Original Article

High-quality Agar and Polyacrylamide Tumor-mimicking Phantom Models for Magnetic Resonance-guided Focused Ultrasound Applications

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Abstract

Background: Tissue-mimicking phantoms (TMPs) have been used extensively in clinical and nonclinical settings to simulate the thermal effects of focus ultrasound (FUS) technology in real tissue or organs. With recent technological developments in the FUS technology and its monitoring/guided techniques such as ultrasound-guided FUS and magnetic resonance-guided FUS (MRgFUS) the need for TMPs are more important than ever to ensure the safety of the patients before being treated with FUS for a variety of diseases (e.g., cancer or neurological). The purpose of this study was to prepare a tumor-mimicking phantom (TUMP) model that can simulate competently a tumor that is surrounded by healthy tissue. **Methods:** The TUMP models were prepared using polyacrylamide (PAA) and agar solutions enriched with MR contrast agents (silicon dioxide and glycerol), and the thermosensitive component bovine serum albumin (BSA) that can alter its physical properties once thermal change is detected, therefore offering real-time visualization of the applied FUS ablation in the TUMPs models. To establish if these TUMPs are good candidates to be used in thermoablation, their thermal properties were characterized with a custom-made FUS system in the laboratory and a magnetic resonance imaging (MRI) setup with MR-thermometry. The BSA protein's coagulation temperature was adjusted at 55°C by setting the pH of the PAA solution to 4.5, therefore simulating the necrosis temperature of the tissue. **Results:** The experiments carried out showed that the TUMP models prepared by PAA can change color from transparent to cream-white due to the BSA protein coagulation caused by the thermal stress applied. The TUMP models offered a good MRI contrast between the TMPs and the TUMPs including real-time visualization of the ablation area due to the BSA protein coagulation. Furthermore, the *T2*-weighted MR images obtained showed a significant change in *T2* when the BSA protein is thermally coagulated. MR thermometry maps demonstrated that the suggested TUMP models may successfully imitate a tumor that is present in soft tissue. **Conclusion:** The TUMP models developed in this study have numerous uses in the testing and calibration of FUS equipment including the simulation and validation of thermal therapy treatment plans with FUS or MRgFUS in oncology applications.

Keywords: Agar, focus ultrasound, magnetic resonance imaging, phantom, polyacrylamide, tumor

Introduction

Image-guided thermal ablation technology has grown exponentially in the last decade as a minimally invasive therapy and is now frequently used to treat malignant or benign tumors in various tissues or organs.[1-3] Magnetic resonance-guided focused ultrasound (MRgFUS) technology is a representative example of an image-guided thermal ablation technique that is using focus ultrasound (FUS) under MR-guidance and has shown great promise in treating noninvasively many diseases such as cancer, neurological conditions, thrombolysis (clots formed by ischemic stroke) and palliative pain treatment caused by cervical or bone cancer metastasis.[4-6] To specify, MRgFUS technology has been used successfully for the treatment of benign and malignant cancer tumors:[7] early stage prostate cancer,[8] breast cancer,[9] uterine fibroids,[10] adenomyosis,[11] and benign soft-tissue carcinomas;[12] neurological diseases:[13]

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essential tremor,[14] multiple sclerosis, and Parkinson's disease-associated tremor.[15] Other likely treatment contenders being investigated in ongoing clinical trials include brain, liver, kidney, pancreas and thyroid cancers,[16] Alzheimer's disease and epilepsy,[17] and other movement disorders.[18] INSIGHTEC is the only Company that has EU/CE and FDA approval for its MRgFUS technology (ExAblate Body and Neuro models) to be used in humans to treat benign prostate and breast tumors,[19] uterine fibroids,[20] adenomyosis,[21] and essential tremor and tremors caused by Parkinson's disease.[22]

Magnetic resonance imaging (MRI) is utilized in MRgFUS technology for target characterization, treatment planning, and closed-loop control of the acoustic energy deposition delivered to the target by a single-element ultrasonic transducer or a phased array transducer.[23] By combining the FUS and MRI technologies together as a single therapeutic system, enables the operator of the MRgFUS system to achieve high accuracy in terms of beam localization and targeting while monitoring in real-time the treatment process which results in the necrosis of the targeted tissue.[24] FUS can cause necrotic lesions in tumors located in deep-sited healthy tissue through thermal coagulation and cavitation disruption with minimal to no damage to the surrounding tissues.[25]

To completely treat the targeted tissue volume during thermal ablation treatments while minimizing side effects to the patient, accurate control of the temperature magnitude and distribution of the ultrasonic energy being delivered to the target is essential.[23] Consequently, treatment planning is vital before the application of the ablative therapy, in calculating the sonication strength and duration required to produce the appropriate tissue necrosis in the desired tissue volume.[4] Unfortunately, accurately planning and monitoring the tissue heating through MR thermometry in the context of patient-specific and dynamic acoustic characteristics of tissues remains a problem in these types of thermal ablation procedures even today. Testing the MRgFUS technology on *ex vivo* biological tissue or organs such as pig fat, beef liver, or turkey breast, has numerous drawbacks that include high cost, lack of homogeneity, short shelf-life, and their biohazardous nature.[26] To overcome these issues, high-quality tissue-mimicking phantoms (TMPs) are being used for the preclinical development and testing of new FUS and MRgFUS therapeutic techniques.[27,28]

Therefore, TMPs have been employed to test and calibrate newly introduced FUS and MRgFUS systems in preclinical and clinical settings. TMPs are also employed in the premanufacturing of new ultrasound transducers and innovative FUS systems for therapy purposes.[28,29] TMPs have the advantage of allowing for the construction of idealized tissue models with clearly specified acoustic characteristics, dimensions, and internal features, which simplifies and standardizes the treatment protocols and environment.[30,31] TMPs can be engineered to mimic the biological components of interest and help simulate the absorption pattern of the ultrasonic energy delivered by the FUS technique to the targeted volume.[32] TMPs make it possible to conduct biomedical research in an ergonomic and cost-effective manner without the need for animal or human patients. TMPs have better availability and shelf-life than the *ex vivo* models, great structural uniformity, and quality assurance (QA); and can support the training of the operator while helping to optimize the necessary therapeutic MRgFUS protocols.[33] All these advantages can improve QA practices, efficiency, and safety in modern medical systems before and after they enter the market.

In recent years, researchers have used a variety of materials to fabricate TMPs that can simulate as close as possible the properties of the desired targeted biological tissue or organ. Some well-established materials used for producing these TMPs for imaging or thermal ablation purposes are agar,[34-36] gelatin,[37,38] polyacrylamide (PAA),[39,40] poly (vinyl alcohol),[41,42] polyvinyl chloride,[43,44] silicone,[45,46] carrageenan,[47,48] and polysaccharide-based materials (TX-150/TX-151).[27,49] Tissue substitutes used in thermal therapy systems (such as FUS and MRgFUS) must have acoustic properties that are similar to the biological tissue of interest. The most important acoustic characteristics of soft tissue that need to be imitated by TMPs are the compressional speed of sound, characteristic acoustic impedance, attenuation, backscattering coefficient, and the nonlinearity parameter.[30] Furthermore, to effectively mimic real tissue in MRgFUS applications, TMPs should be produced with precise *T1* and *T2* relaxation times.[29] Unlike in the case of biological tissue, the thermally treated TMP material should experience a significant and irreversible change in MR characteristics (*T1* or *T2*) on reaching a threshold temperature that allows thermal coagulation to take place and permit the MR monitoring of the coagulated volume.[50] Many different contrast agents (attenuation components) have been used over the years to successfully replicate some of the acoustical properties necessary for the MR imaging and monitoring of the treatment ablation process such as microbubbles[51] or nanobubbles,[52] silicon dioxide,[33] copper (II) sulfate ($CuSO_4$),[53] and cellulose.[54] Egg whites,[55] egg albumin,[56] bovine serum albumin (BSA),[57] and thermochromatic inks[58] are also some materials used in TMPs to enhance the MRI contrast but at the same time permit permanent coagulation or color change the observation of the temperature distribution into the ablated volume. It should be noted that even though the monitoring of the temperature distribution in a TMPs or a biological volume can be achieved also with MR thermometry, not everyone has access to the advance technology required to do that.[59]

