the range of 0.30–1.49 dB/cm-MHz. A linear increment of attenuation was observed with increasing agar (up to 6%), silicon dioxide (up to 4%), and evaporated milk (up to 30%) concentration.

The attenuation contribution of agar percentage from 2% to 4% was 0.13 dB/cm-MHz, while a more significant (approximately double) contribution with a total attenuation increase of 0.27 dB/cm-MHz was found from 4% to 6%. Agar concentrations over 6% resulted in stiff phantom and did not resemble realistically the stiffness of soft tissue; therefore, agar was limited to 6%.

We have noticed that the attenuation coefficient can best be regulated by varying the amount of silicon dioxide. The addition of 1% w/v of silicon dioxide contributed to an increase of attenuation by 0.101 dB/cm-MHz up to a 4% concentration. Although the scattering of the sample increased with the addition of silicon dioxide for an amount of over 4% w/v, the contribution of silicon dioxide to absorption significantly decreased.[25] Thereby, it seems that for a silicon dioxide concentration of more than 4% w/v, the attenuation is strongly affected by the decrement of absorption,[25] rather than the enhancement of the scattering phenomenon.

The addition of 10% v/v evaporated milk increased the attenuation by 0.127 dB/cm-MHz probably due to absorption. Evaporated milk was found to have a minimal contribution to attenuation (0.013 dB/cm-MHz for every 1% v/v of evaporated milk), compared to the attenuation contribution of silicon dioxide (0.101 dB/cm-MHz for every 1% w/v of silicon dioxide). The concentration of evaporated milk was limited to 30% because although attenuation (and absorption as it was previously reported[25]) can be further raised with increased evaporated milk concentration, increase milk concentration

results in a loose phantom. Evaporated milk has been already suggested as a material that increases attenuation by Madsen *et al*.[11]

Other materials such as glycerol (more than 5%) and graphite powder have been reported to increase the attenuation of TMMs.[14,18] The range values of the attenuation of agar/gelatin-based gels doped with glass bead scatterers were found in the lower range (0.35–0.46 dB/cm-MHz) of the attenuation results of this study.[14] Similar to the range of the estimated attenuation values are the gelatin-based gels doped with graphite.[18] The pure gelatin (with water and alcohol) gel demonstrates an attenuation in the range of 0.2–0.3 dB/cm-MHz. By adding a uniform amount of powdered graphite, the attenuation can be adjusted between 0.2 and 1.5 dB/cm-MHz (at 1.1 MHz) which is similar to the attenuation range values found in this work.

The ultrasonic attenuation coefficient of the sample with 4% agar (0% of silicon dioxide and evaporated milk) was found to be 0.43 ± 0.06 dB/cm-MHz which is close to the value found for human fat, liver, and cardiac tissue.[29,30] In addition, the ultrasonic attenuation with an amount of agar of 6% was found to be 0.70 ± 0.06 dB/cm-MHz which is almost equal to that of the human brain, according to.[30] The recipe with identical agar and silicon dioxide concentration of 2% (0% evaporated milk) was found to possess attenuation close to that of a bovine spleen (0.87 dB/cm-MHz), while a silicon dioxide concentration of 2%–4% w/v induced attenuation similar to the bovine brain (0.97 dB/cm-MHz).[31] Mast *et al*.[29] reported an attenuation coefficient of human muscle similar to the one obtained for the phantom with the optimum recipe of 6% agar and 4% silicon dioxide. Finally, using the recipe with 6% agar, 4% silicon dioxide, and 10% evaporated milk, the attenuation coefficient was found to be 1.22 ± 0.04 dB/cm-MHz which is close to the value of porcine liver (1.25 dB/cm-MHz).[31]

A linear relation between the attenuation coefficient and frequency was estimated for the phantom with 6% w/v agar and 4% w/v silicon dioxide between a frequency range of 1.1–1.9 MHz. In agreement with our findings, other studies for tissue-mimicking phantoms, including polyacrylamide hydrogel-based phantoms[23] and gelatin-based phantoms doped with graphite,[18] have reported a linear relationship between attenuation and frequency, in the range of 1–5 MHz. From the results of this study and those from other researchers,

