Soft Tissue Equivalent Phantom Materials: Experiments on Mechanical Properties of Agar/Gelatin/PPG Based Phantoms

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Abstract

The aim of this research study is to present some different material blends that are suitable for building a physical model that accurately mimics the dynamic mechanical properties of brain tissue. These materials can act as a replacement for actual biological tissue samples for a wide variety of experimental procedures.

The experimental testing setup has been defined for parallel plates oscillatory shear testing, considering both amplitude and frequency sweeps. Biological tissue samples of porcine brain were tested along with several phantoms, built from the aforementioned materials blends.

Results obtained from experiments show that the proposed blends adequately simulate the sought brain properties, though further composition refinement must be done.

1. Introduction

Of all body parts, the head is the most vulnerable, but there is still a huge lack of knowledge in the biomechanics of this area. Nowadays, there is a need to analyze mechanical properties of human brain in order to advance in the field of neurology, working on the diagnosis of neurodegenerative diseases and in the development of neurosurgery simulation tools.

Palpation is a basic and effective clinical examination tool that has been used for centuries. In engineering terms, palpation assesses the tendency of tissue to resist deformation, a physical property called elastic modulus. However, brain tissue or other organs are not accessible to the palpating hand. Magnetic resonance elastography (MRE) is a non-invasive technique that may be a tool for the detection of abnormal stiffness differences in soft tissues, analyzing the physical properties as elasticity of human tissues. A change in elasticity can often represent a pathological change in the tissue and may provide information for the detection of tumors and for the diagnosis of neurodegenerative diseases as Multiple Sclerosis or Alzheimer. However, it is too early to claim limits in the detection ability of MRE. [1], [2], [3].

Valid quantitative measurements of the elastic modulus of cerebral tissue are also of interest in biomechanical studies of neurosurgery simulation, vehicle safety, brain trauma or ballistic injury [3]. In addition to this, a phantom (physical model of brain tissue or other organ) may be useful to develop a surgical medical training or to calibrate MRE equipment.

Although a large collection of data is available, studies on the characterization of brain properties by different laboratories using different testing protocols yield a tremendously wide range of results, already for the linear viscoelastic properties. In this work, biological brain tissue is tested with the aim of knowing the values of mechanical properties as shear modulus. Some gels were prepared and tested, in order to develop a phantom whose mechanical properties are similar to the ones measured in the brain tissue. Heterogeneity, anisotropy and nonlinearity of brain tissue are not considered in this study.

This paper begins with the presentation of the theory of the mechanical behavior of viscoelastic materials. Afterwards, several tests with brain tissue are collected from the literature. In the third section, prior research studies with brain phantoms are discussed. This is followed by the materials and methods used to analyze biological samples and to carry out and test the gel samples. Finally, the obtained results of the shear test are presented, as well as a final discussion and conclusions.

2. Mechanical Testing Of Viscoelastic Materials

Oscillatory Shear Testing (OST) is the most commonly used in vitro technique to characterize dynamic mechanical properties of all kind of viscoelastic materials [4]. It is possible to perform controlled shear strain or controlled shear stress tests. In the first case, a sinusoidal strain is imposed on the viscoelastic material, resulting in a sinusoidal stress of the same frequency but phase-shifted ahead by an angle δ

$$\gamma(t) = \gamma_A \cdot \sin(\omega t) \implies \tau(t) = \tau_A \cdot \sin(\omega t + \delta) \quad \textbf{(1)}$$

Similarly, for controlled shear stress, the input is a sinusoidal stress, resulting on a sinusoidal strain applied to the material, but phase-shifted ahead by δ

$$\tau(t) = \tau_A \cdot \sin(\omega t) \implies \gamma(t) = \gamma_A \cdot \sin(\omega t + \delta) \quad \textbf{(2)}$$

The complex viscoelastic modulus, G^*, is defined (3) as the ratio between shear stress and shear strain amplitudes.

$$G^* = {}^{\tau_A}/_{\gamma_A} \quad \textbf{(3)}$$

From (3) two other material constants are defined: the storage modulus, G', and the loss modulus, G''. The storage modulus is a measure of the deformation energy stored by the sample during the shear process and represents the elastic behavior of a test material. The loss modulus is a measure of the deformation energy used up

by the sample during the shear process and represents the viscous behavior of a test material. G' and G'' are calculated according to (4).

$$G' = G^* \cos\delta \; ; \; G'' = G^* \sin\delta \quad \textbf{(4)}$$

The phase angle, δ, also named damping or loss factor, quantifies the viscous component of the tested material. The value for materials with ideal elastic behavior is $\delta = 0^o$ and for ideal viscous behavior $\delta = 90^o$. So, it is expected that for viscoelastic materials $0^o \leq \delta \leq 90^o$.

