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Biomechanical properties of breast tissue, a state of the art review

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Abstract:	This paper reviews the existing literature on the tests used to determine the mechanical properties of women breast tissues (fat, glandular and tumour tissue) as well as the different values of these properties. The knowledge of the mechanical properties of breast tissue is important for cancer detection, study and planning of surgical procedures such as surgical breast reconstruction using pre-surgical methods and improve the interpretation of clinical tests. Based on the data collected from the analyzed studies some important conclusions were achieved: (1) the Young's modulus of breast tissues is highly dependent on the tissue pre-load compression level (2) the results of these studies clearly indicate a wide variation in moduli not only among different types of tissue but also within each type of tissue. These differences were most evident in normal fat and fibroglandular tissues.
Response to Reviewers:	Revision of the Manuscript “Biomechanical properties of breast tissue, a state of the art review”

(BMMB¬D¬1 5¬001 46R1)

We are grateful again to the Reviewer for his comments and suggestions that hopefully will help to improve the quality of our paper.
We changed the Ackowledgments section of the manuscript by adding the following text,
“The authors would like to acknowledge the outstanding revision work carried out by the reviewers of the paper. Their constructive criticism was a fundamental contribution to elevate the overall quality of the manuscript.”
We tried our best to answer all the comments accordingly. We made revisions based on the comments/suggestions of the Reviewer. They are listed below, followed by our response (clarifications and changes).
The changes performed in the revised manuscript are written in red.
Best regards,
Pedro Martins
Nilza Ramião

Reviewer comments
Reviewer: 1
General comments:
The authors made substantial changes to the manuscript and addressed most issues raised by the reviewers adequately. In addition to minor issues which are listed below, the manuscript still requires substantial editing to correct several stylistic errors.
Authors Reply: The authors are, once more, grateful for the reviewer’s comments and suggestions. The manuscript was reviewed and several stylistic errors were corrected.
Comment 1: Therefore, according to the experimental protocol for measuring the mechanical
properties of breast tissues mentioned by several authors (Krouskop et al. 1998; Samani et al. 2007; Matsumura et al. 2009; Umemoto et al. 2014) Young' s modulus, E, is analyzed using equation (1) (Krouskop et al. 1998)
.
E=(2(1-v^2) qa) /w (1)

The above equation is valid for semi-infinite medium only. A fundamental equation of the
Young' s modulus, e. g. E = \Delta \sigma / \Delta \epslion, should be used instead. The reviewer recommends that this be followed by: In indentation test where part of the specimen' s surface is indented while forces corresponding to applied indentation displacements are recorded, the slope of force vs. indentation displacement (S) is calculated to estimate the Young' s modulus using the following equation:

E - \kappa S (*)

where \kappa is a conversion factor that depends on the indenter' s geometry, specimen' s
geometry and boundary conditions.
Authors Reply: The authors decided to maintain the text and equation (1) since this is the most conventional way of presenting the Young’s modulus for indentation tests. However we agree with the Reviewer’s comment and added the following sentences, “ … The above equation is valid for semi-infinite medium only. Since the sample’s thickness is finite, Samani et al. 2007 developed an interactive inversion Finite Element algorithm used to calculate Yong’s modulus according to equation E=kS, where k is a conversion factor that depends on the indenter's geometry, specimen's geometry and boundary conditions and S is the slope of force vs. indentation displacement.”

Comment 2: In "The most common mechanical analysis performed is the indentation test discussed ahead in this section. " change ahead to next
Authors Reply: The authors agree with the reviewer’s correction of the word, but used the word further instead of next.
“The most common mechanical analysis performed is the indentation test discussed ahead in this section” The most common mechanical analysis performed is the indentation test further discussed in this section.