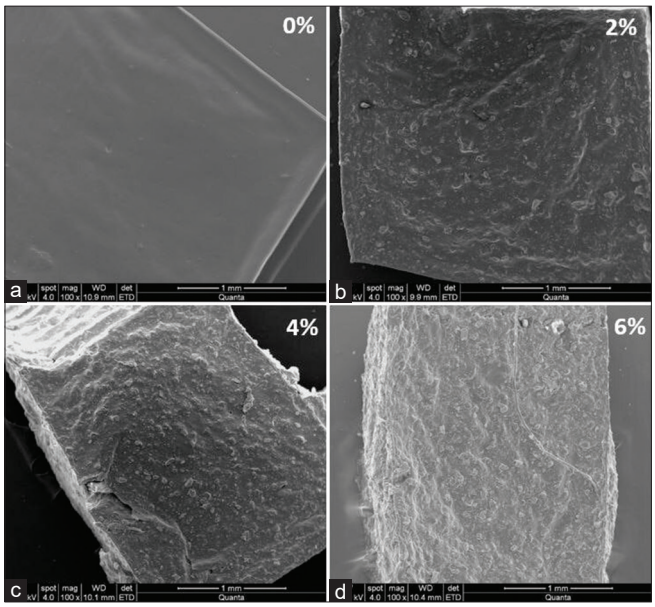

Figure 11: Scanning electron microscope images of the internal structure of phantoms with 6% w/v agar and different w/v silicon dioxide concentrations, (a) 0%, (b) 2%, (c) 4%, and (d) 6%

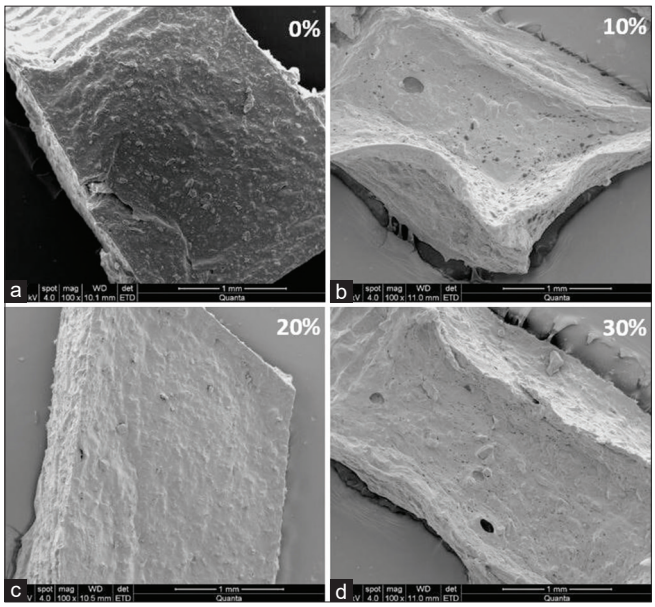

Figure 12: Scanning electron microscope images of the internal structure of phantoms with 6% w/v agar, 4% w/v silicon dioxide, and different v/v evaporated milk concentrations, (a) 0%, (b) 10%, (c) 20%, and (d) 30%

Table 2: The estimated mean value of the attenuation coefficient and the corresponding standard deviation of each rabbit tissue, and the phantom recipes that could be possibly used to mimic each one

Tissue	Mean value of attenuation coefficient±SD (dB/cm-MHz)	Phantom recipe		
		Silicon dioxide (% w/v)	Agar (% w/v)	Evaporated milk (% w/v)
Liver	0.86±0.20	0-4	6	0
Muscle	1.18±0.46	2-4		
Kidney	1.46±0.44	2-4		

SD: Standard deviation

we can assume that the attenuation coefficient of the agar-based phantoms doped with silicon dioxide is almost linear.

Finally, the accuracy of the measurement system was confirmed by measuring the ultrasonic attenuation of freshly excised rabbit tissue, since our findings were in a good agreement with the values reported in[32] for rabbit liver. The suitability of agar-based phantoms in mimicking real tissue was also assessed. Since the estimated values of the attenuation coefficient for both (the phantoms and liver tissues) lie within the same range, we can safely conclude that agar-based phantoms can be used to mimic rabbit tissue. Specifically, the optimum recipe of 6% w/v agar and 4% w/v silicon dioxide concentration, with an attenuation coefficient of 1.10 ± 0.09, was found to be suitable for mimicking rabbit tissue.