There are several types of OST. The most commonly performed ones, especially regarding soft tissue testing, are both amplitude and frequency sweeps.

Amplitude Sweeps (AS) are OST performed at variable strain amplitudes, keeping a constant frequency value. Their purpose is to determine the limit of the Linear Viscoelastic (LVE) range, where the values of the dynamic properties (G', G'') remain steady. Beyond a certain strain, which specifies the limit of LVE, the material exhibits non-linear behavior.

Frequency Sweeps (FS) are OST performed at variable frequencies, keeping a constant strain amplitude value and are used to investigate time-dependent shear behavior.

There are different experimental setups for obtaining in vitro values of G', G'' of the brain, used in prior research studies in literature; Frequency tests of porcine brain tissue ranges from 0.04 Hz to 200 Hz with a fixed strain amplitude from 0.5% to 7.5% [5], [6]. In amplitude tests, frequency can be fixed from 0.1 to 10 Hz with a strain amplitude from 0.01% to 10% [7].

3. Research studies on Brain Phantom Materials

Several research groups have proposed a variety of suitable materials for gel preparation, with the purpose of building soft tissue phantoms. Some of the most recurrent materials which have been successfully used are agar and gelatin based blends, described in [8]; carrageenan has been successfully used in [9] as an alternative to agar; a blend of mineral oil and SEBS copolymer was proposed in [10], with accurate results and providing gels with long durability; polyacrylamide-based phantoms for elastography and imaging modalities (MRI, US) are commercially available (CIRS Inc. Norfolk, Virginia, USA) and show excellent properties, though they seem fragile, complex and expensive compared with water-based phantoms [11].

Several combinations regarding other materials like, polyurethane [12] or PVA Cryogels [13] have been used with less repercussion. In fact, experiments using a blend of PVA, pure water and ethanol homopolymer showed that the obtained phantom did not posses long-term stability and were significantly stiffer than biological soft tissue. Preparation of PVA or silicone gels is time consuming and costly and their sizes and shapes cannot be easily changed.

Propylene glycol-based phantoms were proposed in [14] as an alternative type of gel. This alternative hasn't been developed to its full extent despite the promising results obtained. Conventional materials have been treated extensively in literature; in any case it is still necessary to explore new stable material blends which might be more finely adjustable and which can mimic better the mechanical properties of soft tissues. This is why these experiments have been used as a departure point for our experimental work, where new stable material blends with suitable mechanical properties are proposed.

4. Materials and Methods

The present research study consists of three different stages which are: preparation of brain tissue samples, preparation of material blends and gel samples and testing, given a suitable experimental setup.

4.1. Preparation of Soft Tissue Samples

Porcine brain tissue was chosen as a substitute for the human brain due to its availability and the availability of studies in the literature. Two brains were harvested from 7 to 8 month-old pigs (at this age, the tissue is considered to possess a fully developed micro-structure [15]) in a local slaughterhouse, 24 hours *post mortem*. Brains were refrigerated before being collected and preserved later in a solution of phosphate buffered saline (PBS) at 4°C, to slow down their degradation and dehydration. All tests were conducted between 24 and 29 hours post mortem. Post mortem time can vary in the literature from 3 hours to one or two days [7]. Cylindrical probes were cut from the brain parenchyma. The measures of the probes were: 25 mm diameter and approximately 3 mm height. No difference has been made between white and grey matter or between different functional brain regions.

4.2. Preparation of Sample Gel Probes

Materials used for making the testing probes for the experimental stage were as follows: Agar, food-grade from Scharlab; Gelatin, from porcine skin; Poly Propylene Glycol, (PPG) and Glutaraldehyde (GTA) from Sigma – Aldrich. On Table 1, the different material blends used for making the gel probes are presented.