	Comment 3: In "Thus, according to the main structure of the breast tissues and the main objectives of each study, several authors opted by the compression (Sarvazyan et al. 1994) (unconfined or confined) and indentation tests (Krouskop et al. 1998; Samani et al. 2007; Matsumura et al. 2009; Umemoto et al. 2014; Wellman et al. 1999) . " a. main structure is ambiguous. b. opted by should be opted for. Authors Reply: a. The authors agree with the reviewer's comment. Therefore, we change the expression "main structure" to different structures. b. The authors added "for", following the reviewer's suggestions. Thus, according to the main structure of the breast tissues and the main objectives of each study, several authors opted by the compression (Sarvazyan et al. 1994) (unconfined or confined) and indentation tests (Krouskop et al. 1998; Samani et al. 2007; Matsumura et al. 2009; Umemoto et al. 2014; Wellman et al. 1999). Thus, according to the different structures of the breast tissues and the main objectives of each study, several authors opted for the compression (Sarvazyan et al. 1994) (unconfined or confined) and indentation tests (Krouskop et al. 1998; Samani et al. 2007; Matsumura et al. 2009; Umemoto et al. 2014; Wellman et al. 1999). Comment 4: In "The resulting deformation of the external surface is recorded. The slope relating stress with strain (force-displacement) represents the compressive Young' s modulus (E) shown in equation (1) . " should be revised to: "The resulting deformation of the external surface is recorded. The slope relating force with indentation displacement represents the compressive Young' s modulus (E) according to Equation (*) . " Note that Equation (*) is suggested to be added (see item 1) . Authors Reply: The authors included the Reviewer's sugestion in the manuscript, The resulting deformation of the external surface is recorded. In this case, the fluid flow outside the indenter-tissue contact point is possible in both lateral and axial directions. The slope relating stress with strain (force-displacement) represents the compressive Young's modulus (E) shown in equation (1). Another approach as suggested by Samani et al. 2007 corrects equation (1) using E=kS. Comment 5: Change "There are different methods of elastography depending on the tissue response measurement, namely ultrasonography/compression, MR and optical (Fig. 6) . " to "There are different methods of elastography depending on tissue stimulation method and imaging modality used to measure generated displacement field (e. g. quasi-static or harmonic ultrasonography elastography, MR elastography and Optical coherence elastography (Fig. 6)) . Authors Reply: The authors agree with the reviewer and accepted the suggestion. Therefore, the sentence was changed according to the proposal of the reviewer. "There are different methods of elastography depending on the tissue response measurement, namely ultrasonography/compression, MR and optical (Fig. 6)" There are different methods of elastography depending on tissue stimulation method and imaging modality used to measure generated displacement field (e. g. quasi-static or harmonic ultrasonography elastography, MR elastography and Optical coherence elastography (Fig. 6))

Biomechanical properties of breast tissue, a state of the art review

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Abstract

This paper reviews the existing literature on the tests used to determine the mechanical properties of women breast tissues (fat, glandular and tumour tissue) as well as the different values of these properties. The knowledge of the mechanical properties of breast tissue is important for cancer detection, study and planning of surgical procedures such as surgical breast reconstruction using pre-surgical methods and improve the interpretation of clinical tests.

Based on the data collected from the analyzed studies some important conclusions were achieved: (1) the Young's modulus of breast tissues is highly dependent on the tissue pre-load compression level (2) the results of these studies clearly indicate a wide variation in moduli not only among different types of tissue but also within each type of tissue. These differences were most evident in normal fat and fibroglandular tissues.

Key words: breast tissue**,** mechanical properties, compression loading, elasticity modulus

1. Introduction

The breast is an important organ in women´s body, since it contains glandular tissue essential for the production and secretion of milk. The breast is a heterogeneous structure containing different layers of tissue (Fig. 1), however, the predominant types of tissue within the breast are fat and glandular tissue. Each breast contains 15–25 lobes of compound glands that are embedded in fibrous and adipose tissues. These lobes, each containing an excretory duct that drains into the lactiferous sinus, radiate from a central nipple-areolar complex.

The breast is firmly attached to the skin and underlying structures by fibrous bands referred to as suspensory ligaments (Cooper's ligaments), which provide the functions of support, hold the breasts in place and contribute to determine the shape and contour of the breast.

The distribution of various tissues during women's life cycle undergoes cyclic changes that depend on factors such as age, menstrual cycle, pregnancy / lactation, hormone therapy, menopause, among others. Some of these changes have profound effects in tissue's structure and morphology.

Fig. 1. Anatomy of Breast.

Such alterations are expected to have an effect on the biomechanical properties of breast tissue. For instance, a stretching of Cooper's ligaments and a weakening of the coupling between the breast and the surrounding tissues are observed with increasing age. An important property of soft tissues is their intrinsic elasticity, which may change under the influence of pathophysiologic processes, such as tumour development (Bogonoletz 1986). Breast cancer is the most common female disease worldwide, currently affecting approximately 1.38 million women per year. Worldwide, breast cancer comprises about 25% of all types of cancer in women (Stewart and Wild 2014). Their prognosis and survival rates depend mostly on the type and stage of breast cancer. Early detection leads to a more effective treatment and improvement of the survival rate (Society 2013; World Health Organization 2011).

