Title: **Mechanical behavior of tissue mimicking breast phantom materials**

Authors:

- Hina. M. Ismail (hina.ismail@pg.canterbury.ac.nz)
- Christopher G. Pretty (chris.pretty@canterbury.ac.nz)
- Matthew K. Signal (Matthew.Signal@tiromedical.com)
- Marcus Haggers (Marcus.Haggers@tiromedical.com)
- Cong Zhou(cong.zhou@canterbury.ac.nz)
- J. Geoffrey Chase (geoff.chase@canterbury.ac.nz)

Corresponding author:

- Hina Muhammad Ismail (hina.ismail@pg.canterbury.ac.nz)
- Mobile #: 0064211266910

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Abstract

The mechanical properties of soft tissue have long been of interest in biomedical research and applications, and increasingly for breast cancer imaging. In this paper, the mechanical properties of three different materials used to emulate the mechanical behavior of real breast tissue are measured: agar, gelatine, and silicone, to assess their suitability for use in phantoms in systems assessing tissue mechanics for diagnostics. Two widely recognised measurement procedures are used. Quasi-static uniaxial compression was performed under a strain rate of 0.5 mm/min up to 15% strain with preloads of 0.05 N, 0.1 N, and 0.2 N, was used to measure the elastic moduli. Dynamic testing over a frequency range of 0.1-50 Hz for agar and 0.1-100 Hz for gelatine and silicone with the same preload was used to measure the storage moduli. Elastic and storage moduli were (5-81 kPa, 17-85 kPa, 5-112 kPa) and (3-128 kPa, 10-109 kPa, 5-73 kPa) for agar, gelatine, and silicone, respectively at the three preloads. All materials can be cast into arbitrary shapes and are suitable for tissue-mimicking phantoms. Silicone was the most consistent across the different preloads and frequencies, and can provide a range of stiffness ratios of adipose to tumor tissue that match experimentally reported values. More specifically, silicone samples for skin, adipose and tumour tissues show nonlinear stress–strain characteristics at 3 preloads characterized using hyperelastic parameters by fitting Neo-Hookean, Mooney Rivlin and Ogden models. Silicone also does not contain water, so environmental influences do not affect its mechanical properties as much as the other materials, and is thus more durable for consistent re-use. Finally, breast shaped mimicking silicone phantoms were fabricated for in vitro trials of a Digital Image Elasto Tomography breast cancer screening system assessing changes in mechanical properties.

Keywords:

Agar; Breast mimicking phantom; Silicone; Gelatine; Elastic; Viscoelastic;

1.0. Introduction

Breast cancer is a worldwide problem, and approximately 232,000 new cases were expected to be diagnosed in the United States in 2015 [1]. Traditionally, breast cancer has been diagnosed via manual palpation, but mammography has become the mainstream method of screening. However, there are concerns about mammography's performance in detecting smaller tumours [2] and radiation exposure for subjects [3]. To detect deeper and/or smaller tumours, an alternative modality such as elastography might offer a solution [4, 5].

Due to demand for better screening modalities has driven growing interest in phantom materials to mimic breast tissue to aid research and design. A tissue-mimicking phantom should emulate important mechanical properties of biological tissue for the purpose of providing a more clinically realistic imaging test environment. These phantoms in-turn aid development and validation of new imaging techniques, such as elastography [6-8], which have different phantom requirements than non-contact imaging methods, such as MRI. Thus, the phantoms used may well be different and may, or may not, be multi-modality capable.

In this case, the fundamental concept of DIET (digital image elasto tomography) is based on elastography and assessment of mechanical material properties [9]. DIET is non-invasive, portable, inexpensive, comfortable breast cancer screening technique that measures the stiffness of tissue within the breast [6, 10]. The DIET system captures low amplitude surface oscillations in the range 10 – 100 Hz generated by mechanical actuation applied to the breast [6], and examines these images for diagnostic behaviour indicated by the high stiffness contrast of cancerous tissue being evidenced in changes in how shear waves and motion are transmitted through the underlying tissues. Hence, it is difficult to compare the requirements for phantoms in this modality to one for MRI or mammography, for example. However, while such multi-modality phantoms would be of great benefit in comparing modalities, this research focuses on the mechanical properties desired, and over the frequency range of input for the DIET system concept, which has not been fully investigated in past.

A multitude of tissue-mimicking materials and phantoms are described in the literature that have been created using a variety of materials and preparation techniques and that have modelled a range of biological systems. Mixtures of agar and gelatine, Polyacrylamide gel, paraffin-gel waxes, Polyvinyl chloride (PVC), and Polyvinyl alcohol (PVA) were used in phantoms for use in ultrasonography [11-19]. Both agar and gelatine have excellent acoustic properties for ultrasound (US) elastography and would be suitable for both MR and US properties [20]. Sinkus et al [5] describe a breast phantom for MR elastography made from polyvinyl alcohol. The bulk of these phantoms consist of a softer material surrounding a 6-mm cube of harder material, which is a fine standard for non-contact imaging such as MRI and US, as well as for validating acoustic approaches

However, the DIET approach is mechanically based, and thus requires phantoms with mechanical properties to match tissue mechanical properties, as well as being created in a breast shape as shape and location play a role in the phantom. Hence, most of these prior approaches would not be applicable in this more unique case, as well as making comparison difficult to prior phantoms due to differences in shape. Thus, this work focuses on the mechanical properties necessary for making a mechanical tissue mimicking phantom and does not have the scope to enable comparisons to other modalities.

Tissue stiffness has been recognised as playing an important role in diagnosis of breast cancer, as tumour tissues have greater stiffness than the surrounding breast tissues [21]. Unfortunately, only a few studies reporting the elastic properties of real breast tissue are available in the literature. Studies on the mechanical behavior of breast tissue show the elastic modulus (E) ratio of adipose to tumour tissue ranges from 1:5 to 1:15.

Sarvazyan [22] found that cancer can be 7 times stiffer than normal tissue. Whereas, Skovoroda [23] found a normal to tumor tissue ratio of 3:1. It is unclear what strain levels were used during test. Kroupkop [21] recognized that the non-linear behaviour of breast tissues requires the computation of an elastic modulus at more than one strain level. At 5% precompression strain, the ratio of normal to tumor tissue was 1:5, while at 20% precompression strain the ratio increase to 1:15%. In contrast, adipose and tumor tissues have similar elastic moduli at small strain (less than 10%). Thus, while it might not be possible to distinguish malignant tumors from benign tumors at small strain levels alone, it may be possible to do this by considering data at larger strain. These results are used in this work to guide the experimental analysis and use of pre-load to delineate the targeted material properties when formulating phantoms using these materials. Hence, one result of this work will relate the phantom creation process and materials used to the outcome material properties, relative to the desired tissue properties in breast cancer.

Several materials have previously been used to mimic breast tissues and their mechanical properties are close to those of breast tissue [24, 25]. Agar and agar/gelatine combinations have been commonly used to mimic the acoustic and elastic properties of soft tissues [12, 26-28]. Agar has also been used successfully to mimic organs, breast tissues, sinus cavities and vascular systems[26]. In particular, agar, gelatine , and silicone are polymers with nonlinear behavior and can provide similar stress-strain relationships to breast tissue [29]. Agar and gelatine are networks of polymer chains with covalent bonds and water filled interstitial space

[30]. They thus mimic the physiology well. Silicone is comprised of linear chains of dimethylsiloxane and is also used for a range of biomedical products [31].

The aim of this paper is to determine which materials and compositions have appropriate elastic modulus and viscoelastic properties to accurately mimic the actual breast tissues for DIET over its frequency range (up to 100Hz) of inputs, which have not been properly investigated over the full range in past. To investigate the linear elastic properties of tissues, linear stress strain characteristics of agar, gelatine and silicone samples are studied by measuring small strain up to 5%. In this range, the phantom is assumed to exhibit linear elastic behaviour. Using Hooke's law, elastic behaviour of phantoms can be characterized by its Young's modulus, and particularly is specific storage and loss moduli. In particular, the viscoelastic behaviour of each material sample is investigated by subjecting the samples by varying frequencies to assess the storage ($E^{'}$) and loss modulus ($E^{''}$), and thus elastic modulus of these materials using two proven measurement methods. Different compositions of each material are used to approximate breast tissues, as well as to determine hyperelastic model parameters of selected material which have appropriate elastic modulus. This work also examines the effect of preload on strain level on each sample and composition, with results compared to real tissue results for the ratio between healthy and tumor tissues at 0.1Hz input frequency. Finally, the work comments on the reliability and repeatability of these materials, particularly with respect to environmental conditions and storage. The fabrication process of breast shaped mimicking phantom of selected material is presented for completeness and repeatability.

2.0. Materials and methods

To determine the elastic properties of agar, gelatine, and silicone sample tests were conducted using a MTS Criterion model C43.104 (MTS Systems Corporation, USA) and the dynamic viscoelastic properties of agar, gelatine, and silicone samples testing were conducted using a Q800 Dynamic Mechanical Analysis (DMA) (TA Instruments, USA). Procedures for sample preparation and testing are described in the following sections.

