Independent tuning of acoustic and mechanical properties of phantoms for biomedical applications of ultrasound

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***Abstract*— In this work the preparation of tissue mimicking materials (TMMs) with independently tunable acoustic and elastic properties is reported. Although a large number of hydrogel, synthetic polymer, polysaccharides or other natural based materials have been proposed and used for the realization of tissue mimicking materials, both for diagnostic and therapeutic applications of ultrasounds, up to today, simulation of acoustic properties was often performed using solid particles, reducing dramatically the transparency and inevitably affecting the homogeneity and the elastic properties of the TMM. By means of concentrated salts solutions and different polysaccharides, an easy method to prepare these TMMs have been developed. This approach would lead to obtain homogenous tissue mimicking materials with Young modulus ranging over 3 orders of magnitude, i.e. from 2 kPa to 1500 kPa, with independently tunable attenuation properties. An accurate mechanical and acoustic characterization of these TMMs have been performed. Finally, by means of a preliminary trials on protein denaturation induced by a HIFU transducer in a transparent TMMs with different attenuation values, the mechanism underlying on the formation and propagation of lesion, has been investigated. Obtained results suggest that this "chemical" approach would strongly support in vitro investigations on the open issues related to diagnostic and therapeutic application of ultrasounds.**

Tissue-mimicking materials, ultrasonic absorption, mechanical properties, acoustic impedance, HIFU investigations

1. Introduction

Tissue-mimicking materials (TMMs), also known as “phantoms”, used for diagnostic and, more recently, therapeutic applications of ultrasounds, have been studied from many decades. The advantage of phantoms is that idealized tissue models can be constructed with well-defined acoustic and mechanical properties, dimensions and internal features, thereby simplifying and standardizing an environment in which to study the interaction between ultrasounds and tissue substitute media (Culjat et al. 2010).

From a diagnostic point of view, tissue phantoms are available commercially for a range of medical applications, however, commercial phantoms may not be suitable in ultrasound system design for experimental investigations of novel techniques. Moreover, tissue-mimicking materials that are currently available for diagnostic biomedical applications may not be suitable for high-temperature experiments that occur, for example, in high focused ultrasound (HIFU) therapeutic investigations, so that, for studies of these applications, the design and fabrication of different TMMs with tailored thermal, acoustic and optical properties are often studied (Dabbagh et al. 2014). A multitude of tissue-mimicking materials described in the literature have been created using a variety of materials and preparation techniques, developed in liquid, solid, and gel forms, to simulate biological tissues. Gel phantoms have found most popularity because of representing a wide range of thermal, mechanical, acoustic and optical properties. Since one of the main requirements for realizing TMMs useful for biomedical applications of ultrasound (both diagnostic than therapeutic) is represented by the broad interval of attenuation coefficient of human tissues (0.1-1 dB/cm•MHz) which a TMM should have, while keeping, in some cases, good optical properties for visualize the lesions formation induced by HIFU transducers, or, from another point of view, without modifying their mechanical properties, as they are used like "soft phantom" for investigating in advanced diagnostic techniques as sonoelastography. Commonly TMMs based on polysaccharides like Agar (Madsen et al. 2005 3) or Gellan Gum (King et al. 2011) or polymer like Polyvinylalcohol (Surry et al. 2004) or polyacrylamide (Choi et al. 2013, Guntur and Choi 2014) have been studied, but usually, to obtain an attenuation similar to human tissues, the addition of solid particles like alumina, silicon carbide or glass spheres is performed, unfortunately this may cause a lack of transparency and homogeneity, due to particles settling.

From one side, the opacity of TMMs requires special equipments for the assessment of effects induced by HIFU irradiation, like ultrasonic imaging or NMR imaging techniques; from the other side, the presence of solid particles influences the mechanical properties of the TMMs, limiting their reliable use for soft-tissue phantoms with an acoustic attenuation greater than 0.4 dB/(cm·MHz). To our knowledge any alternative methods haven't been explored to modify the ultrasonic attenuation of TMMs. This issue has led us to consider the possibility to exploit a related phenomenon, that is the ultrasonic absorption which occurs in aqueous salt solutions. Ultrasonic absorption, is one of the phenomena to which it is subject to an acoustic wave that propagates through a medium. Attenuation coefficient measurements take account also of scattering and reflection. In pure solutions one usually refers to absorption because of the dissipative phenomena that in a homogeneous polymer matrix surrounded by aqueous salt solution can occur too. However after this short dissertation on ultrasonic absorption we will refer only to attenuation for our TMM samples. Ultrasonic absorption measurement is a well known technique that has been exploited in the past to study the molecular dynamics in liquids, aqueous solutions and liquid mixtures (Tamm and Kurtze 1951, Brai and Kaatze 1992).The first studies regarding absorption in aqueous solutions date back to after the second World War when it was found that the attenuation of sonar signal in sea water is caused by the presence of Magnesium Sulphate, even though it has a low concentration value and the related research on this phenomenon brought M. Eigen to the Nobel prize in chemistry in 1967 (Eigen 1968). Nowadays this techniques is used to determining molecular physical parameters of organic liquid mixtures (Pawarl et al. 2012) or to investigate the equilibrium dynamics in micellar systems (Kaatze and Lautscham 1988, Gopinathb and Ravichandrana 2014). Current studies on ultrasonic absorption from aqueous solutions of inorganic salts are relatively poor, so that the only data available are for a limited, usually low, range of concentration. In this regard, it is also worth to remembering that a TMM preparation with magnesium silicate in hydrogel based matrix has been reported in (Sheppard and Duck 1982), but beyond the attenuation value, no other useful investigations have been further provided. Following the detailed report by Stuehr and Yeager (1962) we have identified the suitable salts through which change the attenuation properties of TMMs. By means of consolidated experimental measurement techniques (Cuccaro et al. 2015) and preliminary results reported here (Troia et al. 2015) we have performed several trials using organic and inorganic salts dissolved in different solutions of polysaccharides and polymers. This led us to prepare homogenous and transparent TMMs with

independently tunable mechanical and acoustical properties. Finally, different trials on protein denaturation induced by an HIFU transducer in transparent phantoms containing an attenuating salt have been conducted. The experiment has showed that a different mechanism underlying the formation of the lesion takes place respect to what usually observed in similar, but low attenuating phantoms. This result could be related to the unpredictable or anomalous heating phenomena, as skin burns, reported from several HIFU clinic trials (Mougenot et al. 2011) and could lead the basis for preparation of new class of tissue mimicking-materials supporting investigation of both diagnostic and therapeutic applications of ultrasounds.