In this context, the mechanical properties of breast tissue play a prominent role in the research related to several clinical, pre-clinical, as well as current applications such as self-diagnosis through palpation. These applications include cancer detection, mechanics of injury, surgical simulators and tumour motion tracking during surgeries. For these, engineering has contributed to improve clinical examination protocols, through improvements on the diagnosis, surgical planning and decision supporting tools. Some studies are based on biomechanics' concepts using finite element modeling (FEM). These numerical models are differentiated, primarily, by how the breast geometry is discretized, application of boundary conditions, and/or knowledge of the breast tissue material properties. In most studies, large deformations were considered, and information on patient-specific breast morphology and on elastic-tissue properties was required. To improve the outcome of breast needle biopsy, Azar et al (2001) developed a model of the breast to predict the tissue deformation during the procedure, and Carter et al (2005) presented a model that can potentially be applied for image guided surgery.

Roose et al (2005) presented a computational model capable of simulating the postoperative shape of the breast with up to 1 cm accuracy after a subglandular breast implantation.

Unlu et al (2010) developed and tested a new computerized finite element method (FEM) based on a 3D non rigid registration of PET and MR breast images. This simple method was proposed to facilitate the nonrigid registration of MRI or CT images of any type of soft-tissue to their molecular counterparts such as those obtained using PET and SPECT.

There are several challenges associated with the localization of suspect lesions in the breast in an MRI exam. These difficulties include patient positioning, visibility of the lesion that may fade after contrast injection, menstrual cycles, and lesion deformation. Stewart et al (2011), Azar et al (2001), Samani et al (2001a), Carter et al (2005), Unlu et al (2010) and Pathmanathan et al (2004) are examples of some authors that developed patient-specific finite element (FE) breast models obtained from diagnostic MR images, with potential for patient-specific therapeutics.

The ideal approach to achieve high quality patient-specific simulations should include the *in vivo*, and ideally non-destructive estimation of the mechanical properties. Han et al (2003) performed *in vivo* material parameter estimation by ultrasonic indentation tests on breast tissues. Another *in*

vivo experimental technique was used by Buijs et al (2011), consisting of a model to predict target displacements using a combination of ultrasound elastography and finite element (FE) modeling. This technique can help pre-operative planning of minimally invasive surgical interventions.

While significant research has been conducted to develop techniques to measure the elastic modulus of breast tissues, little research has been focused on their hyperelastic mechanical behaviour.

Following, the ability of FE models to predict *in vivo* behaviour strongly depends on the accuracy of the mechanical properties of tissue components. An accurate breast model has proved to be very difficult to implement, due to several difficulties associated to breast tissues:(1) the complex morphology; (2) the patient-specific variability, (3) the highly nonlinear (hyperelastic) mechanical behaviour and (4) the difficulty of measuring elastic properties of different types of tissues in the breast (Samani and Plewes 2004; O'Hagan and Samani 2009; Samani and Plewes 2001a; Ruiter et al. 2006; Pathmanathan et al. 2008; Rajagopal et al. 2008).

However, in order to make the biomechanical models predict more realistically *in vivo* behaviour and help improving clinical and pre-clinical applications (for example, cancer detection), several authors studied the mechanical properties of the different breast tissues. Thus, throughout this article we provide a review about the techniques to measure the mechanical properties *in* and *ex vivo*. These measurement types are subdivided in two categories *in vivo* and *ex vivo*. Regarding *ex vivo* techniques, it is given a comprehensive review of the test protocols (compressive tests) used in various studies as well as the obtained biomechanical results (Krouskop et al. 1998; Wellman et al. 1999; Samani and Plewes 2001a; Samani et al. 2001b; Samani et al. 2004; Samani et al. 2007). Considering *in vivo* testing the approach is elastography, a noninvasive by nature and based on an imaging technique (Harrigan and Konofagou 2004; Doyley et al. 2001; Housden et al. 2010; Sayed et al. 2013; Burnside et al. 2007).

All these studies have the purpose of simulating and assisting the diagnostic methods used in clinical breast examination. In most breast examination methods, compression is applied to help detecting lesions, and sometimes it becomes necessary to apply higher compression loads to investigate stiffer regions. This situation creates the need to know more about the mechanical

properties of breast tissue under compression. Despite the importance of compressive loading and its contribution to the characterization of tissue in applications of cancer detection, there are few studies that have focused on the mechanical behaviour of breast tissue in response to compression. Widespread adoption of such techniques (*in vivo* and *ex vivo*) associated with biomechanical modeling and imaging techniques of the breast have the potential to significantly reduce the numbers of misdiagnosed breast cancers and enhance surgical planning for patient treatment.

The main objective of this review is the gathering of the mechanical properties of breast tissues (adipose, glandular, and tumour), available to date in the literature, through different *in vivo* and *ex vivo* tests, enabling as well the identification of the relationship between tissue properties and pathological mechanics.