2.1. Sample preparation

Three different "tissue equivalent" material samples were prepared for testing mechanical properties. Each material is commercially available, but may require mixing. The key materials used were agar, gelatine, and silicone. Sample fabrication procedures were followed from published reports [32-34].

While the literature provides a range of formulations for similar tissues in these materials [35-37], they were varied here to better obtain the desired properties. In particular, for Agar, the amount of solution (n-propanol and deionized water) containing agar powder (concentration ranges from 2-6 g) is varied. For silicone, the composition is varied by changing the percentage of silicone used from 40-299 g. For gelatine, a bloom value of 125 is used instead of 200, as is used in most literature cases the authors are aware of [38, 39], which should provide more accurate outcome elastic moduli in this case.

Each different recipes material were poured by injecting a same amount of prepared solution into a cylindrical Perspex mould. When the samples cured, the bottom plate was carefully removed and unmould the samples with a uniform thickness and flat, smooth surface. Each material had three different recipes created to mimic skin, adipose, and much stiffer tumour tissue. The specific materials and constituent quantities are described in Table 1.

Samples size for the MTS compression tests were 30 mm diameter to fit pre-existing moulds and 12.5 mm thickness to match the ASTM standard D 3767. For dynamic measurements, samples were 30 mm in diameter and 5 mm thick for silicone and gelatine. However, 30 mm diameter and 1 mm thickness for agar was used to avoid sliding on the test system compression plate. These sample sizes are the recommended dimensions from the DMA system user manual [40]. Figure 1 shows some typical samples.

2.2 Mechanical property testing

Compression tests of all three groups of materials were performed using MTS and DMA with preloads of 0.05 N, 0.1 N, and 0.2 N at room temperature (18 ^{o}C to 20 ^{o}C). These preloads were applied to improve contact between the compression plate and the surface of the samples. Using the pre-existing mould, there were 10 fabricated samples for each recipe used to mimic skin, adipose and tumor tissues. For agar and gelatine, a fresh sample was used for each experiment, to avoid any time history dependent effects on their viscoelasticity. To ensure measurement reliability, each sample was tested five times and the average of the resulting elastic and storage moduli were calculated for each material.

To determine the hysteresis curve, a quasi-static uniaxial compression loading and unloading applied under a strain rate of +/- 0.5mm/min was performed. The elastic modulus of each sample was calculated during this quasi-static uniaxial compression. A 100 N load cell was used with the MTS machine to perform the tests. Due to the brittle nature of agar, initial testing above 15% of strain damaged the samples, so compression tests were stopped at 15% strain for this material. While silicone and gelatine are more ductile, the compression tests were also stopped at 15% strain to simplify comparisons with agar samples.

DMA testing is used to characterize the viscoelastic behavior of materials. To measure the storage modulus, oscillatory tests using a frequency sweep method at room temperature was applied to each sample. The frequency was varied from 0.1-100 Hz with increments of 12 Hz for gelatine and silicone, and 0.1-50 Hz with increment 25 Hz for agar due to water content beyond 50 Hz samples slides.

Polymers like agar, gelatine and silicone are nonlinear elastic behaviour is represented by a hyperelastic model. Hyperelastic models namely, Neo-Hookean, Mooney Rivilin and Ogden models are fitted to measure the nonlinear characteristics of selected phantoms materials which provides insight into tissue stress strain curve nonlinearities. The parameters of hyperelastic are extracted which could be imaged using nonlinear elastography.

Neo-Hookean (NH) is the simplest hyperelastic model which is the reduced version of the Mooney Rivlin model. The stress-strain relationship is derived from strain density energy function denoted W. For the Neo-Hookean model, the strain density energy is given by the following equation:

$$W = C_{10}(I_1 - 3) \text{---------------} (1)$$

Where I_1 is the first deviatoric strain invariant and C_{10} is the material constant which is related to shear modulus.

Mooney Rivlin [41, 42] is the material model to represent incompressible, isotropic and elastic materials. For the Mooney Rivlin model, the strain density energy is given by the following equation:

$$W = C_{10}(I_1 - 3) + C_{01}(I_2 - 3) \text{---------------} (2)$$

Where C_{10} and C_{01} are the material constant for a 2 parameter model which are determined empirically.

The Ogden model [43] is popular used to fit isotropic biological tissue. Ogden strain density energy function is written in terms of principal stretches instead of the invariants. For the Ogden model, the strain density energy is given by the following equation:

$$W = \sum_{r=1}^{N} \frac{\mu_r}{\alpha_r}(\lambda_1^{\alpha_r} + \lambda_2^{\alpha_r} + \lambda_{13}^{\alpha_r} - 3) \text{---------------} (3)$$

Where $N = 1,2,3,...$ and λ is the stretch ratio and μ_r and α_r are constants. The initial shear modulus is given as $2\mu = \sum_{r=1}^{N} \mu_r \alpha_r$.

3.0. Results and Discussion

The elastic and viscoelastic properties of skin, adipose, and tumour sample made from all three materials are compared with preloads 0.05 N, 0.1 N, and 0.2 N. Elastic modulus, and storage modulus and loss modulus, values for agar, gelatine, and silicone are summarized in Tables 2, 3 and 4. Mechanical properties, of real breast tissue as reported in the literature and mimicking materials with adipose to tumor ratio is summarized in Table 5 for comparison.

3.1. Effects of preload on strain

In biological tissue the Young's Modulus cannot be assumed constant as a function of preload. The results in Table 6 show the preloads effects on strain on each sample of agar, gelatin and silicone material. At all preload 0.05 N, 0.1 N and 0.2 N pre-compression strain ranges 0.3% - 4.8%, 0.3% - 6% and 0.3% - 14.6% for agar, gelatine and silicone respectively. It can be noted that agar material is stiffer as compared to silicone and gelatine. For all three materials, each adipose sample tissues have higher pre-compression strain percentage as compared to tumor sample tissues because adipose sample tissues are softer than tumor tissue.

3.2. Elastic characterization using MTS

Figure 2 shows the upper and lower portions of quasi-static uniaxial compression loading and unloading cycle of all three material samples. Hysteresis curves show each material is viscoelastic. The energy dissipated during the loading-unloading cycle is given by the area within the loop. Due to the more ductile nature, gelatine and silicone materials show more energy dissipation than agar.

For elastic modulus measurements, only the loading part was considered. The elastic modulus of all three materials were calculated from the initial linear region up to 5% strain.

Figure 3 shows the stress strain behavior of agar, gelatine and silicone samples for skin with preloads of 0.05 N, 0.1 N, and 0.2 N from which elastic modulus can be determined. Elastic moduli for agar and gelatine for mimicking skin sample increases with the increase of preload. However, the elastic modulus for silicone material for skin is independent of preload up to 0.2 N. This means that a large preload would be required to achieve a greater elastic modulus. The elastic moduli of the skin samples for each group of materials are shown in Table 2. Mechanical properties of human skin depend upon skin thickness, stress applied to the skin during experiments, types of experiments, sex, and age. Elastic moduli of human skin measured in-vivo varies largely from 0.02 MPa to 57 MPa [44]. To obtain mechanical properties of human skin in-vitro is not easy because removing it from its natural environment may cause changes in the mechanical properties.

Figure 4 shows the elastic moduli of agar, gelatine and silicone adipose samples with preloads of 0.05 N, 0.1 N, and 0.2 N. The elastic modulus for silicone remain almost constant for adipose samples with increasing increments of preload, similar to results in Krouskop et.al, where breast adipose tissue has a constant modulus over the strain range [21]. For agar, elastic moduli remains constant for preload 0.05 N and 0.1 N, and then increases with preload 0.2 N. In addition, adipose sample for gelatine significantly decreases with increasing of preload. This means that agar and gelatine are dependent on preload, unlike silicone samples. Results are shown in and Table 2.

Figure 5 shows the elastic moduli of agar, gelatine and silicone tumour sample with preloads of 0.05 N, 0.1 N, and 0.2 N. The stiffness ratio of adipose to tumour tissues plays an important role in how detectable the tumour is using elastography. The elastic modulus of tumour tissues is much greater than adipose tissues [21]. All three tested materials of tumour samples have greater elastic moduli than adipose materials, as shown in Figure 5. The elastic modulus of tumour samples of all materials are preload dependent.

For preloads 0.05 N, 0.1 N, 0.2 N, the adipose to tumour tissues ratio of silicone materials are 1:14, 1:10, and 1:18, for gelatine they are 1:2, 1:3, and 1:5, and for agar they are 1:5, 1:9, and 1:2. The ratios for silicone materials are better than those for gelatine and agar, as seen in Table 2 because the elastic modulus ratio of adipose to tumour tissue ranges from 1:5 to 1:15 [21, 45].

3.3. Viscoelastic characterization using DMA

The viscoelastic behaviour of soft tissues can be measured by applying a periodic compression to a uniform thickness and cross sectional cylindrical/disc sample. The complex elastic modulus is given by

$$E^* = E' + iE'' \qquad (1)$$

The real part of the complex modulus (E') is storage modulus, representing the elastic portion, as it measure the stored energy. The imaginary part (E'') is the loss modulus, representing the viscous portion, as it measure the amount of dissipated energy. The storage and loss modulus were obtained by using frequency sweep method. Storage and loss modulus together help define the overall stiffness and dissipative properties of the material.