Each one of the above-mentioned phantom engineering materials has its strengths and weaknesses in simulating perfectly a biological component on thermal ablation applications. For example, agar and carrageenan phantoms have good elastic and stability properties and can be shaped easily into any desired shape. However, gelatin and carrageenan phantoms are only advised to be used in hyperthermia applications because they are unable to endure

high FUS ablation temperatures.[31] PAA, on the other hand, can withstand the high FUS ablation temperatures due to its high melting point, but the acrylamide required for the PAA phantom fabrication is highly neurotoxic so additional care must be taken during its preparation. Alternatively, agar phantoms do not have any toxicity problems and have been shown to be very promising for usage in MRgFUS technology.[34] It should be noted though that the PAA phantoms are safe for handling after the polymerization process is completed and offer better optical transparency in comparison to agar ones, therefore allowing the direct observation of the coagulative lesions during the ablation process. PAA and agar phantoms have shown that can mimic well many of the important thermal, acoustical, and MR relaxation characteristics of different biological tissues or organs.[29,36]

Even though many biological mimicking phantoms have been introduced for use in thermal therapy applications, none of them fully satisfies all the criteria of an ideal tumor phantom. To add to the complexity of simulating a biological tissue or an organ with TMPs, a limited number of them can be found in the literature that can simulate a tumor model for thermal therapy experimentation with MRgFUS technology. After reviewing the available bibliography on TMPs, the materials of choice selected to overcome a variety of issues reported by other scientists are PAA and agar. Our purpose is to use PAA and agar materials with the appropriate MR contrast and heat-sensitive agents to engineer multi-modal TMPs that can simulate and monitor almost perfectly a tumor model. PAA material can be used to prepare a spherical tumor-mimicking phantom (TUMP) with the appropriate agents that can simulate the malignant tissue. The spherical shape TUMP can be then added into the center of a secondary square-shaped TMP, fabricated by either PAA or agar materials that can simulate the healthy tissue surrounding the TUMP. The PAA material was chosen to fabricate the TUMPs due to its high melting point, good mechanical strength, and competence to fabricate high optical transparent TUMPs and TMPs at room temperature and any desired shape.[39] BSA was selected as the heat-sensitive and MR contrast agent to simulate and monitor the thermal ablation of a malignant tissue, while simultaneously measuring the thermal dose applied to it through the FUS application.[25] Silicon dioxide and glycerol were also used as contrast agents in both TUMPs (PAA and agar) to assist with the MR monitoring of the tumor model during thermal application.[33] Therefore, for this study, we will prepare a TUMP model that will consist of 2 parts: A normal square TMP and a spherical TUMP that will be placed in the center of the TMP. In a previous study,[60] also published by the same team, a similar TUMP model was engineered where both parts of it (TMP and spherical TUMP) were fabricated with only agar material. In the spherical TUMP though, silicon dioxide was additionally added to provide the necessary contrast between the two types of phantoms during the MRI experiments. The novelty that the TUMP model described in this study has, is that the spherical TUMP merged in the center of a square TMP is fully transparent and is fabricated with PAA mixed with BSA protein that was adjusted to have specific thermosensitive properties (change color due to coagulation after a critical temperature point is passed, e.g., 55°C) therefore giving the advantage to the user to track the ultrasonic ablation focusing area and the thermal changes in the TUMP model caused by the FUS application with "naked eyes" without the necessity of using advance equipment and techniques such as MRI and MR-Thermometry.

Materials and Methods

General methodology

Three cuboid shapes 6 cm × 6 cm × 6 cm phantoms were prepared consisting of a tissue-mimicking base that integrated into their center a 2 cm spherical shape TUMP. The phantom as a whole can mimic a TUMP model, where the tumor phantom is surrounded by the base TMP simulating the surrounding healthy tissue. The phantoms were fabricated in triplets by both PAA and agar materials with the use of cuboid and spherical molds. First, the spherical TUMP was prepared and then placed in the center of the cuboid mold by hanging horizontally by a thread. The tissue-mimicking PAA or agar solution was then added in the cuboid mold, therefore surrounding the spherical PAA TUMP. Once the polymerization phase was completed, by the addition of polymerization initiators-activators for the PAA solutions, the TUMP models were ready for use and testing with FUS, MRgFUS, and MR-thermometry technologies.

Preparation of tumor-mimicking phantom models

Experiment 1–agar/polyacrylamide tumor-mimicking phantom model with bovine serum albumin protein

Methodology

The agar TMPs for Experiment 1 were prepared based on the methodology and formulation followed by Antoniou *et al.*[31] Filippou and Damianou[61] and Menikou and Damianou[34] where in their experiments they used 6% (w/v) agar and 4% (w/v) silicon dioxide to prepare agar-based TMPs to measure their acoustic, scattering, and thermal properties, including their MR relaxation times. The preparation of the PAA *(acrylamide*/bis-*acrylamide)* TUMPs was prepared based on the methodology followed by Bu-Lin *et al.*[40] and McDonald *et al.*[50] where they used PAA solutions with BSA protein and adjusted pH (4.3-4.7) to prepare multi-modality TMP to monitor and visualize the temperature effect of the FUS ablation to the PAA-mimicking phantoms due to the coagulation properties of the BSA protein emerged by a specific pH value and the thermal stress applied. The PAA formulation was also based on the research of Zhong *et al.*[26] where they used silicon dioxide as an MR scatterer and BSA protein as a coagulation agent to monitor through MRI the FUS ablation and the impact on the TMPs.

Preparation of the polyacrylamide polymerization initiators-activators

L-ascorbic acid, iron (II) sulfate heptahydrate ($FeSO_4$), and hydrogen peroxide (H_2O_2) were used as catalysts to initiate the

polymerization of the PAA solutions. The reason the specific combination was chosen as a catalyst for the polymerization of the PAA solutions is because citrate buffer was used in the mixture to lower the pH of the solution to approximately 4.5 and the above combination is more efficient and "friendlier" with the citrate buffer than other existing ones (e.g., TEMED and APS).[50]

The polymerization of the PAA solution is initiated by the addition of 0.1 g (0.001% w/v) of L-ascorbic acid, 0.25 ml (0.0025% v/v) of 1% $FeSO_4$ (add 0.1 g of $FeSO_4$ in 10 ml of deionized water) and 0.3 ml (0.0030% v/v) of 3.0% v/v H_2O_2 (dilute 1.0 ml of 30% w/v stock H_2O_2 in 9.0 ml of deionized water). The ascorbic acid is photosensitive; therefore, it must always be stored in a dark place. The prepared $FeSO_4$ solution should be kept at 4°C and the H_2O_2 solution must always be made fresh before each experiment as it degrades over time due to its weak peroxide bond into water and oxygen.[50,62]