An accurate knowledge of the breast tissues' mechanical properties allows realistic simulations by finite element modeling and improvement in clinical exams for breast cancer (screening, diagnosis and monitoring tests), thus it opens possibilities for medical applications such as surgery planning and surgery outcome simulation.

This paper is organized in five sections that evolve from the simplest concepts of breast tissue characterization to the state of the art testing techniques used. Section 2 details the characterization of breast tissues, and addresses the main experimental challenges involved. Section 3 analyses the differences and specificities of in vivo and ex vivo mechanical techniques. Section 4 summarizes the mechanical experimental results of each breast tissue, and Section 5 includes a discussion and reached the conclusions.

2. Characterization of soft tissues – basic concepts

This section presents fundamental concepts for the understanding of the biomechanical studies presented. The biomechanical properties of tissue (ex. stiffness/elastic modulus) vary markedly between organs and tissues, and are inherently related to tissue function (Fig. 2).

Breast tissue has a unique rheology and optimum biomechanical properties, changing over the course of development in response to function (as during mammary gland lactation) or in

pathological situations (such as tumours). Although breast tumours are much stiffer than normal breast, the material properties of breast tumours remain significantly softer than those of muscle or bone (Butcher et al. 2009).

An important characteristic of breast tissue is their nonlinearity at high deformation (Price et al. 2010). For example, the tensile response of breast tissue exhibits a nonlinear stiffening while undergoing high deformations.

Fig. 2. Stiffness in different soft tissue**.** Adapted from (Cox and Erler 2011)**.**

The mechanical characteristics of soft tissues, consists, in general, of a complex combination of elastic and viscous components (Fung 1993). This combination controls the deformation of tissue (Shiina 2013).

Regarding the classic elasticity theory, this represents the linear relation between stress (σ) and strain (ε), given by Hooke's Law: $\sigma = E\varepsilon$. In this case, the constant of proportionality (E) represents the elasticity modulus, which is the slope of the stress-strain curve in the linear section (see Fig. 3) – corresponding to the elastic region – and constitutes the mechanical parameter which indicates the stiffness of a material (Fung 1993). To characterize the tissue stiffness, there are three types of elastic modulus defined by the tensile (Young's modulus), shear (shear modulus) and volumetric elasticity (bulk modulus) respectively.

The Young´s modulus is the most commonly used to quantify stiffness, and will be used throughout article to characterize breast tissue. Therefore, according to the experimental protocol for measuring the mechanical properties of breast tissues mentioned by several authors (Krouskop et al. 1998; Samani and Plewes 2007; Matsumura et al. 2009; Umemoto et al. 2014) Young's modulus, *E*, is analyzed using equation (1) (Krouskop et al. 1998).

$$E = \frac{2(1-\nu^2)qa}{w} \qquad (1)$$

where ν is the Poisson´s ratio, q is the load density (force per unit area), a is the radius of the loaded area, and the w is the maximum displacement in the direction of the load. The above equation is valid for semi-infinite medium only. Since the sample's thickness is finite, Samani et al. 2007 developed an interactive inversion Finite Element algorithm used to calculate Yong's modulus according to equation $E = kS$, where k is a conversion factor that depends on the indenter's geometry, specimen's geometry and boundary conditions and S is the slope of force vs. indentation displacement.

The most common mechanical analysis performed is the indentation test further discussed in this section.

Fig. 3. Mechanical behaviour of linear elastic and hyperelastic materials

Poisson's ratio (v), measures transversal deformation relative to the longitudinal direction of load application and is defined as follows:

$$v = -\frac{\varepsilon_d}{\varepsilon_a} \tag{2}$$

where ε_a is the strain in loading direction (axial) and ε_d is the corresponding strain in lateral direction. The Poisson's ratio is an intrinsic parameter of a material, and it is unique for different materials. For soft tissues which are quasi-incompressible due to their high (incompressible) fluid content, Poisson's ratio is ~0.5.

Mechanical properties of a tissue are also dependent on time and strain history. For this reason, stress- strain curves during loading and unloading do not follow the same path, and loading-unloading cycles are always different from one to the other, usually displaying a hysteresis effect, shown in Fig. 4. This can be related to the viscoelastic phenomenon taking place when the load-deformation (stress-strain) diagram curve suffers a path deviation (Fung 1993).

The effect of viscoelasticity results mainly from shear contact between collagen fibers, the proteoglycans and elastin component of ground substance.