The results of frequency sweep tests on the DMA for all three materials are shown in Figures 6-8 with preloads 0.05 N, 0.1 N, and 0.2 N. These figures show all the measured values of storage modulus and loss modulus by varying the frequency from 0.1-100 Hz for gelatine and silicone, and 0.1-50 Hz for agar. Tables 3 and 4 show the storage modulus and loss modulus at 0.1 Hz frequency for all three tested materials.

Figure 6 shows the storage and loss modulus of agar, gelatine and silicone skin samples with preloads of 0.05 N, 0.1 N, and 0.2 N. The storage modulus at 0.1Hz of agar and silicone skin samples gradually increases with preloads of 0.05 N, 0.1 N, and 0.2 N. Whereas storage modulus for gelatine remains constant at 0.1Hz with all three preloads. It is noted that storage modulus of gelatine sample for skin remain stable with frequency over the range of 0.1-10 Hz and unstable near to 100 Hz for every preload value. The storage modulus remains almost same with frequency from 1-90 Hz for silicone, and shows small instability near to 100 Hz for every preload value. In addition, agar sample for skin, the observed storage modulus gradually increases with frequency from 1-10 Hz and then decreases near to 50 Hz.

Figure 7 shows the storage and loss modulus of agar, gelatine and silicone adipose samples with preloads of 0.05 N, 0.1 N, and 0.2 N. The storage modulus at 0.1Hz for gelatine and silicone adipose samples remains constant for all three preloads, whereas, agar adipose sample gradually increases with increasing of preload. The storage modulus for gelatine and silicone for adipose sample remains stable with frequency over the range of 0.1-70 Hz with preload 0.05 N, 0.1 N, and 0.2 N. Agar sample for adipose are gradually increases with frequency range 0.1-25 Hz with all three applied preloads.

Figure 8 shows the storage and loss modulus of agar, gelatine and silicone tumor samples with preloads of 0.05 N, 0.1 N, and 0.2 N and shows greater storage modulus for the tumour sample compared to the skin and adipose sample for all three materials. In addition, as the sample is more compressed, the storage modulus increases at frequency 0.1Hz. Gelatine material for tumour sample show stable results with frequency range of 0.1-10 Hz, but become unstable near to 100 Hz. For agar, tumour sample gradually increase from 0.1-25Hz and decreases near to 50 Hz. There is no noticeable variation in storage modulus of silicone tumour samples with frequency.

The storage modulus ratios over all three preloads of adipose to tumour sample for silicone materials are 1:5, 1:11, and 1:12, for gelatine are 1:3, 1:3, and 1:11, and for agar are 1:4, 1:8, and 1:3. The ratios for silicone materials are better than those for gelatine and agar, due to relatively close to what has been reported in real breast tissues as seen in Table 5.

It can be noted that storage modulus is always ~20 times larger than the loss modulus for all frequencies. This result is similar to the behaviour of biological tissues [21]. For an agar concentration of 2g (adipose tissue) and 4g (skin tissue) at preloads of 0.05 N, 0.1 N and 0.2N the loss modulus values are uniformly lower than 30 kPa, which represents low amount of energy damping for agar samples but matches results in [46]. In general, the loss modulus increases with the increase of agar and gelatine concentration. For silicone, the loss modulus of adipose sample is smaller than in the skin and tumor sample, which matches expectations and prior reports.

Mechanical properties, of real breast tissue as reported in the literature and mimicking materials with adipose to tumor ratio is summarized in Table 5 for comparison. The absolute values of elastic modulus and storage modulus are not very comparable to that of real tissue, because of the different precompression and preload values applied in their experimental methods and also different measurement methods [21, 45]. It is obvious that real human breast tissues are more complex and heterogeneous, thus it is very hard to achieve the same absolute values. However, the ratio of moduli for adipose to tumour sample in this work compares very well to that of real breast tissue.

According to [21], adipose breast tissue has a constant modulus over the strain range and the elastic modulus of tumour tissues is highly dependent on the level of tissue precompression used in the measurement. For example, the elastic modulus of tumour tissue was found to be 5 and 15 times larger than that of normal adipose tissue when applying precompression levels of 5% and 20%, respectively. This dependence confirms the nonlinear elastic behavior often observed in biological tissues [21].

Similarly, the gelatine and silicone adipose sample data in this study has almost constant storage modulus at all preloads, and tumour samples were also similarly dependent on the preload. However, the effect of preload on agar samples are unlike silicone and gelatine. At all agar concentrations (2-6 g) there is a significant variation in storage modulus at 0.1 Hz frequency at all three preloads. In summary, agar materials can be used as tumour tissue with gelatine concentration to obtain the right material properties and ratios.

Overall, elastic moduli and storage moduli of all three materials in mimicking of skin, adipose and tumor tissues is summarized in Tables 2 and 3. It is notable that for agar and gelatine, the mechanical properties depend on the concentration. Thus, increasing the agar and gelatine concentration increases the stiffness of the sample. This control allows different tissue types to the mimicked based on their different tissue stiffness in vivo. In gelatine based phantoms, apart from increasing the concentration of gelatine, it is possible that the use of formaldehydes to increase the melting point of the gel simultaneously increases the resulting material stiffness, as formaldehyde can be used to increase cross-linking among collagen fibres. Gelatine modulus and mechanical properties depend not only on the dry-weight concentration used in the mixture, but also on the Bloom value of the gelatine used. Agar mechanical and imaging characteristics can be achieved similar to those of soft tissues by adjusting concentration and amount of liquid solution, where its main limitation is its low toughness, making it fragile during handling. For silicone, the properties are a function of the creation process, such as curing time and the amount of silicone used as a percentage. The results presented provide guideline ranges for custom tailoring the material properties towards the intended values and/or ratio of tumor to healthy tissue properties, where specific outcomes or formulations depend on the range of factors presented. However, overall, the results show what is possible and how these properties vary across frequencies not typically considered in these phantom materials. All material samples were stored in a controlled environment to avoid dehydration during experiments.

3.4. Hyperelastic characterization

The stress strain curve of polymers material have two region; an elastic which is the initial portion of the curve and a hyperelastic region where material exhibits more stress for a small increment in strain. After results and discussion silicone material is selected because of its appropriate elastic and viscoelastic properties to find hyperelastic parameters by fitting uniaxial compression experimental data of skin, adipose and tumor sample tissues into Neo-Hookean, Mooney Rivlin and Ogden models and the results are shown with mean experimental stress strain data in Figure 9 and computed parameters with goodness of fit (R^2) are shown in Table 7.

The Ogden and Mooney Rivlin model appeared to be the most suitable choice for predicting the behaviour of given silicone composition because of its ability to match experimental data points at small and large strain values. It can be noted that the goodness of fit (R^2) of Neo-Hookean model is less than the other two models. Since we consider uniaxial study isotropic material, The Mooney Rivlin model could be used for fitting the experimental curve of the prepared skin, adipose and tumor tissue samples.

Overall, we established hyperelastic models for characterizing constitutive relations of silicone based samples and computed parameters. These parameters could be used as input in finite element hyperelastic simulation of silicone based breast phantoms and modelling of silicone breast tissue.

3.5. Environmental Effects on Samples

When agar material samples were stored in a controlled environment at approximately 20 ^{o}C, within a week all samples developed a fungal growth. This growth is due, in part, to the availability of water in the samples. Agar is more brittle than gelatine and silicone and has a relatively small elastic region. Thus, different concentrations of agar in solution results in increased storage modulus and elastic modulus.

Gelatine samples for skin, adipose, and tumour were mixed with different water and oil concentrations to change the elastic modulus and storage modulus values. Gelatine materials show appropriate mechanical properties in terms of stability in the storage modulus with the range of frequencies in DMA testing. However, because of its high water content, relative humidity will effect mechanical properties over time, as evaporation over longer term storage means its mechanical properties change. Thus, such a phantom cannot be used for long term, reuse, and comparison.

Silicone materials are thus more attractive than agar and gelatine because of their stable material properties and fungal resistance. Greater consistency means they can be used for long term testing and reuse. Hence, they could be used for repeated testing over longer periods, so different elastographic systems could be compared with the same ground-truth. Figure 10 shows the environmental effects on agar, gelatine, and fungal resistance silicone samples.

In summary, the overall advantages and disadvantages of each material are outlined in Table 8.

3.6. Breast shaped silicone phantom fabrication

Symmetric breast shaped phantom was fabricated by using core and cavity mold. For skin total amount of 55g of 100% A341 solution has been used. Vacuum chamber was used to remove air bubbles from the silicone. The solution is poured onto the breast shaped cavity and the mold is placed on top of it. This produces a thin and uniform 1 mm thick skin layer. The skin is cured at 60 ^{o}C within an hour.

Once skin layer is cured the core can be removed and Perspex plate is placed on top of the cavity with a support of two plates. The nylon bolts are attached for ease of handling and clamping during experiment on DIET machine.

For adipose 192g of A341 and 299g of DC 200 solution has been used and mixed properly. The mixture is then placed inside the vacuum pump to remove bubbles and then poured into the cavity mold. The adipose material is cured at 60 ^{o}C within two hours. Once the adipose silicone is cured, the breast shaped phantom can be easily unmold from the cavity.