2. Materials And Methods

2.1 Preparation of tissue mimicking materials

As we found from preliminary investigations (Troia et al. 2015), different salts can be used to obtain high absorption/attenuation in TMM. We found that concentrated solutions of Manganese sulphate or Zinc acetate, exhibit the desired ultrasound attenuation for the frequency range that usually concern the TMMs (1-10 MHz). Zinc Acetate appears as ideal candidate since it is not toxic, it is not paramagnetic, so that can be included for preparation of TMM suitable in NMR equipped HIFU apparatus, unfortunately zinc ions bind the proteins causing immediate denaturation. For this reason for the experiments on the visualization of HIFU lesions we have used Manganese Sulphate, which shows similar attenuation.

As polymeric matrices, three polysaccharides and two synthetic polymers have been used. Respectively: Agar, Carrageenans, Gellan Gum, Polyvinilacol (PVA) and Polyacrilamide (PAA). Agar is maybe the most used polysaccharide for the realization of TMMs. Due to its easily preparation, different Agar based TMMs have been realized to investigate the effect of the different concentrations of $Zn(CH3COOH)_2$, between 0.3 M and 1 M, on attenuation properties. Agar samples (2% by weight) have been dissolved in Zinc acetate solutions, using a glass bottle heated on a hot plate up to boiling temperature (around 100 °C) while is kept under stirring; a sealing plug is used to prevent water content evaporation. After that, a careful procedure of casting into a cylindrical mould (diameter 5 cm, height 3 cm), has been followed to avoid bubble formation and to obtain very smooth and flat surfaces which are essential features for limiting uncertainty factors during the acoustic and mechanical characterization

measurements. Carrageenans are a sulphated polysaccharides extracted from red edible seaweeds. In our preparations, a commercial product from Sigma Aldrich mainly composed of k-carrageenans, which forms strong gels (Hermansson et al. 1991 , Campo et al. 2009), has been used. In this case Carrageenans (2% in weight) has been dissolved in a 0.4 M solution of $Zn(CH3COOH)_2$ and then heated up to boiling following the same procedure adopted for Agar.

Gellan Gum is a more complex polysaccharide, which consists of two residues of D-glucose and one of each residues of L-rhamnose and D-glucuronic acid. During last five years it has received more attention because it has a higher temperature stability, higher mechanical strength and better clarity besides a substantially lower cost which makes it an attractive polymer for realizing TMM matrix. Gellan Gum, as Agar or other polysaccharides, forms a physical gel by transforming from random coil to double helix transition upon heating and cooling treatment. Stronger gels are formed if cationic ions, in particular divalent, are present during the sol-gel transition, however the presence of cationic species immediately activates the polymerization of Gellan Gum solution, especially with the concentration required in our preparations (i.e. 0.4M). Thus, in this case, a completely different experimental procedure has been adopted. Firstly Gellan Gum (2% in weight) has been dissolved in pure water kept at a constant temperature of 60°C. Once a clear yellowish solution has been obtained, it has been carefully cast into the cylindrical mould and left cooling. After 2 h the formed soft gel has been dipped in a 0.4 M solution of Zinc Acetate which is absorbed slowly into the polysaccharide matrix increasing its mechanical strength and its absorption/attenuation properties. Since this process requires more than 72 h to let the salt diffuse into all the sample, its reproducibility has been evaluated by monitoring the electrical conductivity of the host salt solution. After a rapid decrease of conductivity, a lower limit has been reached, due to the saturation effect, which has indicated that the process was completed. Conductivity has been measured with a conductivity meter r (HQ-40 Hach Langhe) which has detected a decrease of 30% respect to the initial value, more exactly form 16.8 mS/m to 11.4 mS/m, for all the TMMs prepared with this method. Finally, the preparation of TMM based on synthetic polymers has been triggered by two different purposes. In one case the purpose is to explore the lesion formation induced by HIFU in a transparent TMM containing the attenuating salt and an heat sensitive protein, Bovinum Serum Albumin (BSA); in the second case the possibility of obtaining very soft and homogenous phantom with an attenuation coefficient higher than 0.4 dB/(cm·MHz) was investigated.

For the first case, since BSA cannot be dissolved into a polysaccharide based TMM because of the heating treatment required to obtain the gel, a well-known recipe for the preparation of PAA based TMM containing BSAhas been used. Briefly: 5 g of BSA has been dissolved in 100 ml of acrylamide solution (10% in weight) containing 0.526 gr of N,N'-metilen-bis-acrilammide. Then 1 ml of ammonium perfsulphate (10% in weight) has been added to the solution and finally 0.5 ml of Tetramethylethylenediaminehas been used to initiate the polymerization reaction. As mentioned above, to reach high attenuation values MnSO4, 2.5 M, has been added to the solution before the ammonium persulfate step. This TMM will be hereafter referred as PAAMn. In order to compare the different lesion formation and heat propagating phenomena underlying the HIFU irradiation,identical TMM of PAA without MnSO4 has been also prepared and it will be hereafter referred as PAA. Lastly the preparation of a very soft phantom which exploits the attenuation mechanism given by salt solutions aimed to modulate its acoustic properties has been investigated. For this purpose a well known procedure of freezing-thaw technique using PVA based TMM has been employed. PVA (5% in weight) has been dissolved upon heating at 70 °C in a 0.4 M solution of Zinc Acetate. After the complete dissolution of PVA the solution has been poured in special 3D printed moulds, in order to prevent the yielding of the TMM during the acoustical characterization (see figure 3d), and cooled at -25 °C for 18 h.