Shear stress causes energy dissipation due to the recovery of the tissue after elongation or contraction, a behaviour that creates a hysteresis cycle, during loading and unloading stages of the test (Lemaitre 2001). Moreover there is also a microscopic sliding among collagen fibers while the tissue undergoes an axial stress (Silver et al. 2008; Silver et al. 2003).

Fig. 4. The dashed is a hysteresis loop and shows the amount of energy lost (as heat) in a loading and unloading cycle. Adapted from (Fung 1993).

All tests presented in this review contain repeated loading and unloading of the tissue sample which can reduce hysteretic effects, and can also soften the tissue. Pre-conditioning involves the repeatedly loading and unloading of the tissue so that a steady state is achieved for a given load cycle (Fung 1993). Pre-conditioning was performed by Krouskop et al (1998), Samani et al (2007), Samani and Plewes (2001a) and Wellman et al (1999). Both Wellman et al (1999) and Krouskop et al (1998) found viscous effects to be negligible, although Wellman et al (1999) did note that some long time scale force relaxation was likely to occur. This behaviour results from complex interactions of collagen fibers, elastin, proteoglycans and water within the tissue, and can provide us with additional insight into the composition of tissues and allow us to build sophisticated models of tissues.

The structure and mechanical properties are quite different in several soft tissues, vary significantly from one individual to another and can take different values whether measured *in vivo* or *ex vivo*.

Thus, according to the different structures of the breast tissues and the main objectives of each study, several authors opted for the compression (Sarvazyan et al. 1994) (unconfined or confined) and indentation tests (Krouskop et al. 1998; Samani et al. 2007; Matsumura et al. 2009; Umemoto et al. 2014; Wellman et al. 1999). The unconfined compression consists on the application of a compressive load on the specimen which is fixed between two plates (as seen in Fig. 5a). The compressor size should not be inferior to the size of the material sample tested. The tissue is then deformed in a parallel direction to the applied force (lateral).

Confined compression is similar to unconfined compression (see Fig. 5b), but in this case the specimen is additionally constrained in the radial direction to the applied load. The specimen is placed in a chamber and a constant compressive load is applied to it. The additional constrain avoids free lateral tissue deformation developing a lateral pressure. On the tissue sample interstitial fluid can only flow axially through the surface into the filter in chamber, shown as in Fig. 5 (b).

Indentation test is similar to compression test, a procedure where an indenter applies a compressive load to the tissue with a cylindrical, typically plane-ended or spherical-ended indenter. The resulting deformation of the external surface is recorded. In this case, the fluid flow outside the indenter-tissue contact point is possible in both lateral and axial directions. The slope relating stress with strain (force-displacement) represents the compressive Young's modulus (E) shown in equation (1). Another approach as suggested by Samani et al. 2007 corrects equation (1) using $E = kS$.

The indentation machine uses a linear motor programmed to apply a user defined displacement. Therefore, deformations can be obtained directly from the test parameters. The load, applied with the indenter and the contact area indenter-tissue are known parameters. The main difference between indentation and compression test (see Fig. 5c) is that indenter surface is smaller than the specimen testing surface.

Fig. 5. (a) Unconfined compression, (b) Confined compression and (c) Indentation test

In punch indentation experiments the piston has a diameter of around 5mm which allows a tissue of a homogenous type to be tested, although the technique presented in Samani et al (2007) is suitable for samples which contain both normal and pathological tissue.

When breast tissue is compressed the strain increases rapidly corresponding to the elimination of free fluid. The elastic modulus becomes progressively higher with increasing strain (Wells and Liang 2011).

The practical implication of this mechanical behaviour is that breast tissue needs to be statically preloaded and accurate measurement requires small increments in stress (i.e. in the linear region),

to obtain reproducible and useful values of Young's modulus. In addition, the testing conditions as well as tissue characteristics must be specified (for example: age of the tissue, its temperature, type of test…).