Fabrication of spherical polyacrylamide tumor-mimicking phantom

Under a fume hood and at room temperature, a 0.2M citrate buffer solution was prepared with a pH of 4.5 ± 0.1 by dissolving 2.09% (w/v) of citric acid monohydrate and 2.96% (w/v) of sodium citrate tribasic dihydrate in 100 ml of deionized water. Sodium hydroxide or hydrochloric acid was gradually added to the solution (while monitoring the solution with a pH meter), until it reaches the exact pH value of 4.5. BSA protein was then added to the citrate buffer solution at a concentration of 2% (w/v) and stirred slowly until a homogeneous solution was formed. It is important to avoid rapid mixing of the solution once the BSA protein is added to avoid any bubbles formation. Acrylamide (6.65 w/v) and N, N'-methylene-bis-acrylamide (0.35 w/v) were then added into the solution and stirred gently until a clear and homogenous solution is achieved. Safety equipment must be always used during the handling and preparation of the PAA solution as it is neurotoxic before its polymerization. After a clear PAA solution is achieved, 6% (v/v) of glycerol and 1.1% (w/v) silicon dioxide were added and the formed solution was top up with deionized water to the appropriate volume while stirring gently. Finally, the polymerisation initiators-activators were added to the PAA tumor solution: 0.3% (v/v) of 3% H_2O_2, 0.1% (w/v) L-ascorbic acid, and 0.25% (v/v) of 1% $FeSO_4$ and transferred immediately the final solution into 20 ml syringes to load up the spherical 2 cm molds (x^3) before the polymerization process is completed. The spherical molds were filled through a 2 mm hole, each holding around 4.2 ml of PAA solution, and sealed with plasticine to prevent any unwanted leaks. A 10 cm thread with knots at each end was also placed in the center of the spherical molds before injecting them with the PAA solution. As the polymerization process is exothermic, the spherical molds loaded with PAA solution were immediately transferred into sealed freezer bags and placed at around 4°C for a minimum of 30 min to avoid premature coagulation of the thermally sensitive BSA protein. The transparent spherical PAA TUMPs were then carefully removed from their molds and placed in water-filled freezer bags (to prevent dehydration or swelling) until used. The TUMPs preparation took around 90 min.

Fabrication of the tumor-mimicking phantom model

The agar tissue-mimicking solution that surrounds the TUMP was prepared by following a similar methodology as Filippou and Damianou.[61] Under a fume board, 800 ml of distilled water were added into a beaker and placed into a hot plate until it was heated to 50°C. Then, 48 g of agar powder (6% w/v) was added slowly into the beaker and stirred with a magnetic stirrer for 5 min. Finally, 32 g of silica dioxide (4% w/v) were added into the agar solution and continued stirring for 15–20 min until a temperature of 95°C was reached. The temperature was monitored constantly with an electronic thermometer with an accuracy of 0.1°C (Model: HH806AU, Omega Engineering, USA). The agar-silica solution was left to cool down to around 45°C. While waiting for the agar-silica tissue solution to cool down, the previously prepared transparent spherical PAA TUMPs are removed from the water-filled freezer bag and placed in the center of three 6 cm × 6 cm × 6 cm cuboid molds with the help of a 10 cm thread embedded in the center of their spherical structure. The TUMP spheres are held in place by the thread that was fixated at the side of the cuboid molds with plasticine. Once the agar-silica tissue solution cools down to 45°C (to avoid coagulation of the BSA protein in the PAA TUMPs), it is poured slowly into the three cuboid molds that hold 216 ml of solution each, then sealed and placed immediately at 4°C overnight. Finally, the Agar/PAA TUMP models are carefully removed from the cuboid molds and are placed in freezing sealed bags at 4°C with deionized water until use. Table 1 shows the transparent TUMP model formulation and Figure 1 summarizes the methodology followed to prepare it.

Experiment 2–polyacrylamide/polyacrylamide tumor-mimicking phantom model phantom with bovine serum albumin protein

Methodology

For Experiment 2, the opaque agar TMP material that was used to fabricate the Agar/PAA TUMP models in Experiment 1 is replaced with the transparent PAA material, while the TUMP formulation is kept the same. The PAA TMPs and TUMPs for Experiment 2 were prepared based on the same methodology followed in Experiment 1, and both were mixed with BSA protein to help monitor and visualize the temperature effect of the FUS ablation to the PAA TUMP models due to the coagulation properties of BSA protein emerged under the thermal stress applied. To fabricate the transparent and clear TUMP models, the concentration of the PAA solution and the BSA protein were selected to 7% (w/v) and 2% (w/v), respectively, similar to Experiment 1. To fabricate the PAA TMPs surrounding the PAA TUMPs, the agar solution was replaced with a 7% (w/v) PAA solution concentration with the BSA protein concentration remaining at 2% (w/v). The 7% PAA tissue solution was prepared with the same methodology used for the fabrication of

Table 1: Formulations used for the preparation of the Agar tissue-mimicking phantom (opaque) and the spherical polyacrylamide tumor-mimicking phantom (transparent) with bovine serum albumin protein. The TUMP is inserted in the centre of the TMP to give the final Agar/PAA TUMP model

Materials	Product code**	Quantity (%)	
		Tumour phantom*	Tissue phantom
Deionized water	-	90.00 (v/v)	100.00 (v/v)
Citric acid monohydrous	1.00244.1000	2.09 (w/v)	-
Sodium citrate tribasic dehydrate	S4641	2.96 (w/v)	-
BSA	A9647	2.00 (w/v)	-
Acrylamide	A8887	6.65 (w/v)	-
N, N-methylene-bis-acrylamide	M7256	0.35 (w/v)	-
Glycerol	G7757	6.0 (v/v)	-
Agar	1.01614.1000	-	6.0 (w/v)
Silicon dioxide (silica/SO_2)	83340	1.1 (w/v)	4.0 (w/v)
		Top up with deionized water to 0.1 L	-
Polymerization initiators/ activators			
L-ascorbic acid	A5960	0.10 (w/v)	-
1% $FeSO_4$	F7002	0.25 (v/v)	-
3% H_2O_2	1072090250	0.30 (v/v)	-

*Before adding the polymerisation agents, the pH of the PAA tumour solution is adjusted by monitoring it with a pH meter to 4.5 (55°C) by gradually adding NAOH or HCL, **All the materials were purchased from Sigma Aldrich (Merck KGaA, Darmstadt, Germany). BSA: Bovine serum albumin, $FeSO_4$: Iron (II) sulfate heptahydrate, H_2O_2: Hydrogen peroxide, PAA: Polyacrylamide, TUMP: Tumor-mimicking phantom, TMP: Tissue-mimicking phantom

the 7% TUMPs. In addition, the silicon dioxide was only added to the PAA TUMPs and not to the TMPs.

The tumor and tissue PAA solutions formulation used in Experiment 2 are shown in Table 2. The final 7% (w/v) PAA tissue solution was transferred into cuboids molds, where transparent spherical PAA TUMPs were already placed in their center with the help of a horizontal nylon thread that was fixed through the center of the PAA TUMP. Figure 2 shows the placement of the transparent PAA TUMP fixated in the square mold before pouring the agar or PAA tissue-mimicking solutions, including rendered images of the opaque Agar/PAA and transparent PAA/PAA TUMP models.