2.2 Measurement of acoustic properties of TMM

The similarity to biological tissues of TMMs has been investigated starting from the study of their acoustic properties, in particular of the attenuation coefficient. This quantity represents the amount of energy an acoustic wave loses passing through a medium and, for the samples under study, it will be hereafter indicated with the symbol α_s. In this work, α_s has been measured by means of the insertion technique, using demineralised water as reference material. Thus, the attenuation coefficient can be determined as (Cuccaro et al. 2015)

$$\alpha_s = -\frac{20}{l_0} \cdot \mathrm{Log} \frac{A_s}{A_w} + \alpha_w , \quad (1)$$

in which A_s and A_w are respectively the measured amplitude in presence or in absence of the sample in the acoustic path, l_0 the thickness of the sample and α_w attenuation coefficient of water (Pinkerton 1949). Samples investigated in this work have a cylindrical shape with a diameter of about 5 cm and a thickness of 3 cm.

The description of the experimental apparatus employed for determining the attenuation coefficient and further details on the analysis method of the signal and on the measurements conditions can be found in (Cuccaro et al. 2015). Since the same apparatus has been also used to determine TMMs speed of sound, to assume far-field propagation conditions a distance of about 23 cm has been chosen between the ultrasound source and the sample holder. Attenuation and speed of sound measurements have been carried out in a thermostated room to maintain the temperature of the water in the tank at a constant value. Slight water temperature variations have been controlled through a temperature control system consisting of a PT100 platinum resistance thermometer and an heating foils placed in the tank. The heater was controlled through a proportional–integral–derivative (PID) controller. An additional PT100 thermometer has been placed in the tank on opposite side to the previous one to verify the homogeneity of the water temperature.

2.3 Measurement of for mechanical properties of TMM

The mechanical properties of TMMs, in terms of Young's modulus, E, yield strength, σ_y, and tensile strength, σ_T have been measured on the basis of engineering uniaxial tensile test in compression until failure and complete stress-strain diagrams have been achieved. Measurements have been performed at 20 °C and at a constant strain rate of 0.03 mm·s^{-1}. The complete measurement set-up is well described elsewhere (Schiavi, et al. 2016).

Young's modulus values have been calculated using the expression reported below:

$$E = \frac{\Delta\sigma}{\Delta\varepsilon} = \frac{F}{A} \cdot \frac{l_0}{\Delta l} \qquad (2)$$

where E is the Young's modulus (Pa), $\Delta\sigma$ is the incremental stress (Pa), $\Delta\varepsilon$ is the incremental strain (dimensionless), F is the compression force (N), A is the surface area of the sample (m^2) on which the force acts, l_0 is the initial thickness of the sample (m) and Δl is the deformation in thickness (m) resulting from the application of F within which the strain/stress curve of sample shows an elastic behaviour.

E has been determined applying a linear fit to the measured stress-strain curve. The line angular coefficient allows the Young's Modulus value to be calculated. Due to the high viscosity of hydrogels, the stress-strain curve in compression is no longer linear until yield strength, as a consequence the Young's modulus should be considered as a function of strain range. Moreover, the effect of lateral expansion (that inevitably occurs during compression), has been also taken into account in terms of surface area variation dA (at boundary, dA/dt = 0) in order to accurately evaluate Young's modulus E, as a function of the actual surface area expansion, A+dA, due to the lateral poissonian deformation. In order to prevent samples degradation, due to possible water loss, TMMs have been stored in a sealed and isolating membrane to avoid water evaporation during the conditioning time. Samples have been placed between two parallel aluminium plates and have been compressed by a linear displacement acting perpendicularly on the upper surface of gels. Parallel plates have been lubricated with a thin layer of glycerine in order to prevent any friction between samples and plates and also to guarantee a homogeneous uniaxial deformation. (Schiavi, et al. 2016).

2.4 Experimental set-up for investigation of HIFU lesion formation and heat propagating effect

To perform experimental investigations on lesion formation induced by HIFU transducers in transparent TMMs with different attenuation properties, a dual frequency operating transducer has been employed (Sonic concept HR-101) with working frequency of 1.1 MHz and 3.3 MHz (fundamental and third harmonic, respectively). The HIFU transducer has been driven by a waveform function generator (Agilent mod 33250A) coupled with an high frequency amplifier (A&R mod. 800A3B). A power of 100 W and an irradiation time of 5s has been used for all experiments, except for the ones reported in Fig. 10, in which longer irradiation time have been investigated. PAA based TMM has been poured in cubic glass cuvette (dimension 5 centimeters) and positioned at the same distance from the emitting surface for all the trials. A scheme of the whole set used for these experiments is shown in figure 1. Water temperature of the tank have been controlled through a PT100 thermometer .

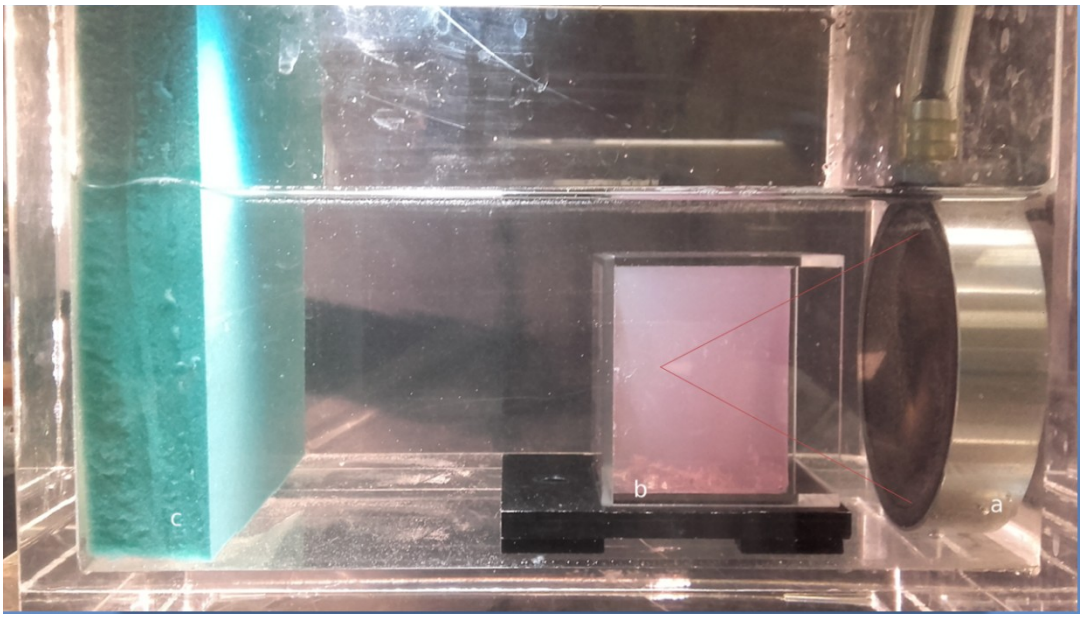

Figure 1. Experimental apparatus used for HIFU induced denaturation in PAA based TMM: a) HIFU transducer HR101; b) glass square cuvette filled with PAAMn TMM (the pink color is due to manganese sulphate); c) rubber adsorber to prevent reflection from the wall of the Plexiglas chamber. The chamber is filled with deionized water or a 70% solution of Glycerin in order to assess the reflection given by impedance ratio at liquid/gel interface..