3. Experimental Techniques to characterize Breast Tissue

This section reviews the available literature on the stiffness of the breast tissues obtained experimentally. The discussion is divided in two parts: (3.1) *in vivo* techniques and (3.2) *ex vivo* techniques. With *in vivo* techniques, tissues are tested with small strains or loads and all the changes induced are reversible. In contrast, *ex vivo* protocol involves larger strains or loads inducing non-reversible changes to the tissues. The relationships between the strain (a measure of deformation) and the stress (internal pressure) are reviewed for each breast tissue type (Wellman et al. 1999; Samani and Plewes 2001a; Samani et al. 2001b; Samani et al. 2004; Samani et al. 2007; Krouskop et al.1998; Sinkus et al. 2005, 2000; McKnight et al. 2002; Van Houten et al. 2003; Srivastava et al. 2011; Kruse et al. 2000; Sarvazyan et al. 1995; Scaperrotta et al. 2008; Umemoto et al. 2014). Several authors have reported that mammary tissue has a nonlinear mechanical response (Unlu et al. 2010; Samani and Plewes 2001a; Samani et al. 2001b, 2004, 2007; Krouskop et al. 1998; McKnight et al. 2002; Van Houten et al. 2003; Srivastava et al. 2011; Kruse et al. 2000; Sarvazyan et al. 1995; Wilson et al. 2000; Mariappan et al. 2010; Ophir et al.1991; Cespedes et al.1993; Korte and Steen 2002; Manduca et al. 1997; Mehrabian et al. 2010; Ralph Sinkus et al. 2005; Muthupillai et al. 1995): the *in vivo* (elastography) and *ex vivo* (compression and punch indentation tests) testing methods found in literature are summarized in Table 1. The mechanical properties of the breast constituents have been characterized considering linear elastic Young's moduli to quantify the stiffness.

3.1. *In vivo* Techniques

Palpation is an effective method for breast cancer diagnosis. It's a technique based on a qualitative assessment of the low-frequency stiffness of tissue, which is primarily useful to detect relatively large and superficial tumours.

However, this technique is not sufficiently sensitive with cases where the tumour is too small and/or its location deep in the body, precludes its detection and evaluation by palpation. In some cases, the examiner may be inexperienced or the signs are not clear, so the result of palpation becomes doubtful. Therefore, other qualitative methods are required to detect the presence of abnormalities.

To help detecting relatively large and superficial tumours, an imaging technique, called elastography, has been developed from the late 1980s to the early 1990s (Lerner and Parker 1987; Lerner et al. 1988; O'Donnell et al. 1994; Ophir et al. 1991). This technique provides quantitative information on tissue stiffness and is characterized by estimations of the elastic modulus (Samani et al. 2001b; McKnight et al. 2002; Van Houten et al. 2003; Garra et al. 1997; Sinkus et al. 2000). Elastography helps estimating or assessing the non-invasively changes in the mechanical properties of the tissues under compression at a microscopic level (Srivastava et al. 2011). This technology can be understood as imaging-based counterpart to palpation, commonly used by physicians to diagnose and characterize diseases.

Elastography is characterized by having a higher degree of specificity and sensitivity. It has the ability to detect the type of abnormality (benign or malignant tumour) and separate it from healthy tissues, for example separating the tumour from the adipose and fibroglandular tissue (Wilson et al. 2000).

This technique provides an insight into the elastic properties of biological tissues (when applying a small axial uniform compression) and of the strains resulting on site (Ophir et al. 1991; Cespedes et al. 1991; Garra et al. 1997). The goal of this technique is to create an image of the distribution of physical parameters related to the mechanical properties of the tissue by measuring the response or strain of the tissue resulting from the applied stress. The elasticity imaging methods consist in applying some form of stress or mechanical excitation to the tissue, and measuring the

tissue response to this stimulus, and from this response calculating parameters that reflect the mechanical properties (see Fig. 6) (Mariappan et al. 2010).

There are different approaches to elastography, either quasi-static or dynamic (transient and harmonic). The following references provide a comprehensive overview of these techniques (Bamber et al. 2002; Greenleaf et al. 2003; Manduca et al. 1998; Ophir et al. 2000; Parker et al. 2011; Doyley et al. 2013).

There are different methods of elastography depending on tissue stimulation method and imaging modality used to measure generated displacement field (e. g. quasi-static or harmonic ultrasonography elastography, MR elastography and Optical coherence elastography (Fig. 6)).

After the measurement of tissue responses to applied stress, using the elasticity imaging processes and the acquired data, it's possible to estimate the mechanical properties of the tissue. Typically, soft tissue is assumed to be isotropic, linear elastic, and Hookean when elasticity imaging techniques are employed.

Some studies intend to characterize the type of abnormality and increase the specificity of elastography, in an attempt to avoid unnecessary biopsies (Mehrabian et al. 2010).

Changes in the stiffness of soft tissues are generally associated with the presence of pathology; malignant or benign breast tumours are usually stiffer than normal breast tissues, and malignant breast tumours are significantly stiffer when compared to benign tumours. Thus, the mechanical properties of breast tissues, measured by elastography, can help to detect the presence of abnormality in the breast (sensitivity), and also to classify the type of the detected abnormality (specificity) (Mehrabian et al. 2010).