Characterization of the tumor-mimicking phantom models

Density calculation of polyacrylamide tumor-mimicking phantom by water displacement method

The water displacement method[34] was used to calculate the PAA TUMP density by immersing it in a known volume of water and measuring the difference in water level. Beforehand, the PAA phantom mass M (in grams-g) was measured in a high accuracy balance. Using the formula $V = V_f - V_i$ where V_f = final water volume and V_i = initial water volume, yields the volume V (in cm^3) of the PAA phantom submerged in water. Finally, to find the density D (in g/cm^3) of the PAA phantom submerged in water the formula D = M/V is used, where M (in g) is the mass of the phantom and V (in ml) is the water volume displacement (1 ml of water takes up 1 cm^3 of space). The experiment to measure the mass density of the PAA phantoms was repeated in triplicate. The density of the Agar phantom material is already measured by the team in previous studies.[61]

Transmission through method for measuring acoustic attenuation coefficient

To measure the acoustic attenuation coefficient of the PAA phantoms the same methodology as the research of Menikou and Damianou[28,34] was followed, where two immersion planar transducers were used to measure it. One of the transducers was used to transmit the signal (operating at 4 MHz) and the other one was used to receive it. To ensure a consistent response, the two transducers run at the same central frequency and gain. A PAA phantom was fabricated with the same properties as in sections 2.2.1 and 2.2.2 using a custom-made mold but with a size of 2.5 cm × 2.5 cm × 5.0 cm (LxWxH). The PAA phantom was placed halfway between the two transducers, ideally outside of the transmitting transducer's far field, where the constructive interference of waves generated at the transducer's face produces a uniform front that smoothly fades away with increasing distance. The experiment to measure the acoustic attenuation coefficient of the PAA phantoms was repeated 4 times. The agar's phantom acoustic attenuation coefficient was not measured for this experiment as it is already known by previous studies of the group.[61]

Focus ultrasound application – demo of necrosis

The FUS setup and parameters used to ablate the TUMP model were based on previous methodology carried out by Drakos *et al.*[63] A FUS transducer (MEDSONIC LTD, Limassol, Cyprus), with an operating frequency of 2.75 MHz, was used to sonicate the TUMPs, and the experiment was repeated in duplicate to verify the FUS ablation in the preset focal point in the phantom (FUS Experimental parameters: Spatial Peak Temporal Average Intensity-I_{SPTA} = 0.042 W/cm^2, Electric Power = 200 W, Ablation Time: 60 s). The transducer, which is responsible for the thermal ablation in the spherical TUMP, is used to deliver the ultrasonic energy required to increase the temperature at a preset FUS focal point above 55°C which is in the range that causes tissue necrosis. The transducer operates at 2.75 MHz and has a focal length of 6.5 cm and diameter of 4 cm. A 3D-printed (F270, Stratasys Ltd., Minnesota, USA) experimental setup was used to hold the transducer and the phantom stable at fixed positions [Figure 3]. The whole setup was immersed in an acrylic water tank with a size of 23 cm × 15 cm × 18 cm (HxWxL). Degassed distilled water was included as a coupling media between the transducer and the phantom. The positioning device's arm held the transducer, which was submerged in the water tank to provide a good acoustical coupling with the phantom. The focal depth was set at 3 cm in the phantom.

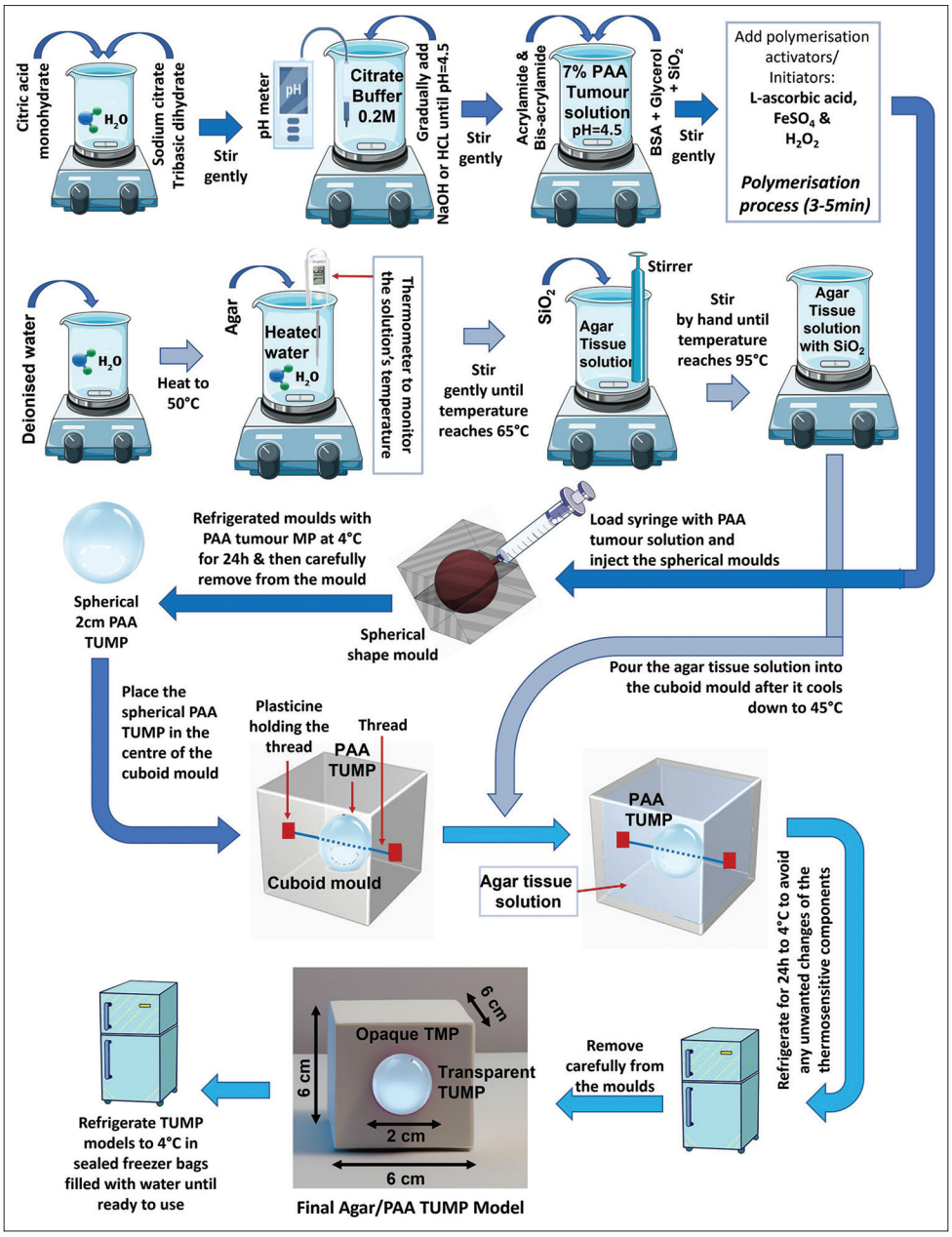

Figure 1: Methodology followed in Experiment 1 showing the preparation of the transparent polyacrylamide tumor-mimicking phantom (PAA TUMP) and the opaque agar TMP, including the fabrication of the final Agar/PAA TUMP model with bovine serum albumin protein

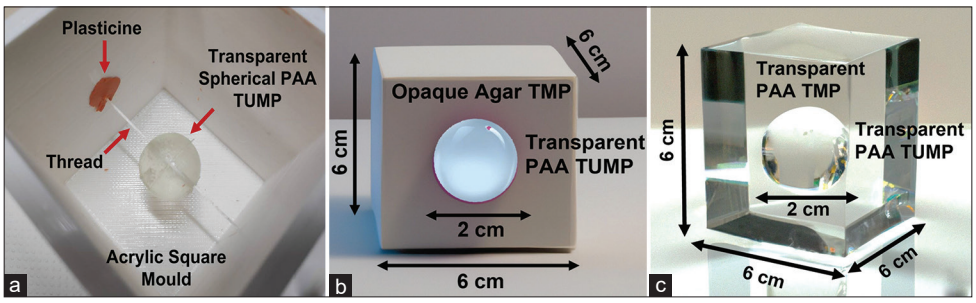

Figure 2: Shows (a) the placement of the spherical polyacrylamide tumor-mimicking phantom (PAA TUMP) in the acrylic mould before adding the TMP solution, (b) a rendered cross-section image of the opaque Agar/PAA TUMP model and (c) a rendered image of the transparent PAA/PAA TUMP model (rendered in OPENAI DALL-E online software)

The purpose of the experiment was to evaluate and visualize the temperature increase through FUS sonication in the Agar or PAA TUMP models containing a PAA tumor and assess if the BSA protein is coagulating due to the temperature rise above 55°C in the PAA TUMP, therefore changing color from transparent to cream white.