Gel Type	Gelatin [%]	Agar [%]	PPG [%]	GTA [%]
A	–	0.5, 0.75, 1.0	–	–
B	0.3	0.5	1	–
C	0.5	0.4	0.5	–
D	0.5	0.4	0.5	1

Table 1. *Material blends for sample probe preparation*

Gels were prepared using with Milli-Q water. Adequate proportion of water was poured in a glass beaker and components were added to the mixture and stirred at 580 rpm, 85ºC, using a magnetic hot-stirrer in order to get a homogeneous mixture. Samples were stored at room temperature until gelification. GTA was added to the blend as temperature dropped to 36ºC. After the

gelification, cylindrical probes were cut using the same geometry as for tissue samples. Probes were molded on suitable cylindrical PPE recipients and preserved at room temperature.

4.3. Setup and Testing Procedures

In order to determine the limit of the Linear Viscoelastic (LVE) range, where the values of the dynamic properties (G', G'') remain constant, amplitude sweeps oscillatory shear tests were performed on a rotational rheometer (Anton Paar Physica, MRC 301) with a parallel plate configuration. Also, some frequency sweeps are realized to compare our values with the literature ones.

Cylindrical samples were placed between the parallel plates of a rheometer. It was irrigated with PBS to prevent dehydration during testing. The top plate of the rheometer was lowered until it contacted the upper surface of the specimen. All measurements were performed at 20ºC.

All tests were realized at low frequencies that are the similar to the ones that could be found during the interaction with the tissue in a surgical procedure. Amplitude sweeps were performed at 1Hz.

5. Experimental Results

Figures 1 and Figure 2 show the experimental values of the biological tissue and the A-type agar gels (see Table 1) obtained from the amplitude sweep.

It can be concluded that the LVE limit of the porcine brain tissue is approximately 1% strain. For the strains above 1%, G' and G'' are no longer constants.

None of the gels has simultaneously the same values of G' and G'' as the brain tissue. Gel 0.5% Agar has a value of G' similar to that of the biological tissue, while the 0.75% Agar approximates better the value of G''. In spite of this, it seems that 0.5% Agar is the gel which better approaches the behavior of brain tissue. If higher concentrations of 0.5% - 0.75% Agar are used, the obtained results for storage and loss modulus are at least one fold higher than those of porcine brain tissue.

Figures 3 and 4 present the storage and loss modulus respectively for all types of gel probe. Figure 5 depicts the relation between strain and stress for these gel probes.