The study of the phantoms' internal structure through the SEM images demonstrated that a higher agar concentration leads to a denser and smoother phantom matrix. The incorporation and the percentage increase of silicon dioxide create phantoms with a rougher texture while the addition of evaporated milk, especially above 20% v/v leads to a significant increase in voids (hundreds of microns) and nano/micro-pores (nm-μm).

A phantom with 6% w/v agar and 4% w/v silicon dioxide was tested using a HIFU protocol. A temperature elevation of around 35°C (34.5°C using the thermocouple and 35.2°C calculated through the MR thermometry) was achieved in the agar-based phantom.

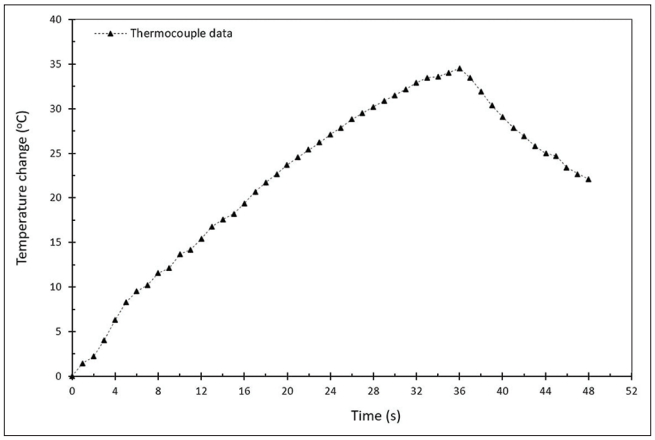

Figure 13: Temperature change versus time as recorded at the focal point of an agar-based phantom (6% agar and 4% silicon dioxide) using a thermocouple for acoustic power of 25 W

Conclusion

Taken together, the findings of this study have been compared to the attenuation coefficient values found in the literature for human and animal tissues, as well as to the ones obtained for the freshly excised rabbit tissue in the current study. Our work has led us to conclude that agar-based phantoms with attenuation coefficient values similar to those of human and animal tissues can be developed with the proper selection of percentage of agar, silicon dioxide, and evaporated milk.

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Ευρωπαϊκή Ένωση
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Διαρθρωτικά Ταμεία της Ευρωπαϊκής Ένωσης στην Κύπρο

Conflicts of interest

There are no conflicts of interest.

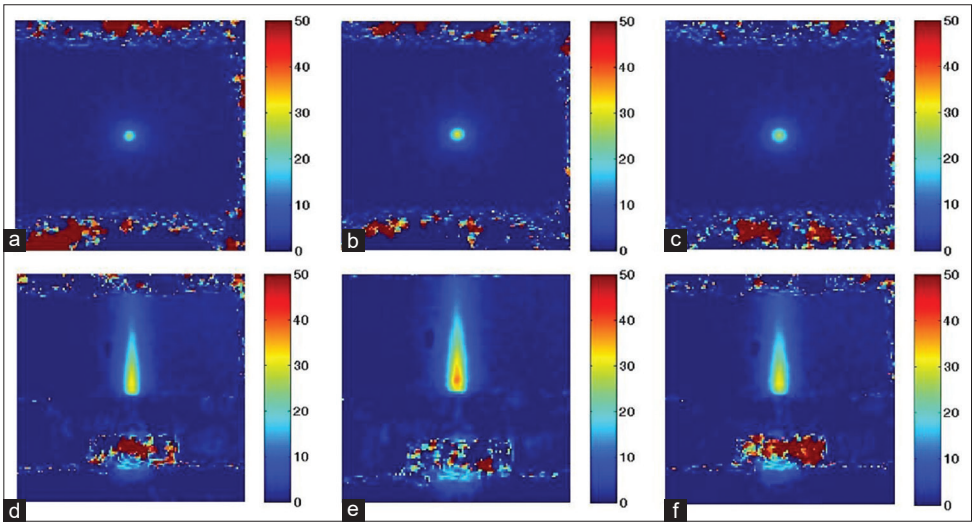

Figure 14: Magnetic resonance thermometry maps of an agar-based phantom in a plane perpendicular to the ultrasound beam at the time of (a) 24 s, (b) 36 s, (c) 48 s and the corresponding thermometry maps d, e, and f in a plane parallel to the ultrasound beam

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