Table 2: The formulations used for the preparation of the polyacrylamide tissue-mimicking phantom (transparent) and the spherical polyacrylamide tumor-mimicking phantom (transparent), both incorporated with bovine serum albumin protein. The TUMP is inserted in the centre of the TMP to give the final PAA/PAA TUMP model

Materials	Product code**	Quantity (%)	
		Tumour phantom*	Tissue phantom
Deionized water	-	90.00 (v/v)	90.00 (v/v)
Citric acid monohydrous	1.00244.1000	2.09 (w/v)	2.09 (w/v)
Sodium citrate tribasic dehydrate	S4641	2.96 (w/v)	2.96 (w/v)
BSA	A9647	2.00 (w/v)	2.00 (w/v)
Acrylamide	A8887	6.65 (w/v)	6.65 (w/v)
N, N-methylene-bis-acrylamide	M7256	0.35 (w/v)	0.35 (w/v)
Glycerol	G7757	6.0 (v/v)	6.0 (v/v)
Silicon dioxide (Silica/SO_2)	83340	1.1 (w/v)	-
		Top up with deionized water to 0.1 L	Top up with deionized water to 1 L
Polymerization initiators/ activators			
L-ascorbic acid	A5960	0.10 (w/v)	0.10 (w/v)
1% $FeSO_4$	F7002	0.25 (v/v)	0.25 (v/v)
3% H_2O_2	1072090250	0.30 (v/v)	0.30 (v/v)

*Before adding the polymerisation agents, the pH of the PAA tumour solution is adjusted by monitoring it with a pH meter to 4.5 (55°C) by gradually adding NAOH or HCL, **All the materials were purchased from Sigma Aldrich (Merck KGaA, Darmstadt, Germany). BSA: Bovine serum albumin, $FeSO_4$: Iron (II) sulfate heptahydrate, H_2O_2: Hydrogen peroxide, PAA: Polyacrylamide, TUMP: Tumor-mimicking phantom, TMP: Tissue-mimicking phantom

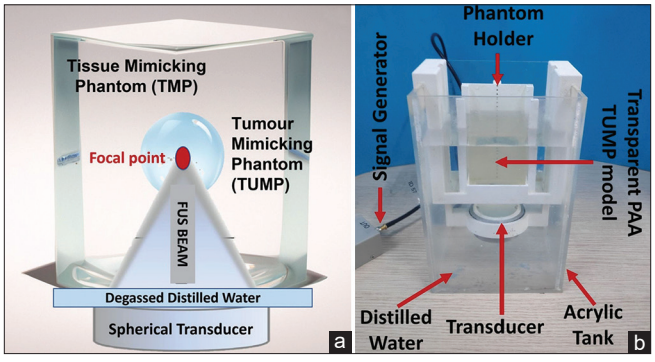

Figure 3: Shows (a) a schematic of the focus ultrasound (FUS) ablation to the polyacrylamide (PAA)/PAA tumor-mimicking phantom model and (b) the realistic custom-made FUS setup used for the thermal ablation

Magnetic resonance-guided focus ultrasound application-magnetic resonance thermometry

An MRI-conditional FUS setup which can create controlled thermal lesions under MRI guidance previously developed by the team[36] was used to estimate the temperature elevation and pinpoint the thermal focal point in the Agar/PAA and PAA/PAA TUMP models produced by FUS sonication. The purpose of the experiment was to evaluate the temperature increase through sonication in the Agar or PAA TUMP models containing a PAA tumor while monitoring and evaluating the thermal process under a 3T MRI scanner.

Each of the TUMP models (Agar/PAA and PAA/PAA) was placed in the square phantom holder of the custom-made FUS setup that was set atop a specially designed plastic plate. The plate was then partially submerged in a tank of distilled water that had been degassed (coupling media between the transducer and the phantom). A 50 mm diameter with a 100 mm radius of curvature spherically focused high-intensity single-element ultrasonic transducer (MEDSONIC LTD, Limassol, Cyprus) was submerged in the water tank beneath the phantom. The transducer was mounted on a piece of plastic that allowed for manual vertical and horizontal positioning. An RF generator (HP 33120A, Agilent technologies, Englewood, CO, USA) powered the transducer. A GPFLEX coil (GPFLEX, USA instruments, Cleveland, OH, USA) was wrapped around the TUMP models.

The parameters set for the Agar/PAA TUMP model experiment were as follows: Water tank with transducer parameters: Frequency = 2.6MHz, Diameter = 50 mm, Radius of curvature = 65 mm, Efficiency = 30%, Focal Depth = 30 mm, sonication time: 30s-120s; Amplifier: AG1016 (AG Series Amplifier, T and C Power Conversion, Inc., Rochester, USA): I_{SPTA} = 0.058 W/cm^2, electric power: 250 W; acoustic power: 75W; Experimental set-up [Figure 4]: Water tank with transducer ID 57; MRI scanner: 3T (Healthineers, Siemens); Coil type: Body coil (Body_12_BM).

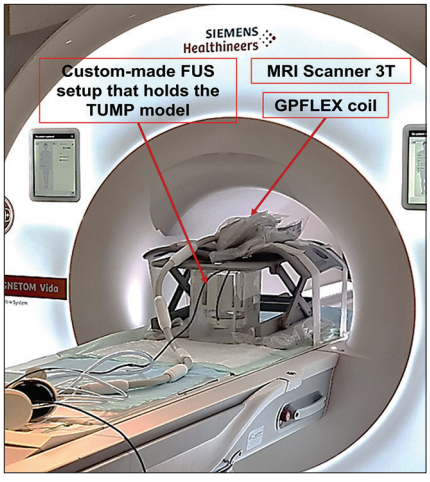

Figure 4: Experimental setup inside the 3T magnetic resonance imaging (MRI) scanner with the focus ultrasound custom-made setup, in which the tumor-mimicking phantom models were placed on the MRI table and a GPFLEX coil placed on top of it to take the MR images

The experimental FUS setup described above was placed in the MRI's magnet isocentre to simultaneously measure the temperature change in the TUMP models using MR Thermometry. The PRF shift technique was used to measure the thermal changes in the phantoms.[32,64] With this technique, the local temperature increase is connected to the accompanying phase shift of the MR signal. The transducer's position in relation to the TUMP phantom was finely adjusted using fast gradient echo sequences by setting the following MRI parameters: echo time (TE) =10 ms, repetition time (TR) = 25 ms, Flip angle (FA) =30°, Receiving Bandwidth (BW) = 501 Hz, Acquisition Matrix = 96 × 96, Field of View (FOV) = 280 mm × 280 mm × 3 mm.

The TUMP models were treated also with an acoustic power of 60W (I_{SPTA} = 0.046 W/cm^2, electric power: 200W) for a duration of 30–120 s in both axial and coronal imaging plane to acquire the MR thermometry high-resolution images. Every 2.4 s seconds while the transducer was turned off, an image was obtained during sonication. The following MRI parameters were applied: Sequence = FLASH 2D, Coil type: Body_12_BM, TR = 25 ms, TE = 10 ms, FA = 30°, acquisition matrix: 96 × 96, slice thickness: 3 mm, acquisition time/slice: 2.4 s, Echo train length: 1, Pixel BW: 501 Hz/pixel, FOV: 280 mm × 280 mm × 3 mm. Each thermometry image that was generated was analyzed using specialized custom software created by the team (written in python) to provide the temperature shift measurements at various intervals.