For tumours, 40g of A341 and 60g of LSR-05 was used. We fabricated three different sizes of tumour in a spherical shape of 20 mm, 10 mm and 5 mm diameter. Both materials are mixed well and placed inside the vacuum pump to remove bubbles. The mixture is then poured with injection into the tumour mould and then placed inside the oven to cure at 60 ^{o}C within three hours. The tumour then suspended from a support in by a wire. Figure 11 shows the overall procedure of fabricated phantom.

3.0. Conclusion

Human breast tissues are complex and replicating their mechanical properties in the laboratory can be very challenging. Our aim was to find the most suitable commonly available material to mimic breast tissue, for use in elastography. In summary, all presented materials are suitable for tissue-mimicking phantoms under different conditions. However, there are various drawbacks of agar regarding its utility in stable homogenous phantoms: (1) Agar is a brittle gel that can fracture with moderate strains; and (2) it exhibits a much more rapidly increasing elastic modulus with preload than normal breast tissues, where, in contrast, agar and gelatine include over 80% of water and have a stiffness similar to that of soft tissue. Thus, agar, and also gelatine can have limited durability due to environmental variations and thus may not be suitable for repeated application over several days or weeks.

Silicone is a soft tissue material with an appropriate elastic modulus ratio of adipose and tumour sample. It is easy to prepare samples with a range of mechanical properties and it is fungal resistance. Measured elastic modulus of silicone samples ranges from 5-112 kPa and storage modulus ranges from 5-73 kPa for all normal and tumour samples. The stiffness ratio of adipose to tumour tissue of silicone samples ranges 1:5-1:12 compared with the real human breast tissues ranges 1:1-1:15. Additionally, experimental data from uniaxial compression tests of skin, adipose and tumour tissue samples were obtained to input into finite element commercial software in order to calibrate hyperelastic model coefficients for given silicone behaviour. Silicone does not contain any water content, which means that relative humidity will not affect the mechanical properties.

4.0. References

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Figures:

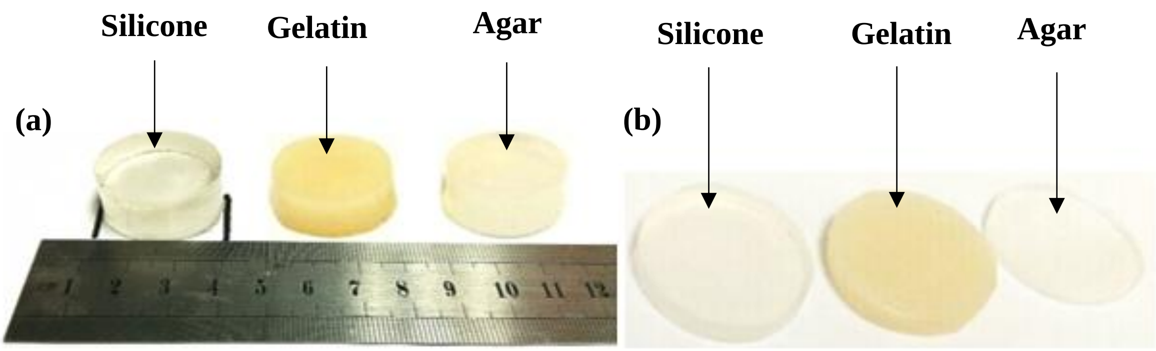

Fig. 1 - Samples size (a) for static property testing using MTS-43, and (b) viscoelatic property testing using DMA

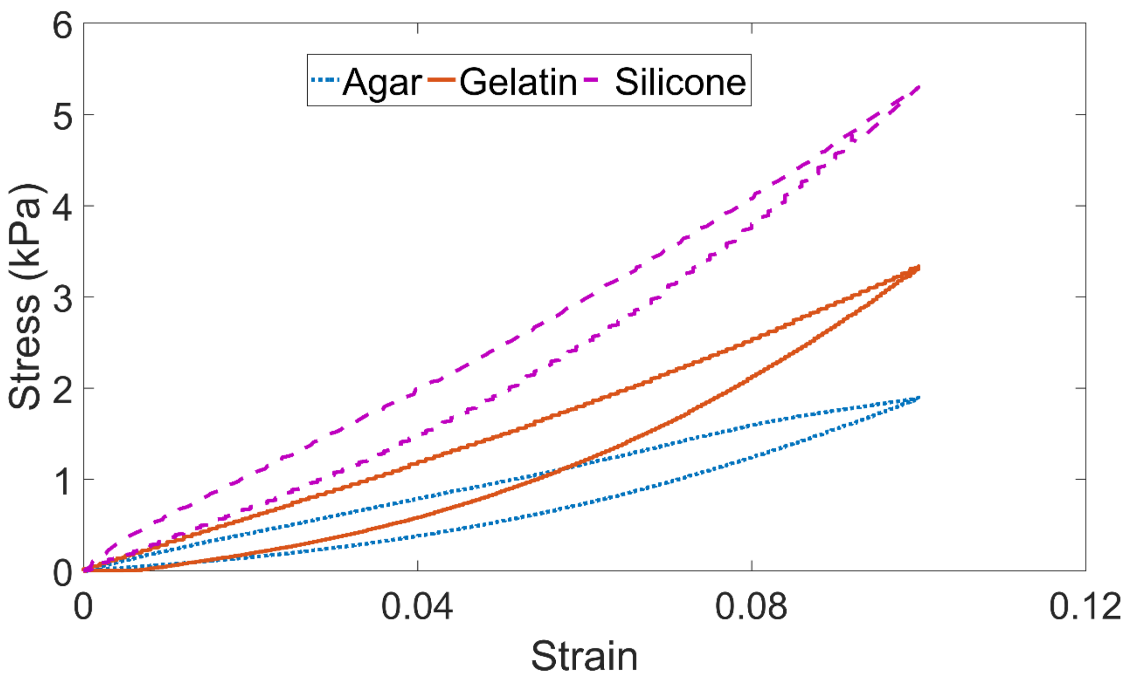

Fig. 2 - Hysteresis curve of agar, gelatine, and silicone for initial testing

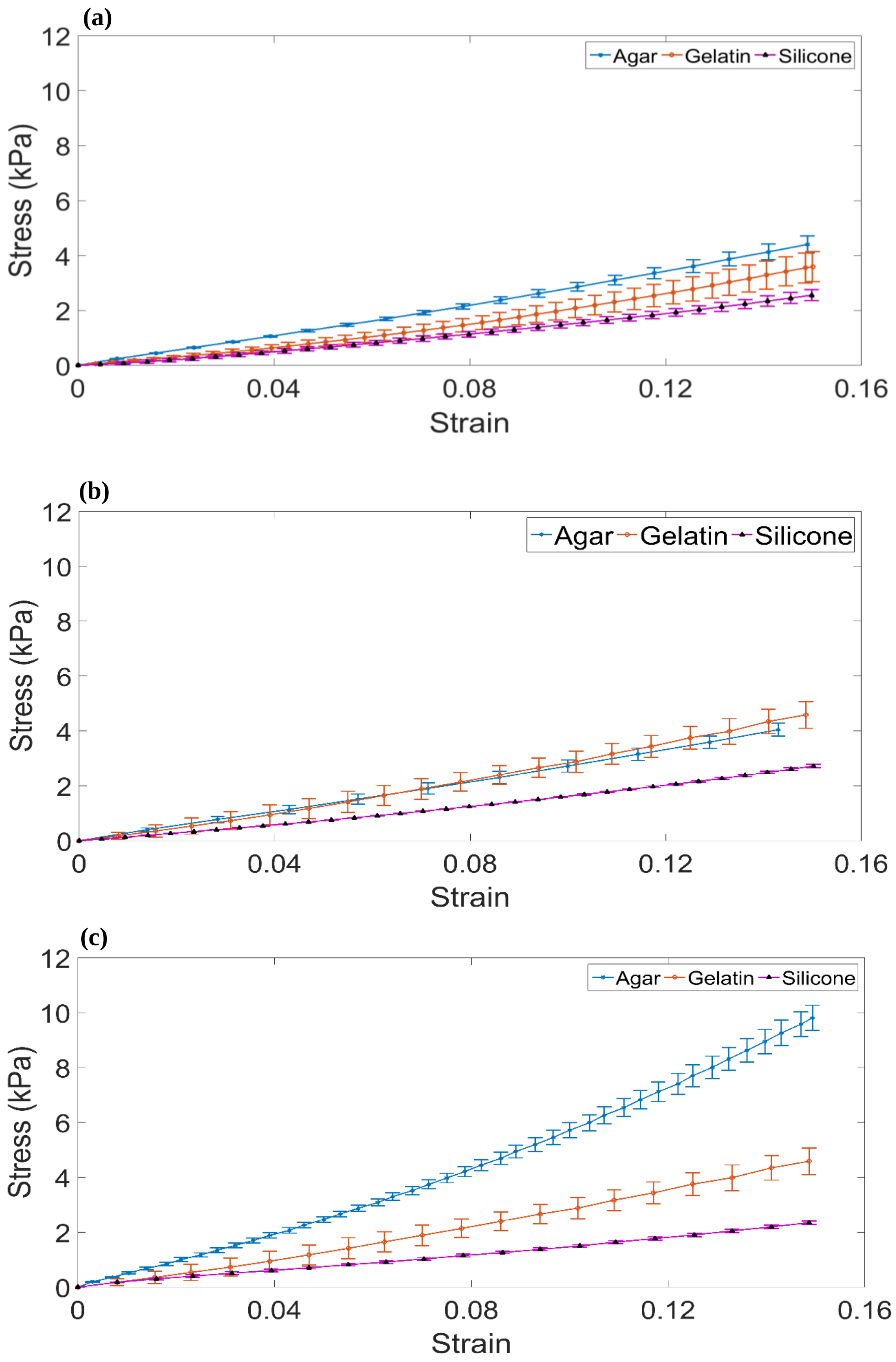

Fig. 3 - Stress-strain behavior for all three materials for skin sample with: (a) preload 0.05 N (b) preload 0.1 N, and (c) preload 0.2 N