3. Results

3.1 Attenuation properties

In figure 2, attenuation values, obtained at the temperature of 23 °C, for Agar based TMMs are shown as a function of the frequency and of the $Zn(CH_3COOH)_2$ concentration. Experimental data have been fitted using with the following expression:

$$\alpha_s = \alpha_0 \cdot f^n, \qquad (3)$$

where *n* is included between 1 and 2. Eq. (3), since, as it is reported in literature, represent the ideal approximation of the attenuation dependence on frequency, for biological tissues.

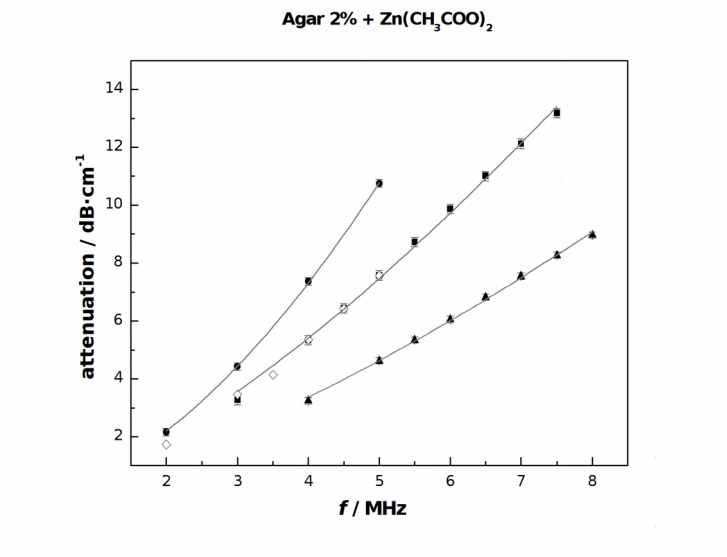

Figure 2. Attenuation values of Agar based TMMs as a function of $Zn(CH_3COOH)_2$ concentration (● 1 M; ■ 0.4 M; ▲ 0.3 M; ◊ 0.4 M zinc acetate solution).

Fitting curve parameters (α_0 and n) result to be α_0= (0.71 ± 0.04) dB/(cm·MHz^n) and n = (1.44 ± 0.03) for the TMM with a zinc acetate concentration of 0.4 M while measurements of attenuation in 0.4 M Zinc acetate solution have evidenced that the attenuation is essentially given by the salt. Carrageenans based TMM maintains a good transparency (see Fig. 3b) and its attenuation curve is shown in Fig. 4. The fitting curve parameters (Eq. 3) result to be α_0= (0.65 ± 0.04) dB/(cm·MHz^n) with n = (1.48 ± 0.04).

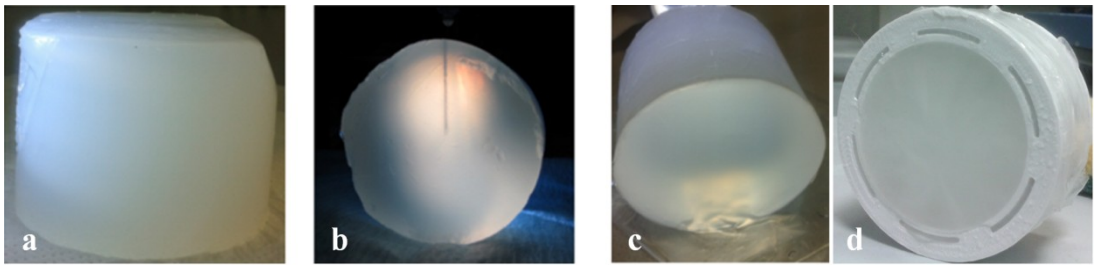

Figure 3. Pictures of the TMM samples prepared with different polymer matrix: a) Agar based TMM, b)Carrageenans, c) Gellan Gum, d) PVA based TMM embedded into a custom 3D printed mould to prevent yielding of the sample for attenuation measurement.

Nevertheless, as it has been recently reported (Yoshida,et al. 2004 22), Carrageenan undergoes liquefaction at low temperature (around 60 °C) which could limit its application for measuring or monitoring the heating effect phenomenon induced by HIFU transducers. On the another hand, Gellan Gum based TMMs have higher temperature stability maintaining a good transparency,see figure 3c . Attenuation curves are shown in figure 4 where they are compared with those ones of Agar and Carrageenans based TMMs, and, as it possible to observe from the figure, the attenuation curves are very similar. The main difference is observed between the sample of Agar and Carrageenan, probably due to the difference of 2°C in temperature of the TMMs during the measurements. In figure 5 attenuation data for polymers based TMMs are shown. In this case two remarkable aspects should be pointed out: firstly that PVAZn based soft phantom reaches an high attenuation value although maintains, as we will show, very low young modulus (less than 3 kPa); secondly the different attenuation curves between the two samples of polyacrylamide, with or without the MnSO4 salt. Attenuation coefficient, α_s, density, ρ, speed of sound, w_s, and acoustic impedance, Z, for all the TMMs investigated in this work have been summarized in Table 1.