Results

General discussion

The concentration of the PAA tumor solutions was selected to 7% (w/v) as it was previously proven that this specific concentration allows the PAA tissue phantoms to be clear and transparent, as the heat released into the PAA preparation solution, due to the exothermic polymerization reaction, is not enough to denature the BSA protein.[57] The concentration of BSA was set to 2% (w/v) as it was demonstrated from previous studies[40,50] that at this concentration the coagulated lesions formed in the PAA phantoms during the FUS ablation offer good thermal visualization with the naked eye due to its transparent structure and also its distinguish contrast on the MR images between the coagulated and uncoagulated regions properties (coagulation of BSA protein results in *T2* relaxation time change).[40,65]

The BSA protein coagulates at approximately 70°C, which is higher than the necrosis temperature of biological tissue (50–60°C), hence a citrate buffer (0.2M with pH = 4.5) was used to lower the pH of the PAA solutions to 4.5.[40] With this specific pH value, the BSA protein starts coagulating at around 55°C. The specific citrate buffer concentration [Tables 1 and 2] was selected for this experiment not only because it can sustain constant pH (4.5) of the PAA solution as other ingredients are added, but also because it can offer the necessary electrical conductivity to the solution essential for FUS ablation.[50] The coagulation temperature of BSA protein was adjusted for the PAA TMPs and TUMPs to 55°C (pH = 4.5) with the addition of an acid or a base, respectively; thus, safeguarding that the coagulation temperature of BSA is within the range of thermal injury to soft tissue of 50°C–60°C.[62] Silicon dioxide was also added to the tumor PAA solution as an MR attenuation agent to monitor through MRI the FUS ablation to the TUMP and distinguish it from the TMP.[26] To further enhanced the contrast of the phantoms in MR imaging, glycerol was also included in the PAA solutions and at the same time made the removal of the phantoms from their molds easier. Glycerol is known to have a relatively long *T1* relaxation time, which can enhance the contrast in *T1*-weighted MR images.

Agar/polyacrylamide and polyacrylamide/polyacrylamide tumor-mimicking phantom models

The cross-sections of the final Agar/PAA and PAA/PAA TUMP models fabricated are presented in Figure 5. The TUMP models were sliced in half carfeully with a sharp blade to identify if the PAA TUMP was in the center of the TMP and if the BSA protein was not coagulated during the experimental preparation steps. Figure 5 clearly shows that the PAA TUMP was entrapped in the center of the TMP and the BSA protein did not show any visible signs of coagulation.

Density and acoustic attenuation coefficient calculation of polyacrylamide tumor MP material

The densities of the 6% (w/v) tissue agar and 7% (w/v) PAA tumor MPs used in the experiments were calculated at 1.060 ± 0.012 g/cm^3 and 1.076 ± 0.011 g/cm^3, respectively. The propagation speeds of the agar TMPs and PAA TUMPs measured at 2.7 MHz were 1537 ± 6 m/s and 1616 ± 7 m/s, respectively [Table 3].

Creation of necrosis

After thermal ablation, the PAA/PAA TUMP models were examined to identify if the ablation area was in the focal region set by the FUS parameters and if it could be visualized by the naked idea. The thermal effect was then evaluated by looking to see if the ablation region covered a significant part of the PAA TUMP. A hot water bath was also used to heat the PAA TUMPs (>55°C) to visualize if there were any color differences between the heated and unheated samples. The PAA TUMP started coagulating once the water temperature was raised above 55°C (as intended) and fully coagulated at around 65°C (from transparent to cream-white color). Figure 6a shows the transparent PAA phantom fabricated for this study before the BSA coagulation process takes place and Figure 6b

Table 3: The densities and propagation speeds for the phantom types prepared

Phantom material	Density (g/cm^3)	Propagation speed (2.7 MHz) (m/s)
Agar (6% w/v)	1.060±0.012	1537±6
PAA (7% w/v)	1.076±0.011	1616±7

PAA: Polyacrylamide

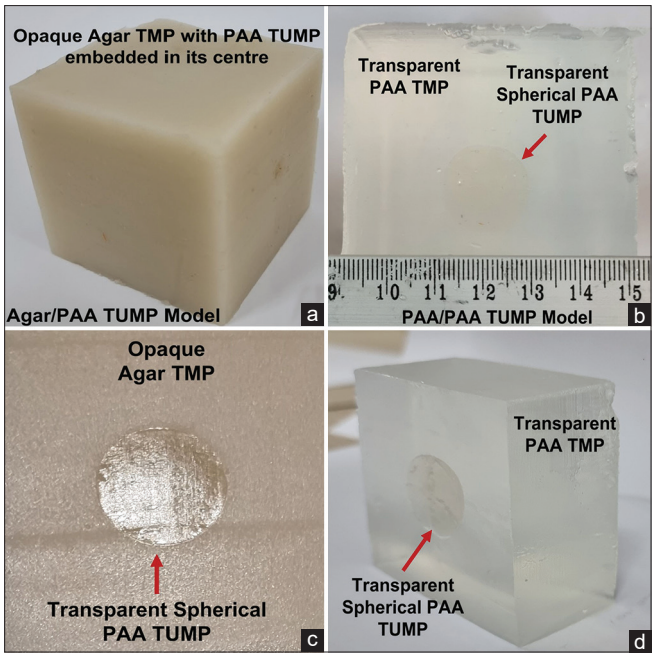

Figure 5: Shows photos of (a) the opaque Agar/polyacrylamide tumor-mimicking phantom (PAA TUMP) model, (b) the transparent PAA/PAA TUMP model and (c) a cross section of Agar/PAA TUMP model and (d) a cross section of PAA/PAA TUMP model

shows the same PAA phantom after heating it above 55°C in a water bath. Figure 6c shows the transparent PAA/PAA TUMP model after sonication, where it can be seen clearly the FUS focal point due to the coagulation of the BSA protein and the optical color change from transparent to cream white, caused by the ablation applied to it.

Magnetic resonance imaging and magnetic resonance-thermometry

The fabricated TUMP models were imaged in a 3 T Siemens MRI scanner to examine their MR properties depending on the effect of the various materials added to their composition. The TUMP models were positioned in the water tank incorporated in the custom-made FUS setup and were imaged with the MRI scanner with conventional T1W FSE and T2W FSE sequences. The transducer's parameters used were as follows: Frequency = 2.6 MHz; Diameter = 50 mm; Radius of curvature = 65 mm; Efficiency = 30%; Focal depth = 30 mm. In addition, the MR-Thermometry PRF shift technique was used in both types of TUMP models to obtain high-resolution thermal images and the temperature evolution observed in a region of interest (ROI) set within the focal spot. The following MR parameters were used: Sequence = FLASH 2D, Coil type: Body_12_BM, TR = 25 ms, TE = 10 ms, FA = 30°, acquisition matrix: 96 × 96, slice thickness: 3 mm, acquisition time/slice: 2.4 s, Echo train length: 1, Pixel BW: 501 Hz/pixel, FOV: 280 mm × 280 mm × 3 mm. The TUMP models (Agar/PAA and PAA/PAA) fabricated were treated with an electric power of 200W for a duration of 60 s in both axial and coronal imaging planes.