Depending on the particular elastography technique used, there is a range of false-negatives (Matsumara et al. 2009). These errors could be reduced or minimized with a better understanding of the testing conditions; such researches can be carried out using *ex vivo* techniques. As pre-compression, required to initiate elastography, influences the test results, an improved knowledge of its influence is critical. A modern and more accurate definition of pre-compression was provided by Umemoto et. al (2009), "pre-load compression".

Fig. 6. An overview of elasticity imaging methods. Adapted from (Shiina 2013; Sudhakar et al. 2014).

3.2. *Ex vivo* Techniques

Other researchers have proposed mechanical tests to measure the mechanical properties of *ex vivo* tissue immediately after it is removed from the body (Samani and Plewes 2001a; Samani et al. 2007; Sarvazyan et al. 1995; Wellman et al. 1999, Krouskop et al.1998).

For example, Krouskop et al (1998) measured the elastic modulus of pathological breast tissues (fibrous, fat, glandular, carcinomas, intraductal carcinomas, and invasive ductal carcinomas) submitted to a uniaxial compressive force with pre-load compression levels of 5% and 20%, respectively. These tissues were tested with a sinusoidal load at three frequencies: 0.1, 1.0, and 4.0 Hz. The strain rate used during compression testing was selected so that viscoelastic effects were negligible. Wellman et al (1999) adopted a similar experimental methodology, but tested more types of breast tissue. Wellman et al (1999) in their study used a test instrument for uniaxial compression and punch indentation of tissue, which applies repeated loads on the sample. Sarvazyan et al (1994) (1995) studied the elastic properties of breast tissues through uniaxial compression test. The authors tested 20 specimens of postoperational material (adipose, fibroglandular and tumour tissue) under compression between two plates. The intent of these studies was to characterize the viscoelastic behaviour and to confirm whether or not the tissue could be modeled as an elastic material within the frequency range of interest.

Samani and Plewes (2001a) and Samani et al (2007) developed a complex system to measure the elastic modulus of normal breast and tumour tissue (without the need to remove the tumours) from slices obtained after surgery. For normal tissue Samani et al (2003) developed a technique where small block shape specimens were indented and the resulting force–displacement slope was converted to the Young's modulus using an FE model. For tumours, Samani and Plewes (2007) used a technique where tumours remained within tissue slices. The tumour is surrounded by normal tissues. The sample is indented and the resulting force displacement slope converted to the Young's modulus iteratively using the tissue slice FE model. One major improvement of

this technique is that the tumour tissue may be tested imbedded on the normal tissue. The comparison between experimental (experimental phantom) and FE simulation (numerical phantom) data has associated errors. As the authors recognize, an ideal solution would include 3D MRI or CT scan, so that the naturally occurring variations in tumour shape and density can be reflected on the FE simulation (detailed FE mesh). This may be the reason why the authors state that they obtained a smaller error while analyzing larger tumours. A relevant thumb rule pointed by the authors is the 1(thickness):4(slice diameter) ratio of tissue slice dimensions. This is the minimal thickness: diameter ratio that allows a comparison between experimental results and the FEM simulation of a semi-infinite body. For practical purposes, if the tumours are small (less than a few centimeters) the errors due to the deformation of the surrounding (normal) tissues will be significant.

Matsumura et al (2009) and Umemoto et al (2014), measured the elastic property, young's moduli, from surgically-resected breast tissue by material testing machine (Instron) with 3mm cylindrical indenter. The breast tissues samples (glandular, adipose and tumour tissue) were cut and soaked in saline and heated for 5 minutes in the thermostatic chamber maintaining 45 C^{o} temperature. Then the samples were removed from the saline and placed on the testing stage which is kept under 37C° temperature (the surface of the sample is kept moist with saline). The authors used different compression protocols, with compression starting from zero-compression (zero-stress – 0kPa) up to a compression strain of 30% (50% in the case of fat or gland) with compression speed of 1mm/min.

In several studies of breast tissue, samples were properly preserved according to standard preservation procedures. Often the time gap between collection and testing did not exceed the period of two hours. There were no measurable changes in the data obtained after allowing the specimens to sit for periods up to two hours (Samani et al. 2007; Krouskop et al. 1998).

The experimental techniques used to estimate the biomechanical properties of breast tissues during the last decades are summarized in Table 1.

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Table 1 Mechanical tests for the breast tissue reported in literature, grouped according with vital state of the subject (*in vivo* / *ex vivo*) and testing techniques.