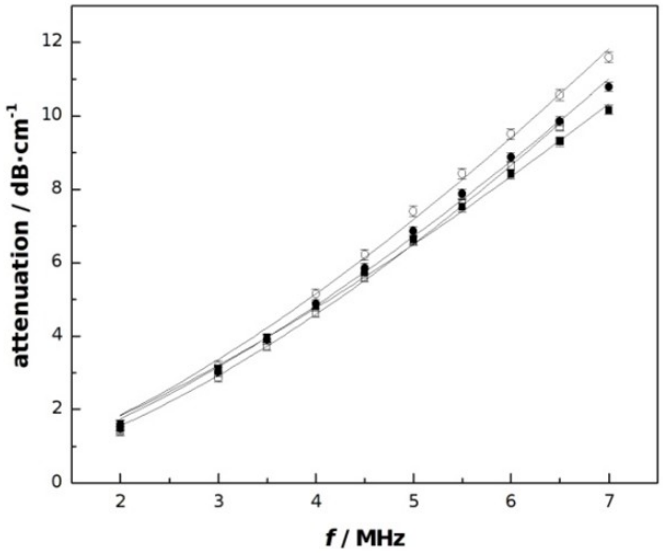

Figure 4. Attenuation values and relative fitting curves for TMMs prepared with a solution of $Zn(CH_3COOH)_2$ 0.4 M.□ Agar based sample; ○ Carrageenans based sample; ■ Gellan Gum 2% based sample; ● Gellan Gum 1% based sample. T = 25 °C.

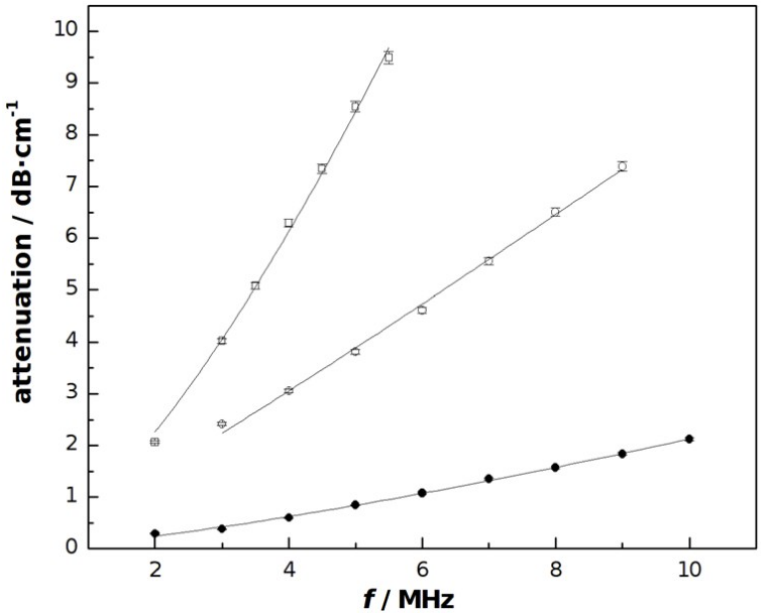

Figure 5. Attenuation values and relative fitting curves for TMMs. □PVAZn 0.4 M;○PAAMn 2M; ● PAA.

Table 1. Attenuation coefficient, α_0, speed of sound, w_s, density, ρ, impedance, Z, values with respective uncertainty of TMM

TMM	$Zn(CH_3COOH)_2$ concentration	$\alpha_0 \pm u(\alpha_0)$ dB/(cm·MHz^n)	$n \pm u(n)$	$w_s \pm u(w_s)$ m·s^{-1}	$\rho \pm u(\rho)$ kg·m^{-3}	$[Z \pm u(Z)]*10^6$ rayl
Agar 2%	1 M	0.66 ± 0.02	1.74 ± 0.02	1524 ± 5	1138 ± 6	1.73 ± 0.01
Agar 2%	0.5 M	0.58 ± 0.03	1.64 ± 0.03	1518 ± 5	1066 ± 5	1.62 ± 0.01
Agar 2%	0.4 M	0.53 ± 0.02	1.56 ± 0.02	1516 ± 5	1058 ± 5	1.60 ± 0.01
Agar2%	0.3 M	0.47 ± 0.02	1.42 ± 0.03	1510 ± 5	1042 ± 5	1.57 ±0.01
Carrageenan 2%	0.4 M	0.66 ± 0.05	1.48 ± 0.04	1511 ± 5	1061± 5	1.60 ± 0.01
Gellan Gum 1%	0.4 M	0.56 ± 0.03	1.55 ± 0.03	1514 ± 5	1002 ± 5	1.52 ± 0.01
Gellan Gum 2%	0.4 M	0.57± 0.03	1.42± 0.03	1513 ± 5	1009 ± 5	1.53± 0.01
PVAZn	0.4 M	0.84 ± 0.06	1.44 ± 0.04	1516 ± 5	1059± 5	1.61± 0.01
PAA	-	0.098± 0.01	1.34 ± 0.03	1546 ± 5	1020 ± 5	1.58± 0.01
PAAMn	2.5 M	0.77 ± 0.08	1.02 ± 0.05	1791 ± 6	1290± 6	2.31 ± 0.01

3.2 Elastic properties

Comments on mechanical characterization require some preliminary considerations. The yield strength point, σ_y, represents the limit of the sample elastic region, that means that, within the correspondent strain range, the sample shows an elastic behavior. However, it is not possible to define unequivocally the Young's modulus of each TMM sample, since a relevant strain range dependence is achieved. Moreover some times, as in the case of PAA samples,

yield strength point corresponds with the tensile strength point, otherwise, as for PVAZn TMM, these points are not observed even if the sample compression of about 50% of its thickness is carried out, see figures 6 and 7. Therefore, the TMMs elastic behaviour can be expressed more accurately as a function of the strain range. The Young's modulus values summarised in Table 2 for all the TMMs investigated have to be considered as the elastic response of Agar between 6% and 11% of strain, Carrageenan between 10% and 15% of strain, Gellan Gum between 12% and 17% of strain and PAA, PAAMn and PVAZn between 10% and 20% of strain.Despite Young's moduli are similar for Agar and Carrageenans (200 kPa) based samples, Gellan Gum shows a complete different value (from 400 up 1500 kPa). Gellan Gum based TMM differ enormously because of the presence of concentrated bivalent cation (Zn) which acts also as increasing strength factor for this type of polysaccharide matrix, although on the other hand affected the elastic behavior of this TMM. For this reason further characterization of this TMM have been carried out on 1% Gellan Gum based TMM, which has a lower Young's modulus but a sufficient elasticity for further characterization. In synthetic polymer based TMMs, we have found a Young's modulus of 25 kPa for PAA and of 2 kPa for PVAZn. Measured mechanical values indicate that the whole set of phantoms simulates quite well the wide range of tissues elastic properties. In particular the measured Young's modulus (~200 kPa) for Agar, Carragenan are typical of strong tissues,muscle, kidney capsule (McKee et al. 2011) while the value for Gellan Gum based TMM are more similar to cartilaginous tissues. Finally PVAZn sample simulates very soft tissue almost close to brain, in which, for example, it should be taken in account that an attenuation value up to 0.7 dB /cm·MHz for gray matter have been reported (Kremkau et al. 1981). In general terms, in the field of tissue mimicking engineering both for diagnostic than therapeutic application of ultrasounds, tuning of acoustic and mechanical properties has been recognized as a fundamental requirement for optimization of ultrasonic techniques and modeling of ultrasound-tissue interaction. From this point of view the possibility of easily tuning independently the acoustic and elastic properties of phantoms can be achieved using these preparation techniques.