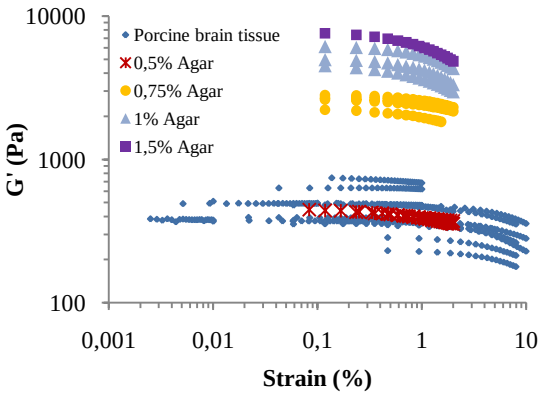

Figure 1. *Storage Modulus: A-type Gels vs. Brain Samples*

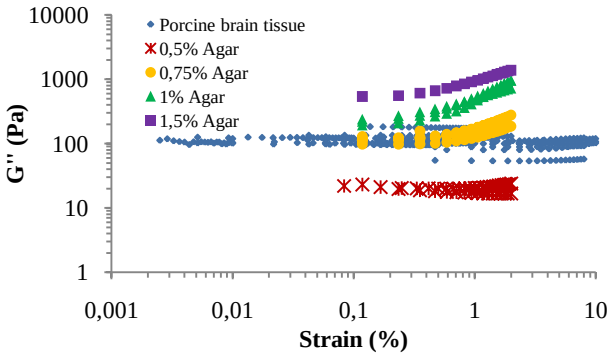

Figure 2. *Loss Modulus: A-type Gels vs. Brain Samples*

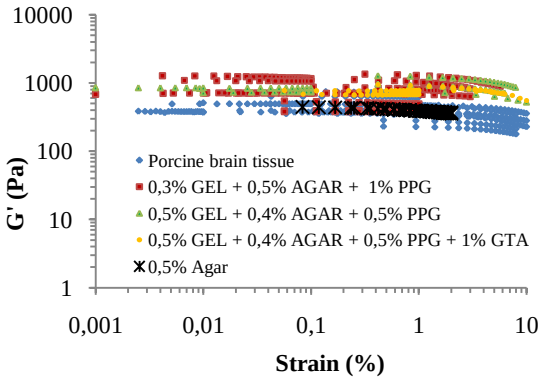

Figure 3. *Storage Modulus: Gels vs. Porcine Brain Samples*

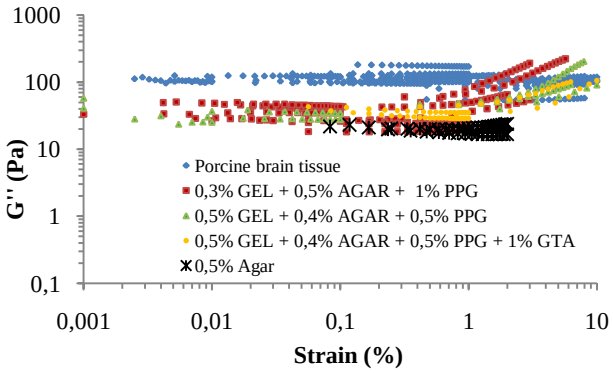

Figure 4. *Loss Modulus: Gels vs. Porcine Brain Samples*

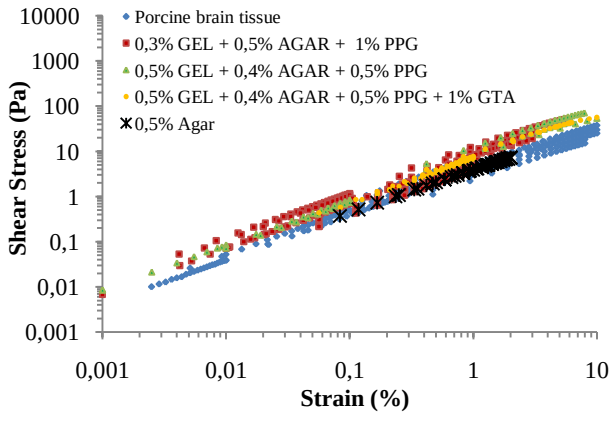

Figure 5. *Strain – Stress relation: All Gels vs. Brain Samples*

The LVE limit of the gels is similar to that of the brain tissue (1% strain). The values of G' and G'' are in the same order of magnitude as the values of the brain tissue.

6. Discussion

Values of LVE limit and shear modulus of tested porcine brain tissue are in the range obtained by other authors [7]. If we compare the values obtained experimentally with the literature, Nicolle found higher values of G' and G'' for porcine brain tissue with an amplitude test at the same frequency of 1Hz [7]. Differences in the values can be caused by the kind and age of the specimen, post mortem time, sample's size or test conditions. However, the experimental values obtained with a frequency sweep in LVE range (at 1% strain) are in the same range of the values obtained by other authors with in vitro tests [6], [16].

The storage and loss modulus of the four gels presented here are of the same order of magnitude as the values of the biological tissue. All of them approach the behavior of brain tissue (see figure 5). For simplicity, the utilization of agar only is more appropriate. However, agar gel concentrations can be altered between 0.5% and 0.7%. Higher concentrations exhibit property values far from those of biological tissue ($G' \approx 4000$ Pa and $G'' \approx 200$ Pa Vs $G' \approx 500$ Pa and $G'' \approx 100$ Pa). The combination of other components like gelatin, PPG or GTA provides a greater range of variability to accurately simulate different soft tissues. Also, with these combinations different textures of the gel can be obtained getting more realistic gels.

7. Conclusions and Future Work

This study presents suitable material blends for manufacturing a phantom that adequately mimics brain mechanical properties in LVE. The non-linear behavior of the brain tissue will be analyzed in further studies for those applications that require it.

There are many differences in the literature about the experimental protocols (post mortem time, age and species of the animal model, sample's size, orientation, composition, tissue conditioning, etc.) when biological tissue is analyzed. Therefore, it is only possible to define a range of values for the mechanical properties of biological tissues. Thus, one of the objectives becomes to establish a range of the mechanical properties of the gels, and not a specific value. In this way, we consider that the gels presented are suitable as phantom material, because the mechanical properties of these gels are of the same order of magnitude as values for biological tissue and they have a mechanical behavior similar to porcine brain tissue. The proposed gels reproduce the behavior of biological tissue, at least in the LVE range and for low frequencies.

A suitable mathematical model is required for the development of interactive real-time surgical simulators. The obtained mechanical properties for brain tissue in LVE range serve as a suitable model to parameterize a mathematical model in the applied conditions.

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