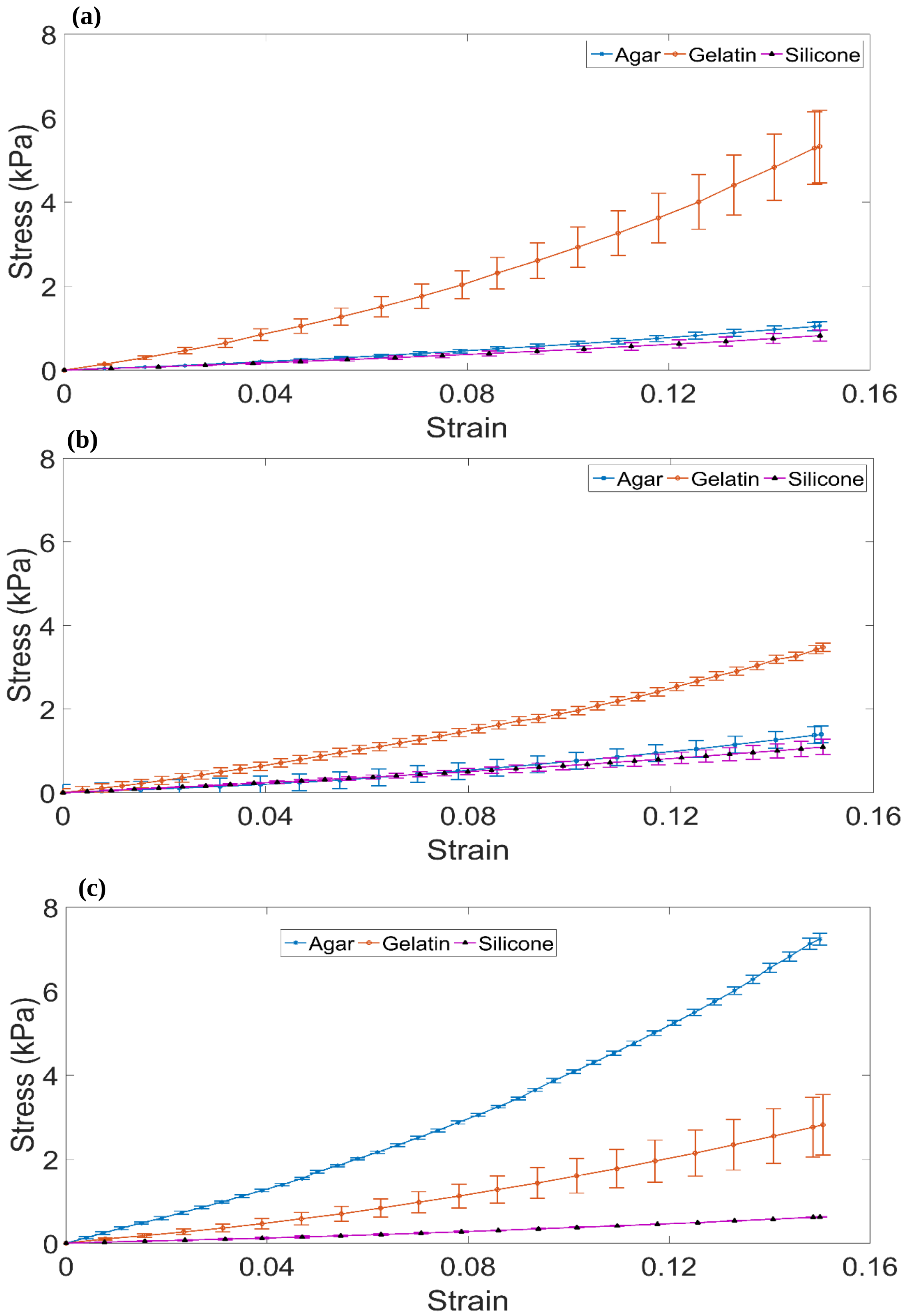

Fig. 4 - Stress-strain behavior for all three materials for adipose sample with: (a) preload 0.05 N (b) preload 0.1 N, and (c) preload 0.2 N

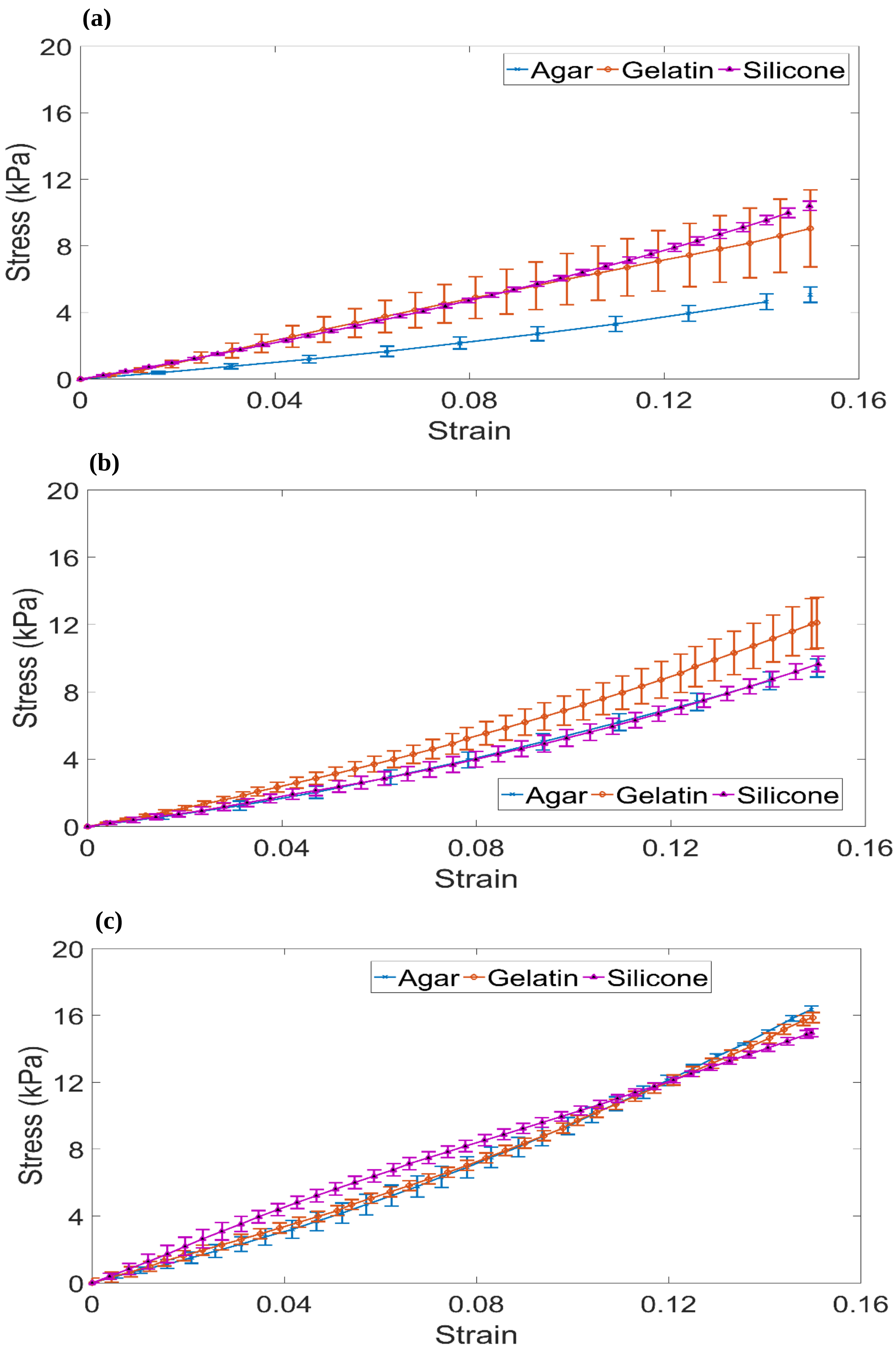

Fig. 5 - Stress-strain behavior for all three materials for tumour sample with: (a) preload 0.05 N (b) preload 0.1 N, and (c) preload 0.2 N