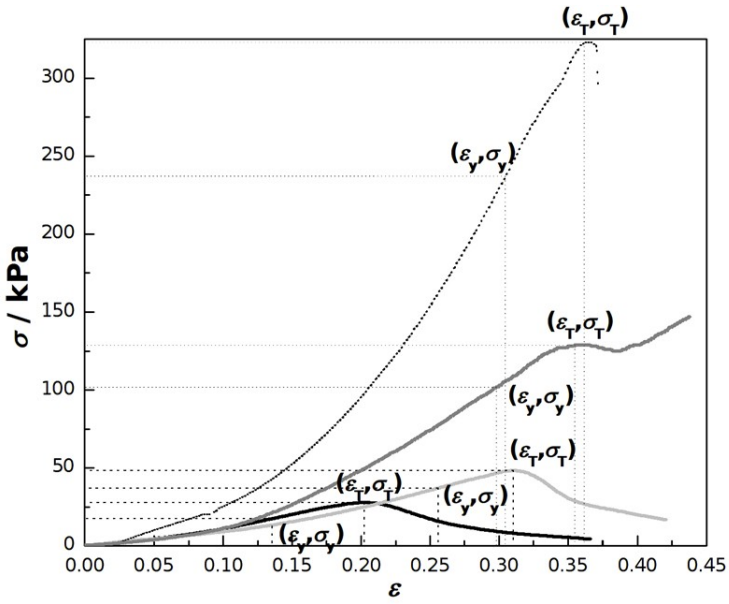

Figure 6. Stress – strain curves in compression test until failure at T = 20 °C and strain-rate = 0.03 mm·s^{-1} of (▬) Agar based TMM with zinc acetate 0.4 M, (▬) Carrageenans based TMM with zinc acetate 0.4 M, (▬) Gellan Gum 1% based TMM with zinc acetate 0.4 M and (▪▪▪) Gellan Gum 2% based TMM with zinc acetate 0.4 M. Respective tensile ε_T and yield ε_y strain, tensile σ_T and yield σ_y stress are shown.

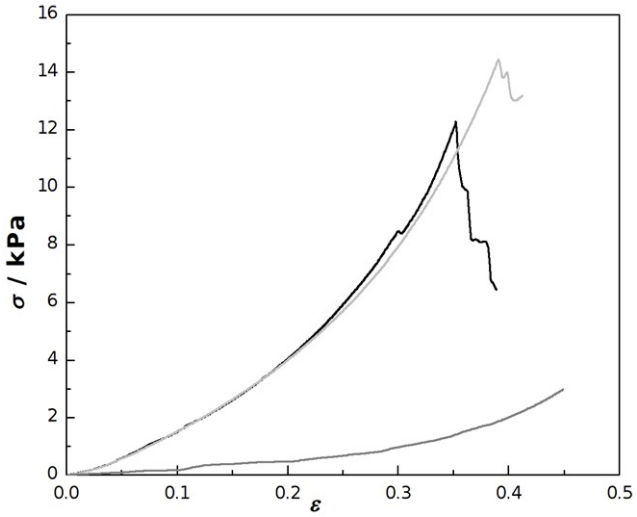

Figure 7. Stress – strain curves in compression test until failure at T = 20 °C and strain-rate = 0.03 mm·s^{-1} of (▬) PAA, (▬) PAAMn and (▬) PVAZn.

Table 2. Fundamental mechanical properties. (E, $u(E)$): Young's modulus and respective uncertainty; ε_y, σ_y: yield strain and strength; ε_T, σ_T: tensile strain and strength at 20 °C and 0.03 mm s^{-1}

TMM	E	$u(E)$	ε_y	σ_y	ε_T	σ_T
	kPa	**kPa**		**kPa**		**kPa**
Agar	184	13	0.14	17	0.20	28
Carrageenan	228	17	0.26	37	0.31	48
Gellan Gum 1%	394	29	0.36	129	0.30	102
Gellan Gum 2%	1550	115	0.30	237	0.36	323
PAA	25	2	-	-	0.35	12
PAAMn	25	2	-	-	0.39	14
PVAZn	2.4	0.2	-	-	-	-

3.3 HIFU investigations

Experiments on HIFU irradiations on heat sensitive TMM have revealed some unexpected results. As it is possible to note from figure 8, in PAA based TMM, the well-known lesion shape (cigar - tadpole), similar to those ones observed by other authors (Choi et al. 2013) has been obtained. A slight difference on the shape of the lesion can be observed as a function of the HIFU frequency used which confirms, as reported also by the manufacturer, the different shape of the ultrasonic field induced by the transducer respect to the two frequencies. At 3.3 MHz the shape of the acoustics field is more focused respect 1.1 MHz, so that it is reasonable to expect a smaller lesion. However, since the attenuation of this TMM is very low, further investigations on propagation and formation of the lesion would be of little use since real tissues have, as it is known, an higher attenuation coefficient.