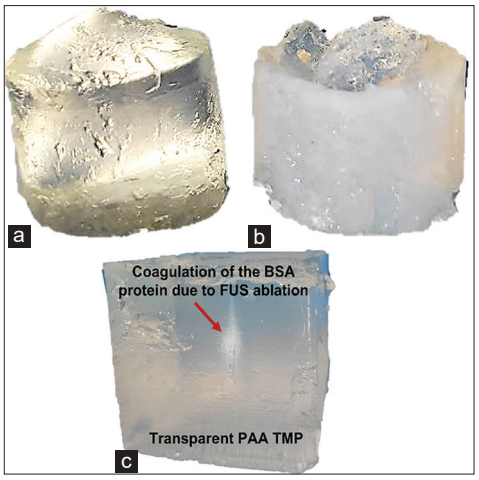

Figure 6: Shows photos of (a) the transparent polyacrylamide tumor-mimicking phantom (PAA TUMP) before heating it in a water bath, (b) the coagulated PAA TUMP after immersing it in a water bath with temperature >55°C and (c) the coagulated region in the centre of the PAA/PAA TUMP model after focus ultrasound ablation

Agar/polyacrylamide tumor-mimicking phantom model

The MRI images acquired by the T1W FSE and T2W FSE MRI sequences (the MR parameters used were stated above) for the opaque Agar/PAA TUMP model as shown in Figure 7, clearly reveal the excellent contrast achieved between the TMPs and the TUMPs. This was due to the lowered MR relaxation times of the PAA TUMPs achieved by the addition of silicon dioxide and glycerol. Figure 7a and b shows MR images obtained using the T1W FSE sequence and Figure 7c and d shows the MR images obtained using the T2W FSE sequence for the opaque Agar/PAA TUMP model.

Figure 8 shows coronal and axial thermal images obtained with MR thermometry in the MRI scanner during the thermal ablation of the Agar/PAA TUMP model. The thermal images obtained and the temperature evolution observed in a ROI set within the focal spot, show that the focal point of FUS sonication was in the spherical PAA TUMP region as planned. Figure 8a and b show thermal maps in coronal and axial plane, respectively, and depict the temperature evolution over time of the Agar/PAA TUMP model with a sonication power of 200W for 60 s.

Polyacrylamide/polyacrylamide tumour-mimicking phantom model

The FUS application to the transparent PAA TUMP models shows the successful coagulation of the BSA protein after the temperature exceeded 55°C in the transparent PAA TUMP that is surrounded by the transparent PAA TMP [Figure 9]. This was also confirmed by the MR thermal images acquired by MR-thermometry in the 3 T Siemens MRI scanner.

Figure 10 shows coronal and axial thermal images obtained with MR Thermometry in the MRI scanner during the thermal ablation of the PAA/PAA TUMP model. The thermal images obtained and the temperature evolution observed in a ROI

set within the focal spot, show that the focal point of FUS sonication was in the spherical PAA TUMP region as planned. Figure 10a and b show thermal maps in the coronal and axial plane, respectively, and depict the temperature evolution over time of the PAA/PAA TUMP model with sonication powers of 200W for 30 s (axial plane) and 250W for 120 s (coronal plane).

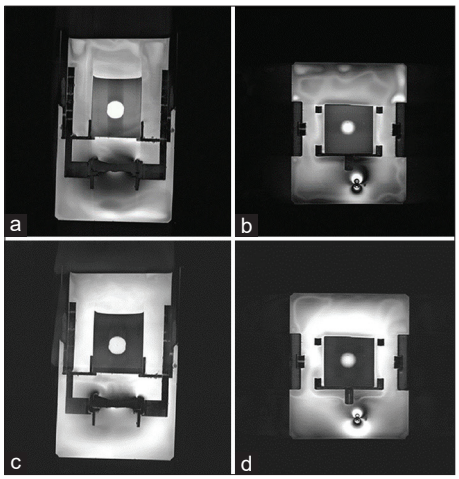

Figure 7: Shows magnetic resonance imaging images of the opaque Agar/polyacrylamide tumor-mimicking phantom model acquired by using. (a and b) The T1W FSE sequence and (c and d) the T2W FSE sequence

Discussion

The study presented in this article aimed to fabricate and evaluate two types of TUMP models for use in the development and optimization of FUS and MRgFUS ablation treatments for different cancer types. The specific TUMP models were designed to have properties similar to spherical tumors surrounded by healthy tissue and were fabricated by using agar and PAA materials. The PAA and Agar materials were favored to make the TMPs and TUMPs with BSA protein because they are easy to prepare, they offer long-term stability, they can be fabricated to have a similar thermal conductivity to that of tissue and additionally, the PAA TMPs and TUMPs can change from transparent to cream white when heated above 55°C. This specific temperature point of 55°C is important as it was reported by previous studies also working with the applications of high-intensity FUS that a temperature above that point and held for 1 s or more can lead to coagulative necrosis and cell destruction.[66,67]

BSA protein was incorporated in the PAA TUMPs that were inserted in the center of the TUMP models due to its thermosensitive coagulation properties. The BSA protein was used as the heat-sensitive indicator to assist the visual monitoring of the coagulation process taking place during thermal ablation, which was clearly shown by the experiments performed here. In both types of TUMPs models (with Agar or PAA) prepared

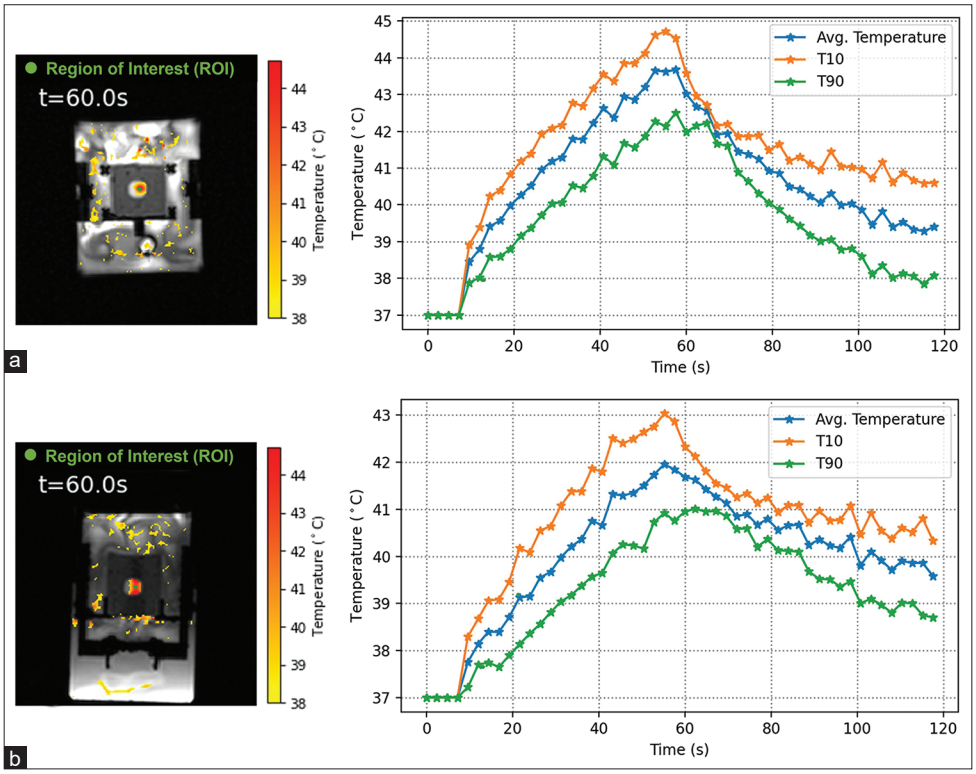

Figure 8: Shows magnetic resonance (MR)-Thermometry images acquired for the opaque Agar/polyacrylamide tumor-mimicking phantom model and the temperature evolution observed in a region of interest set within the focal spot with (a) a coronal thermal map with the sonication power set to 200W for 60 s and (b) an axial thermal map with the sonication power set to 200W for 60 s. MR parameters used: Sequence = FLASH 2D, Coil type: Body_12_BM, TR = 25 ms, TE = 10 ms, FA = 30°, acquisition matrix: 96 × 96, slice thickness: 3 mm, acquisition time/slice: 2.4 s, Echo train length: 1, Pixel BW: 501 Hz/pixel, FOV: 280 mm × 280 mm × 3 mm

here, silicon dioxide was added in the spherical PAA TUMPs as a contrast agent to aid in the MR monitoring of the TUMP model during thermal ablation and help distinguish the TMPs from the TUMPs. Glycerol was also added as a contrast agent in all the phantoms fabricated with PAA to further enhance the contrast in the MR images (due to *T1* relaxation time change).