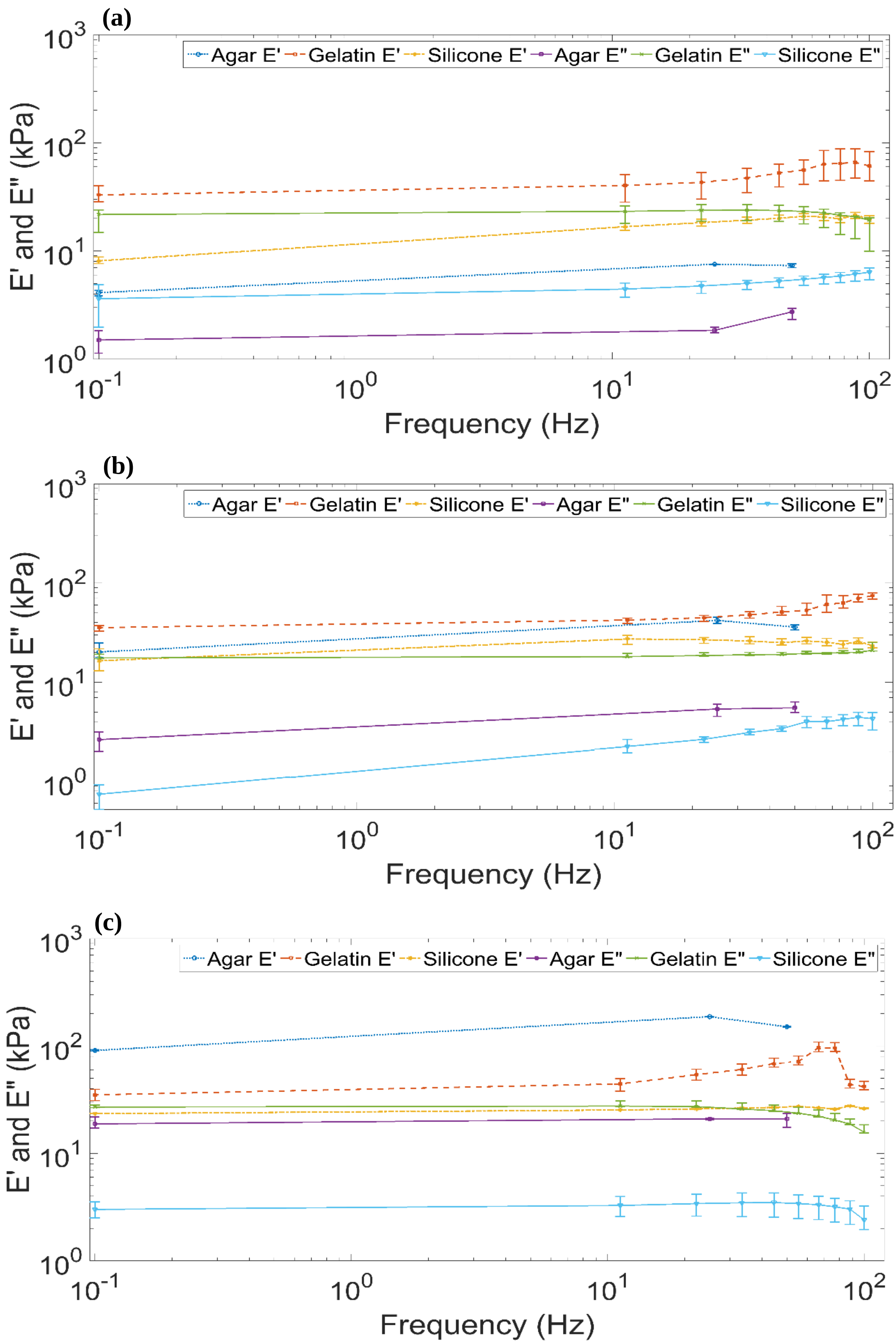

Fig. 6 – Variation of storage and loss modulus for all three materials for skin sample with: (a) preload 0.05 N (b) preload 0.1 N, and (c) preload 0.2 N

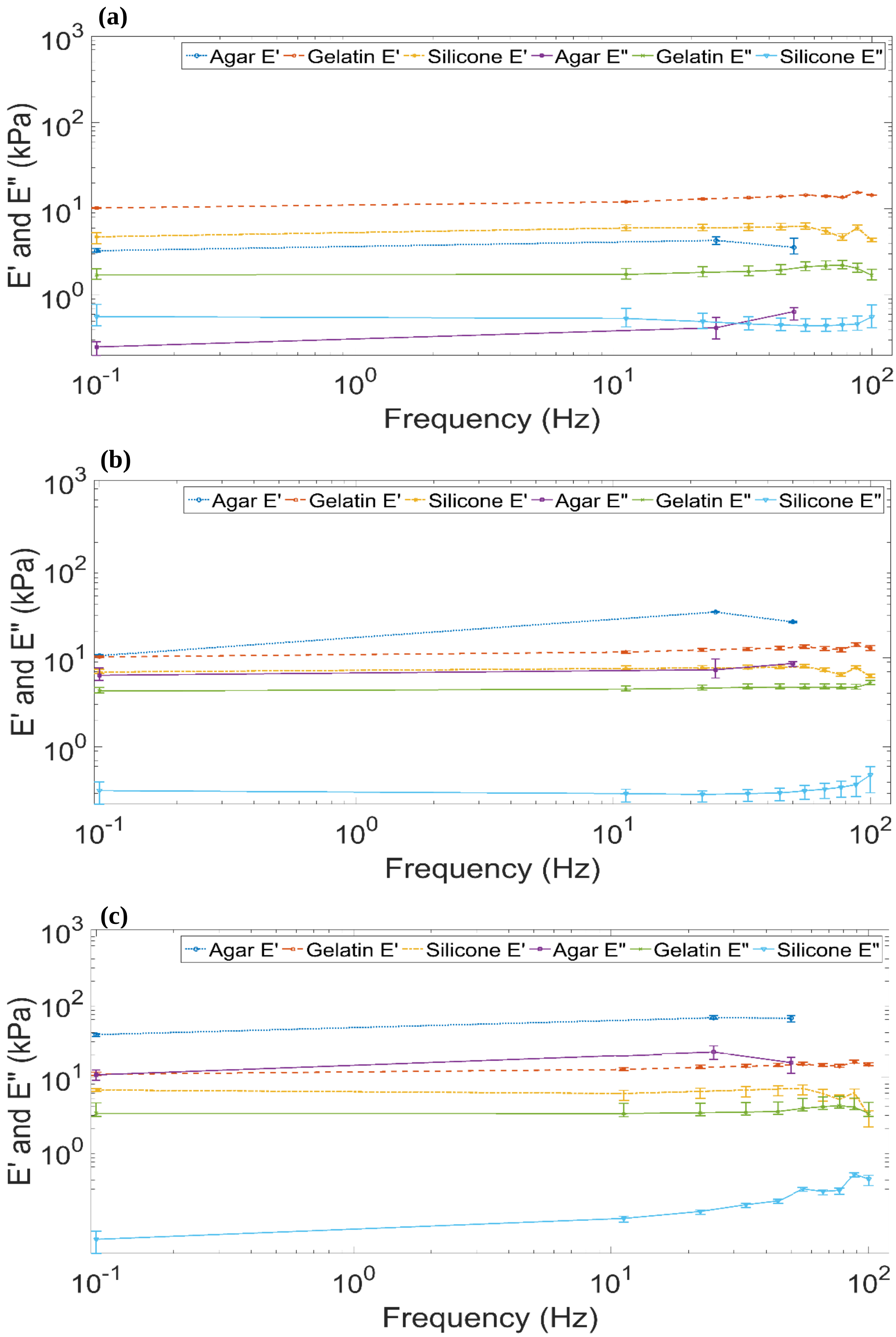

Fig. 7 – Variation of storage and loss modulus for all three materials for adipose sample with: (a) preload 0.05N (b) preload 0.1N, and (c) preload 0.2N