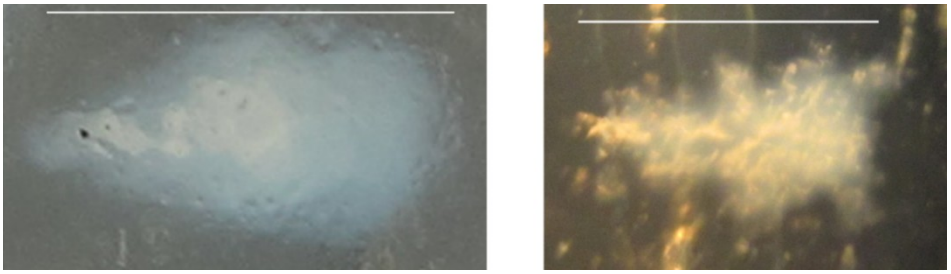

Figure 8. Close up view of the denaturation induced in PAA TMM in the focal region by HIFU transducer operating at: right) 1.1 MHz, 100 W, operating time 5 s, scale bar 7 mm; left) 3.3 MHz 100 W, 5 s, scale bar 3 mm

On the contrary, when the PAAMn based TMM has been irradiated, completely different lesions have been obtained, see figures 9a-9d . In general it appears that in TMM with higher attenuation value, the lesion shows an evident propagation towards the transducer surface in addition to a larger dimension of the lesion into the focal region. It is generally accepted that the focused acoustic field in pre-focal region is not sufficiently intense to induce heating and necrosis effects, which is also one of the peculiar features of HIFU treatments . However this “anomalous” heat

propagation effects are an un-expected and unwanted side effects in medical treatments, that have caused skin burns and damage of healthy tissue. Heat propagation effects, are clearly evident, in particular in figures 9c and 9d, showing that a remarkable denaturation can occur at pre-focus region, very close to the liquid/TMM interface. although in these cases a longer insonation time has been performed (10 s).

Paying attention to the acoustic properties of this TMM, we have to consider that a possible reason for these effects can also be ascribed to acoustic reflection phenomena. The reflection coefficient, *R*, is defined as:

$$R = \frac{Z_2 - Z_1}{Z_2 + Z_1} \quad (4)$$

where Z_1 is the acoustic impedance of the coupling liquid, while Z_2 is the impedance of the TMM. The acoustic impedance of the PAAMn TMM is about $2.31 \cdot 10^6$rayl,almost 60% greater than that one of the PAA which is $1.58 \cdot 10^6$rayl, that lead to obtain a reflection coefficient at liquid/TMM interface of 0.03 and 0.22 respectively for PAA and PAAMn if water is used as coupling medium. This means that at the water/TMM interface, using PAAMn TMM, the reflected wave is “in phase” respect to transmitted one, thus that the acoustic pressure at gel/liquid interface reaches a maximum and causes, probably, these anomalous heating effects . To address this phenomenon, we have performed further experiments with PAAMn using an aqueous solution of glycerin (70% in vol) as coupling media, which has an impedance of about $2.18 \cdot 10^6$rayl (Glycerine Producers' Association, New York, 1963), to obtain a reflection coefficient similar to that one of the PAA/water interface.

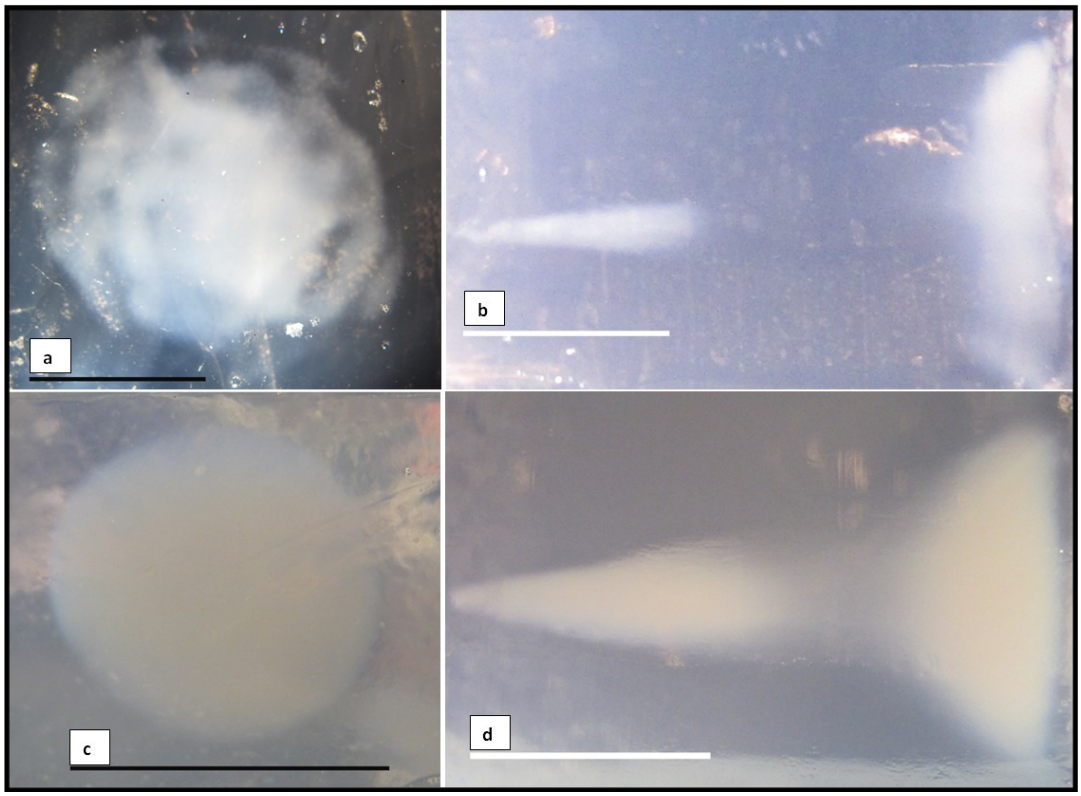

Figure 9. Pictures of the anomalous BSA denaturation in PAAMn induced by HIFU transducers for different operating conditions: a) front view of the lesion produced by HIFU operating at 1.1 MHz 100 W, 10 s, scale bar 1 cm; b) HIFU operating at 3.3 MHz, 100 W, time 10 s, scale bar 4 mm; c) front view of image d, scale bar 1 cm; d) HIFU operating at 3.3 MHz, 100W, time 15 s, scale bar 6 mm.