To identify the creation of necrosis after FUS ablation in the spherical PAA TUMP incorporated in the TUMP models, the models were examined to ensure that the ablation area was in the focal region set by the FUS parameters and could be visualized by the naked eye. The experimental results showed the visible coagulation of the BSA protein from transparent to cream-white in the PAA TUMP caused by the thermal stress applied to the focal point targeted with FUS ablation. In addition, a hot water bath was used to heat the PAA TUMPs to temperatures above 55°C, which caused the BSA protein to coagulate, leading to a change in color from transparent to cream-white. The coagulation of the BSA protein also served as an indicator of the FUS focal point, which was clearly visible in the PAA/PAA TUMP model after FUS sonication.

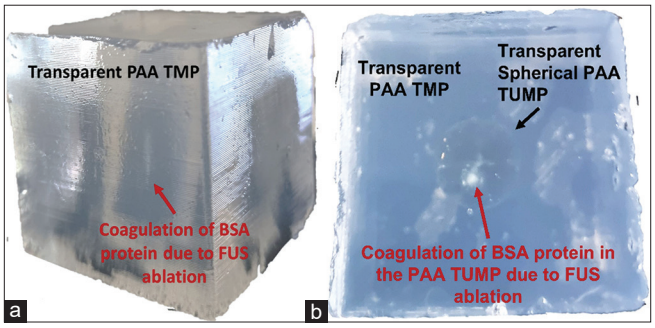

Figure 9: Shows photos of (a) the coagulation of the bovine serum albumin (BSA) protein from transparent to cream white in the (PAA)/PAA tumor-mimicking phantom model (TUMP) model formed by the thermal stress applied with FUS ablation and (b) the coagulation of the BSA protein in the FUS focal spot located in the transparent TUMP fused in the centre of the also transparent PAA TMP material

The phantoms were also designed to be visible by MRI for real-time monitoring of the FUS ablation process, therefore the MR imaging features and thermochromic properties of the Agar/PAA and PAA/PAA TUMP models were examined. *T2*-weighted MR images were used to estimate the three-dimensional geometry of the heated volume since the TUMP models showed a significant change in *T2* when the BSA protein is thermally coagulated. MR thermometry maps demonstrated that the suggested TUMP models may successfully imitate a tumor that is present in soft tissue.

An ideal TUMP model for thermal ablation research must have the following requirements: (a) the user should be able to replicate it in a short time and high consistency, (b) it should be safe to be handled by the user, (c) the operator should be

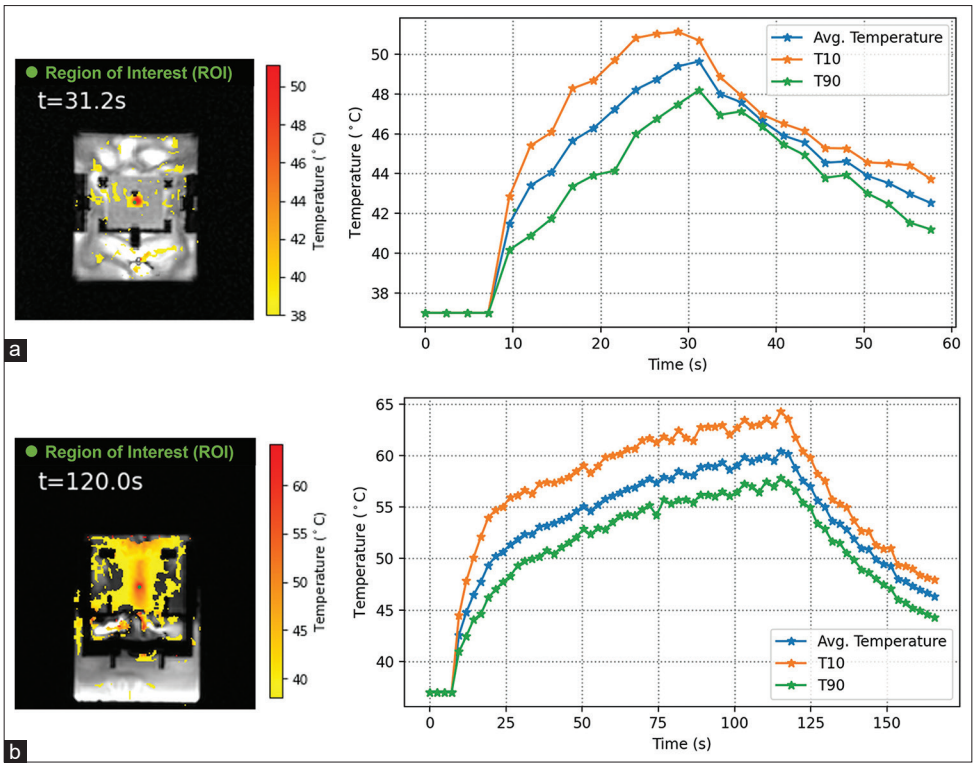

Figure 10: Shows magnetic resonance (MR)-Thermometry images acquired for the transparent (PAA)/PAA tumor-mimicking phantom model model and the temperature evolution observed in a region of interest set within the focal spot with (a) a coronal thermal map with the sonication power set to 200W for 30 s and (b) an axial thermal map with the sonication power set to 250W for 120 s. MR parameters used: Sequence = FLASH 2D, Coil type: Body_12_BM, TR = 25 ms, TE = 10 ms, FA = 30°, acquisition matrix: 96 × 96, slice thickness: 3 mm, acquisition time/slice: 2.4 s, Echo train length: 1, Pixel BW: 501 Hz/pixel, FOV: 280 mm × 280 mm × 3 mm

able to add it into the FUS setup located in the MR scanner with ease, (d) the phantom model should be thermochromic and therefore reveal the ablation region after thermal ablation and (e) it should provide good MR contrast between the TMP and TUMP. All the requirements mentioned here are fulfilled by the TUMP models fabricated for this study. Furthermore, the TUMP models mentioned here are ideal to simulate a breast or a liver tumor, including many other types of deep tumors whose depth is no more than 6 cm as the transducer used in this study has a focal depth of 6.5 cm.

The Agar/PAA and PAA/PAA TUMP models studied here can be helpful models for determining the thermal patterns during FUS ablation application in oncology. The coagulation temperature of the transparent spherical PAA TUMPs can be easily adjusted by changing the pH of the PAA solution that is mixed with the thermosensitive BSA protein. By changing their composition while still keeping the appropriate pH to control the BSA coagulation temperature it is possible to modify their energy absorption properties to match the acoustical and optical absorption of a specific tumor type that is surrounded by a specific soft tissue.

Conclusion

The TUMP models fabricated for this study have numerous uses in the testing and calibration of FUS equipment, the validation of thermal therapy treatment plans in oncology with FUS or MRgFUS applications, including uses in the quality control and QA assessments of FUS therapy systems.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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