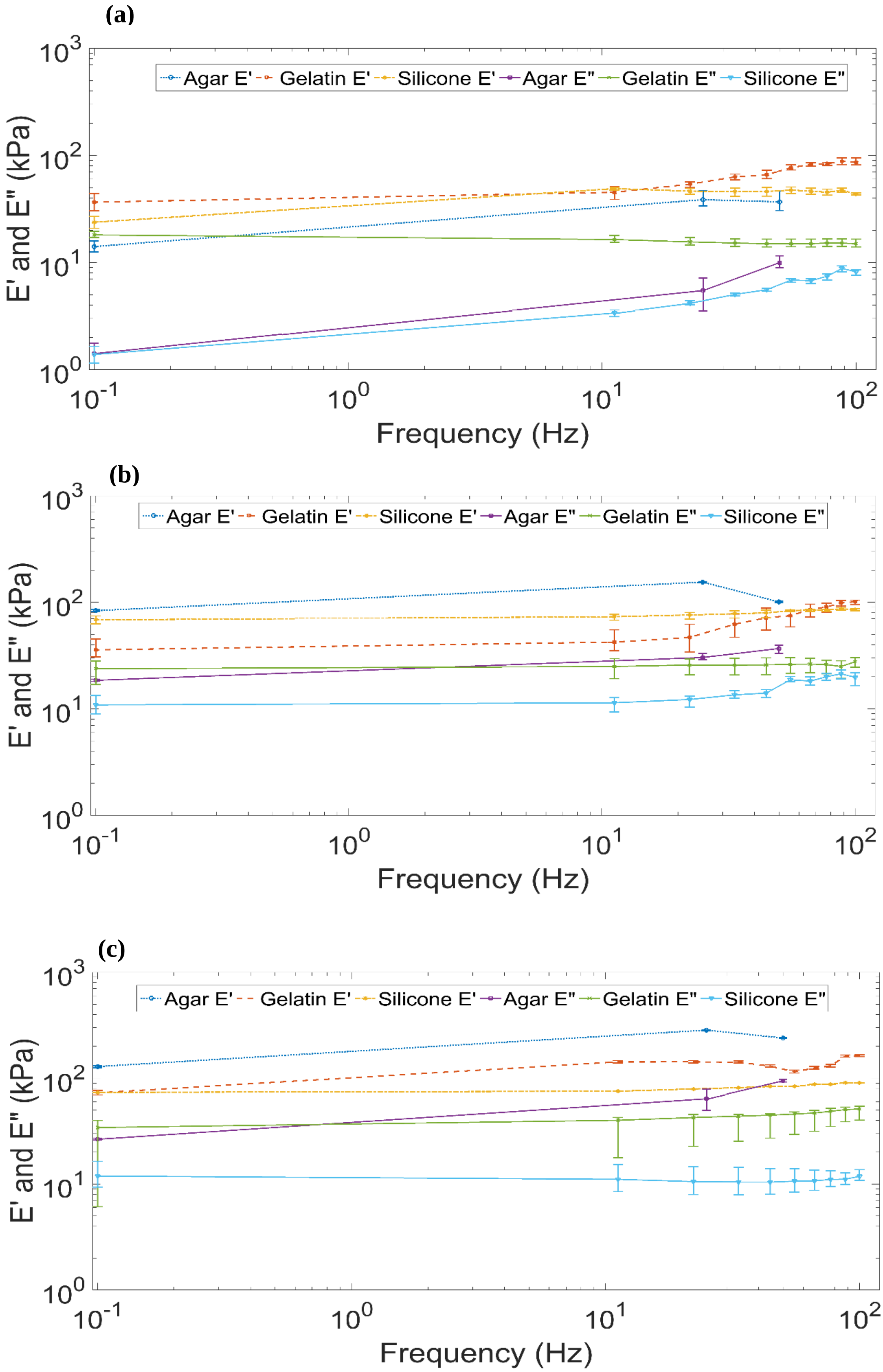

Fig. 8 – Variation of storage and loss modulus for all three materials for tumour sample with: (a) preload 0.05 N (b) preload 0.1 N, and (c) preload 0.2 N

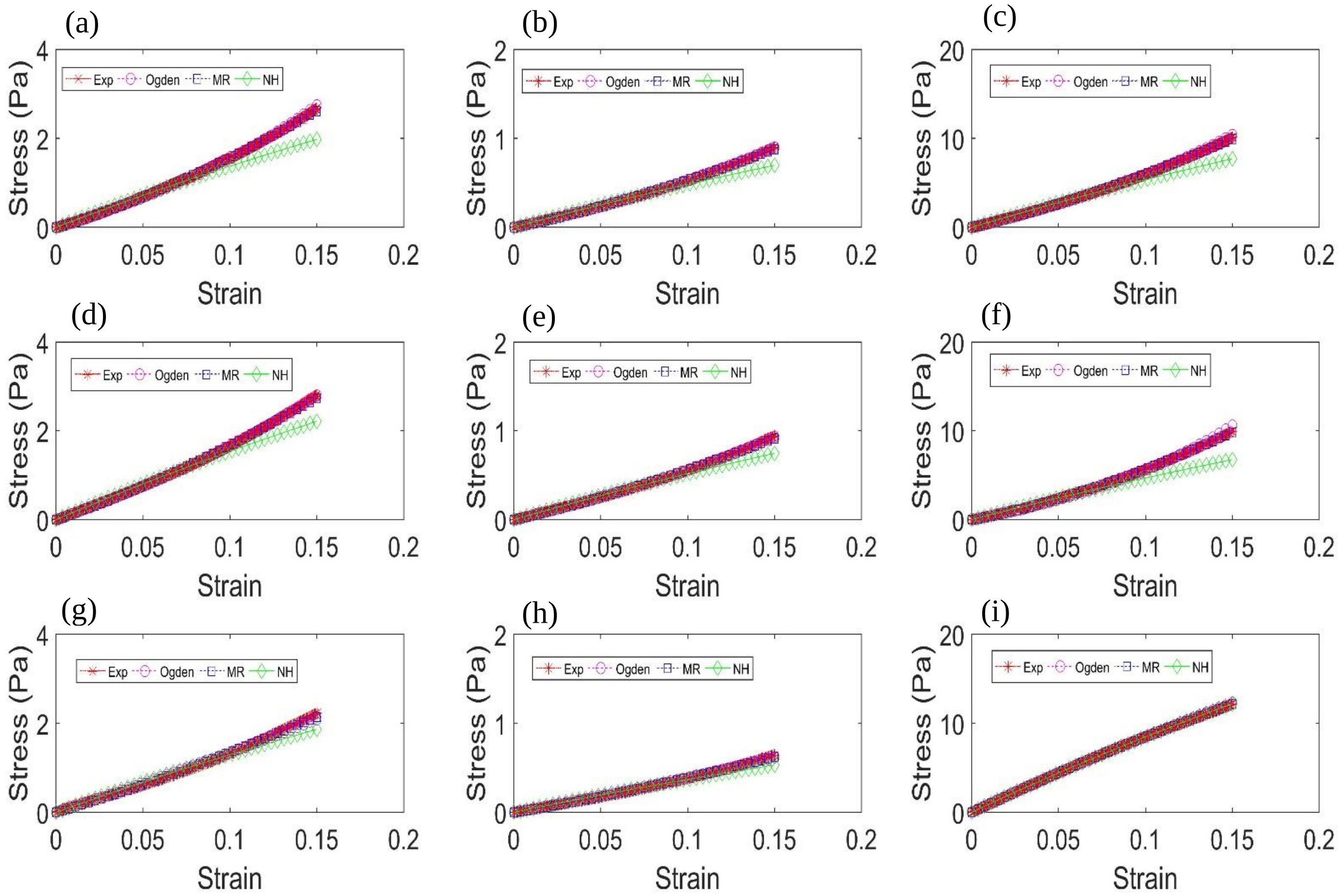

Fig. 9 - (a–i) Neo-Hookean(NH), MooneyRivlin(MR), Ogden models are fitted to experimental stress strain datafor Silicone (a,d,g) Silicone skin tissue sample preload 0.05, 0.1, 02 N (b,e,h) Silicone adipose tissue sample preload 0.05, 0.1, 02 N (c,f,i) Silicone tumor tissue sample preload 0.05, 0.1, 02 N