In this case the heating at the TMM/liquid interface is even absent even if, as it is possible to observe in Figure 10, a remarkable difference on the lesion formation in the focus region is still evident, since the area of the lesion induced in PAAMn TMM is almost the double respect the one obtained in PAA. These trials have been performed only at 3.3MHz, since at 1.1 MHz an higher HIFU driving voltage was necessary to induce the lesions into the focal region. In this case probably the propagation of focused acoustic field was also affected by the slight increase of the attenuation properties characteristic of glycerin solution (WANG X et al. 2015).

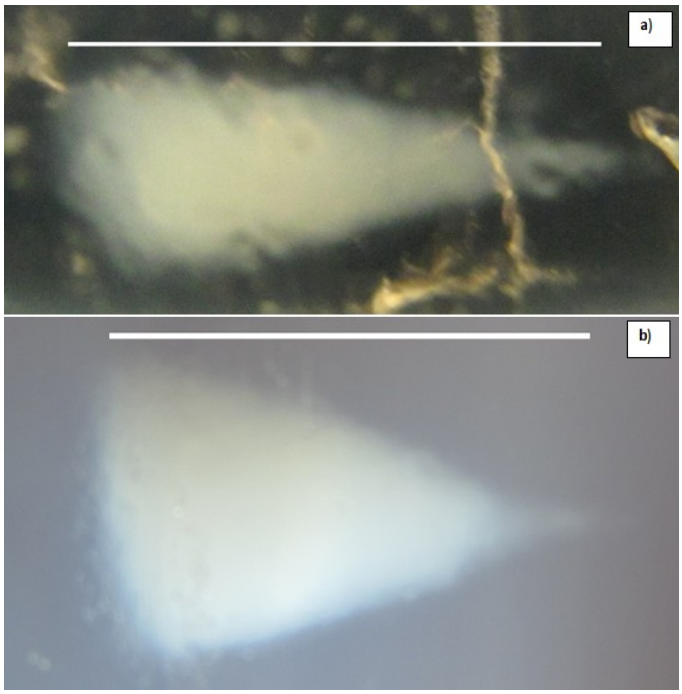

Figure 10. Close up view of the denaturation induced into the focal region by HIFU transducer operating at 3.3 MHz, 100 W , operating time 5 s respectively in: a) PAAMn TMM with 70% glycerine solution as coupling medium, scale bar 7 mm ; b) PAAMn TMM with water as coupling medium, scale bar 6 mm.

4. Discussion

Early works related to ultrasonic absorption of polysaccharides solutions have pointed out that the gel attenuation is given mainly by water molecules trapped into the polymer network and from the free portion of the cross-linked polysaccharides chains . In particular it has appeared that the attenuation given by polysaccharides is very low and the influence of their concentration is negligible (Gettins, et al. 1978). The ultrasonic attenuation of concentrated salts solutions is so high (see figure 2) that for some samples, no useful acoustic signal beyond 5 MHz was detectable by our measurement apparatus. The results obtained with Agar based samples evidenced that 0.4 M represent the ideal concentration, to obtain a TMM with attenuation coefficient similar to most of human organs and tissues. Attenuation values of pure 0.4 M solution of Zinc acetate suggested that its acoustic properties can be applied to different preparations of polysaccharides based TMMs, as we performed in the cases of Carrageenans and Gellan Gum. Ultrasonic absorption of zinc acetate solutions at lower concentrations values i.e. 0.001M, have been already studied, following the hypothesis suggested by Pancholy and Singal (1963), we suppose that, since the amount of

undissociated salt is surely higher, the formation of water-based complex, and the equilibrium reactions which are the mechanisms at the origin of ultrasonic attenuation, allows tuning the attenuation properties of the TMMs . Similar considerations can be done for the case of MnSO4. Studies of the ultrasonic absorption of aqueous solution of MnSO4 have been performed in the past (Jackopin and Yeager 1970) at lower concentration, in which the formation of water-based coordination complex have been hypothesized as responsible for the absorption mechanism and the presence of non-linear effects on ultrasonic absorption, with respect to the concentration, have been already observed. As highlighted above, this method has led us to obtain a set of phantoms in which the acoustic attenuation can be tuned independently respect the mechanical properties, allowing the preparation of a very soft phantom with an acoustic attenuation similar to the real human tissue. To our knowledge it is the first time that a PVA based phantom has reached this attenuation without containing any solid particles dispersed inside. The experiments performed with PAA based phantoms on protein denaturation induced by HIFU transducer have revealed that the formation of lesion is strongly influenced by the acoustic properties of the phantom and also that reflection phenomena, which obviously occur in real medical treatments, should be taken in account. The acoustic power and insonation time used in these experiments was of the same order of magnitude (100-200 W, 5 -15 s) to that used in MRI guided HIFU clinical treatments. . The observation of different lesions formation, has revealed that further “in vitro” trials are advisable in order to define a proper HIFU “safe dose” in order to prevent side effects. A very recent exhaustive review on tissue mimicking gel phantoms for thermal therapy (Dabbagh et al. 2014) has stressed out how the issue of TMMs with attenuation value similar to human organs in which to observe the heating effects induced by HIFU transducers is still unsolved. By these new types of TMMs, the anomalous effects, like near-field heating in fat tissue (Mougenot et al. 2011), up to cases of skin burns, caused by focused ultrasounds could be investigated in order to shed some light on the drawback related to this treatment technique, which still limits its application (Hipp et a.l 2012).

5.Conclusion

In this work we have shown how some of the most common chemical reactions like aqueous equilibrium reactions of coordination complex, or chemical properties of aqueous solutions can be exploited to fine tuning the acoustic properties of phantoms, or for the coupling media, in order to carry out more accurate investigations in vitro on the

mechanisms relative to the interaction between ultrasounds and tissues. By means of the attenuation properties of concentrated salt solutions, a new approach for the realization of tissue mimicking materials would open the possibility to realize different TMMs in which study the most advanced biomedical applications of ultrasounds both for diagnostic than for therapeutic fields. Although many TMMs have been already proposed, most of them have shown some drawback, like low attenuation, inhomogeneity, opacity, high temperature instability and further investigations have been often auspicated. The results reported here have put in evidence how through this mutual relationship between chemistry and ultrasounds, further preparation methods can be still developed to support a better comprehension of the phenomena related to the interaction between ultrasounds and tissues.

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