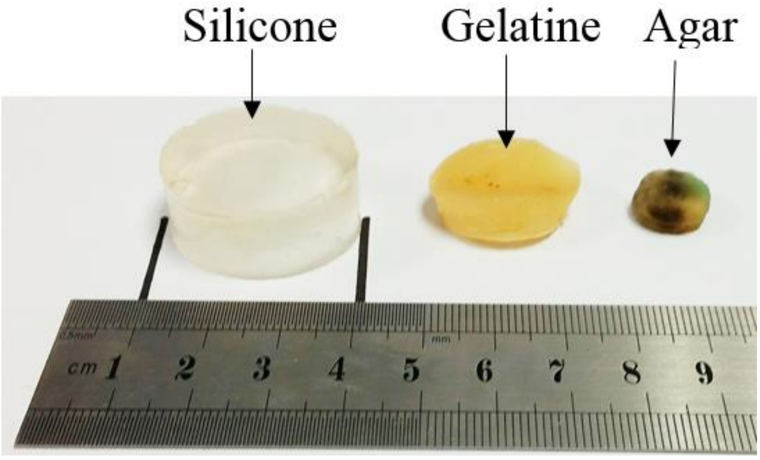

Fig. 10 - Environmental effect on samples

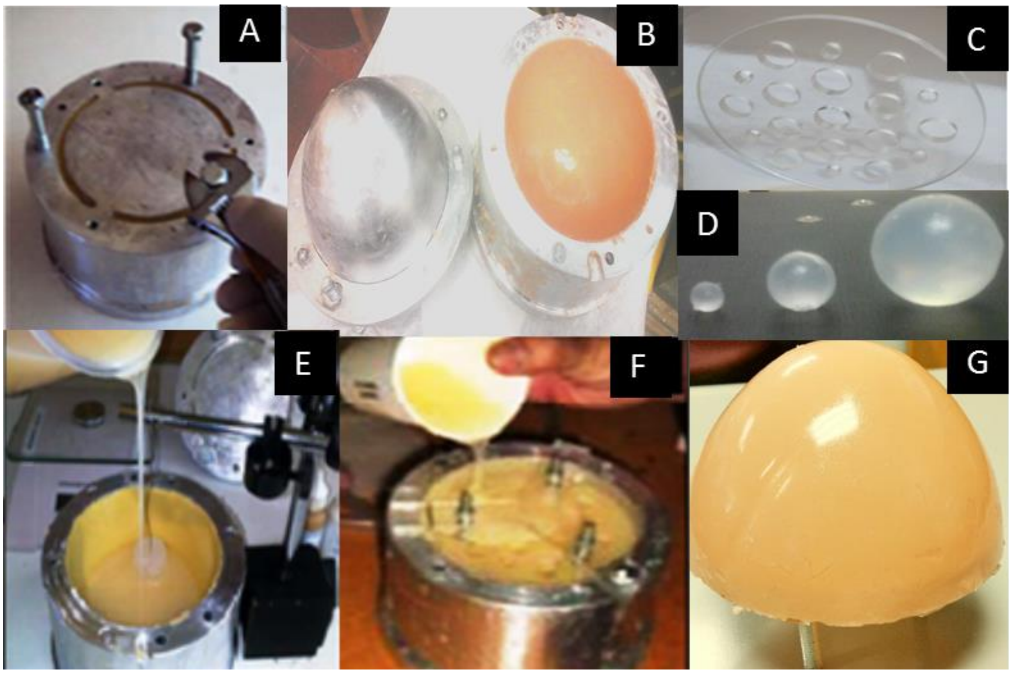

Fig. 11– Fabrication process of silicone breast shaped phantom: (A) Procedure of core extraction (B) Cured skin layer (C) Perspex plate (D) Tumor with different diameter sizes (E) Placing a tumor with a wire (F) Pouring adipose silicone (G) Fabricated breast shaped phantom

Tables:

Table 1 - Material quantities for mimicking different tissue

	Agar [33]	Silicone [32]	Gelatin [34]
Skin	• Agar = 4 g • n-propanol = 20 ml • Deionized water = 97 ml	• SoftGel A-341C = 55g	• p-toluic acid = 0.294 g • n-propanol = 28.69 ml • Deionized water = 279.5 ml • 125 Bloom gelatin = 35 g • Formaldehyde (37% by weight) = 3.33 g • Oil (50% Safflower + 50% Kerosene) = 98.6 ml • Detergent = 5.86 ml
Adipose	• Agar = 2 g • n-propanol = 20 ml • Deionized water = 97 ml	• SoftGel A-341C = 192g • DC 200 Silicone (50cst) = 299g	• p-toluic acid = 0.133 g • n-propanol = 6.96 ml • Deionized water = 132.7 ml • 125 Bloom gelatin = 10 g • Formaldehyde (37% by weight) = 1.53 g • Oil (50% Canola + 50% Kerosene) = 265.6 ml • Detergent = 12 ml
Tumour	• Agar = 6 g • n-propanol = 20 ml • Deionized water = 97 ml	• A-341C = 40g • LSR-05 A and B = 60g	• p-toluic acid = 0.346 g • n-propanol = 17 ml • Deionized water =328 ml • 125 Bloom gelatin = 100 g • Formaldehyde (37% by weight) = 3.72 g • Oil (50% Safflower + 50% Kerosene) = 38.4 ml • Detergent = 2 ml

g = gram, ml = milli litre, percentage (%) = by weight, SoftGel A-341C (*Factor II, Inc., USA)*, LSR-05 A and B (*Factor II, Inc., USA)*, DC 200 Silicone *(Dow Corning Corporation, USA)*, Agar *(Sigma-Aldrich, New Zealand)*, n- propanol *(Fisher Chemical, USA)*, p-toluic acid *(Sigma-Aldrich, New Zealand)*, 125 Bloom geletin *(Sigma-Aldrich, New Zealand)*, Formaldehyde *(Sigma-Aldrich, New Zealand)*, Canola *(Pure Oil New Zealand Ltd, New Zealand)*, Kerosene *(Sigma-Aldrich, New Zealand)*, Detergent *(The Sun Products Corporation, USA)*

Table 2 - Static property summary for all tests. Data shown as mean ±SD

Elastic Modulus (E) in kPa									
Preload (N)	**Agar**			**Gelatin**			**Silicone**		
	Skin	Adipose	Tumour	Skin	Adipose	Tumour	Skin	Adipose	Tumour
0.05	26±1	5±0.4	26±5	17±3	23±5	56±2	14±1	5±0.7	57±1
0.1	27±5	5±0.8	46±7	26±4	18±2	63±7	15±0.3	6±0.6	59±9
0.2	49±3	33±0.2	81±10	28±5	17±3	85±19	15±0.3	6±0.4	112±10

Table 3 - Storage modulus summary for all tests at 0.1Hz. Data presented as mean ±SD

Storage Modulus (E') in kPa									
Preload (N)	**Agar**			**Gelatin**			**Silicone**		
	Skin	Adipose	Tumour	Skin	Adipose	Tumour	Skin	Adipose	Tumour
0.05	4±0.2	3±0.1	14±2	32±6	10±0.1	36±7	8±2	5±0.7	24±3
0.1	20±4	10±0.4	83±7	35±2	10±0.2	35±8	16±4	6±0.4	68±5
0.2	91±10	37±2	128±15	35±4	10±0.6	109±9	23±0.01	6±0.3	73±0.08

Table 4 – Loss modulus summary for all tests at 0.1Hz. Data presented as mean ±SD

Loss Modulus (E'') in kPa									
Preload (N)	**Agar**			**Gelatin**			**Silicone**		
	Skin	Adipose	Tumour	Skin	Adipose	Tumour	Skin	Adipose	Tumour
0.05	2±0.3	0.3±0.04	1.4±0.3	22±4	2±0.8	18±0.8	4±1	0.6±0.1	1.4±0.2
0.1	3±0.5	6±1	18±0.4	17±2	4±0.6	24±5	0.7±0.2	0.3±0.08	11±2
0.2	19±3	11±2	26±0.6	27±1	3±1	34±5	3±0.5	0.06±0.02	12±4

Table 5- Mechanical property summary of real breast tissues from literature and mimicking materials at 0.1 Hz

	Pre-compression/Preload	Adipose	Tumor	Adipose/tumor ratio
Real Breast Tissues [21]	5%	18±7	22±8	1:1
	20%	20±8	291±67	1:15
Real Breast Tissues [45]	3 g	3.25±0.9	16.38±1.5	1:5
Agar	0.05N	3±0.1	14±2	1:5
	0.1N	10±0.4	83±7	1:8
	0.2N	37±2	128±15	1:3
Gelatin	0.05N	10±0.1	36±7	1:3
	0.1N	10±0.2	35±8	1:3
	0.2N	10±0.6	109±9	1:11
Silicone	0.05N	5±0.7	24±3	1:5
	0.1N	6±0.4	68±5	1:11
	0.2N	6±0.3	73±0.08	1:12

Table 6-Effects of preload on samples

	Pre-compression Strain %								
Preload (N)	**Agar**			**Gelatin**			**Silicone**		
	Skin	Adipose	Tumour	Skin	Adipose	Tumour	Skin	Adipose	Tumour
0.05	0.4	1	0.3	0.3	1.5	0.3	0.3	3.3	0.5
0.1	1	2.2	0.5	0.6	3	0.6	0.5	7.3	1.4
0.2	2.2	4.8	1.1	1.2	6	1.3	1	14.6	3

Table 7 - Summary of hyperelastic models for silicone material

Preload	Models	Skin	R^2	Adipose	R^2	Tumor	R^2
0.05 N	**Ogden (kPa)** μ_1	4.11	0.993	1.49	0.998	16.22	0.994
	α_1	11.12		9.61		10.52	
	MR (kPa) C_{10}	12.83	0.998	4.08	0.998	46.78	0.998
	C_{01}	-10.04		-3.41		-39.28	
	NH (kPa) C_{10}	2.51	0.986	0.88	0.992	9.79	0.988
0.1 N	**Ogden (kPa)** μ_1	4.79	0.996	1.62	0.996	13.43	0.989
	α_1	9.17		8.87		13.35	
	MR (kPa) C_{10}	11.95	0.998	4.00	0.998	55.73	0.998
	C_{01}	-9.75		-3.26		-49.74	
	NH (kPa) C_{10}	2.81	0.993	0.94	0.994	8.589	0.974
0.2 N	**Ogden (kPa)** μ_1	4.31	0.998	1.20	0.997	30.78	0.998
	α_1	6.23		7.21		2.33	
	MR (kPa) C_{10}	8.27	0.999	2.49	0.997	14.17	0.999
	C_{01}	-6.38		-1.96		1.50	
	NH (kPa) C_{10}	2.36	0.997	0.67	0.997	15.50	0.998

Table 8 - Mechanical property summary for all tests

Materials	Advantages	Disadvantages
Agar	• Easy to prepare • Takes 20min to solidify in open air • Ranges of moduli can be achieved by varying agar concentration	• Brittle in nature • Effected by environmental influences e.g fungus • Slides and leaks water during experiments
Gelatin	• Inexpensive • Ranges of moduli can be achieved by varying gelatin concentration	• Effected by environmental influences e.g fungus • Take approximately 5 hour to prepare sample and solidify
Silicone	• Easy to prepare and cure • Good mechanical properties • Fungal resistance	• Sticky