Mechanical tests	Experimental Condition	Author
Compression/ultrasound Elastography	*In vivo*	J. Ophir et al. (1991) Garra et al. (1997) Hiltawsky et al. (2001) Thomas et al. (2006)
Magnetic resonance elastography	*In vivo*	Sinkus et al. (2000a) (2000) (2005) Plewes et al. (2000) McKnight et al. (2002) Van Houten et al. (2003) Manduca et al. (1997) Kruse et al. (2000) Lorenzen et al. (2001) Siegmann et al. (2010) Lawrence et al. (1998) Xydeas et al. (2005) Cheng et al. (2011) (2013)
Optical coherence tomographic elastography	*In vivo*	Srivastava et al. (2011)
Uniaxial compression and punch indentation	*Ex vivo*	Krouskop et al. (1998) Sarvazyan et al. (1994) (1995) Wellman et al. (1999) Samani and Plewes (2001a) (2004) Samani et al. (2007) Umemoto et al. (2014) Matsumura et al. (2009)

4. Mechanical Properties of Breast Tissue

Over the past decades, several research works were performed to characterize the biomechanical properties of soft tissues, which are subject to some degree of mechanical activity (Fung 1993). However, very limited quantitative information is available on the biomechanical properties of soft tissues, which do not have an active mechanical function such as the breast (Samani et al. 2007).

Several studies (Cox and Erler 2011; Samani et al. 2007; Unlu et al. 2010; Wellman et al. 1999; Krouskop et al. 1998; Jurvelin et al. 2002) have quantified the mechanical properties of the breast constituents using Young's moduli to relate the stiffness to the type of tissue. These studies have shown that tumours are much stiffer than normal breast tissues. This occurs because the tumour tissues undergo collagen remodeling which leads to stiffening. According to Lopez et al. (2011) "the oriented, thickened collagen fibers along whose mammary gland tumour cells have been seen to migrate are indeed a source of the ECM stiffening". In order to develop tractable mathematical models, from which material properties can be extracted, several researches (Azar et al. 2002, 2001;Krouskop et al.1998; Kruse et al. 2000; Thomas et al. 2006; Wellman et al. 1999; Zhang et al. 1997) assumed that the different types of tissue (fat, glandular and fibrous tissue) can be modeled as homogeneous, and that their behaviour under compression is approximately isotropic and nearly incompressible (Fung 1993). Since soft biological tissue is predominately composed of water - an incompressible fluid - it is considered incompressible (Fung 1993). With these assumptions, it is possible to model the behaviour of the tissue using a single elastic or shear modulus. Several authors (Samani et al. 2007; Wellman et al. 1999; Baki 2000; Krouskop et al. 1998; Azar et al. 2001,2002) considered that the incompressibility condition imply that the Poisson ration is 0.5, which means if compressive load is applied in axial direction the material

expands in other two directions with a ratio of 0.5 with respect to the compression axis (Gefen and Dilmoney 2007).

Under these assumptions, Sarvazyan et al (1994) presented results in which they measured the elastic modulus of 168 *ex vivo* specimens of normal, fibroadenomatous and tumour breast tissues. Reported Young's modulus values ranged from 2.0 kPa for normal tissue and 15.0 kPa for invasive ductal carcinomas. However, these authors did not describe in detail their measurement system, so it is hard to identify the source of the observed differences.

Sarvazyan et al (1995) reported a study of 150 specimens of normal, fibroadenomatous and cancerous tissues. They showed that fibroadenomas are typically 4 times as stiff as normal tissue, while tumours can be as much as 7 times stiffer.

Krouskop et al (1998) measured the elastic moduli of 142 ex-vivo samples of normal and pathological breast tissues. The study concluded that the Young's moduli of the breast tissues is highly dependent on the level of tissue pre-load compression used in the measurement, in other words, the moduli increased significantly with additional compression. For example, at 5% pre-load compression strain he found that the ratio of the elastic modulus of tumour tissue to that of fat was 5:1, while at 20% pre-load compression strain the ratio grew to 25:1. The same authors observed that the modulus of adipose breast tissue is relatively constant over the range of loadings studied. For the ductal carcinoma the modulus is low at low strain; it is indistinguishable from fat at the low strain range but at the high strain range, the modulus is larger than any of the normal tissues. The invasive ductal carcinomas are very stiff and the modulus of this tissue is higher than the other tissues at both strain ranges tested. In conclusion, the modulus dependency on pre-load compression confirms that the nonlinear elastic behaviour is often observed in biological tissues (Wellman et al. 1999; Krouskop et al. 1998). Krouskop et al (1998) found that tumour tissue is not only much stiffer than adipose and normal glandular tissue, but displays a higher non-linear increase in stiffness. Recent studies by Barr and Zhang (2012) contradict the results of Krouskop et al (1998). This evidence demonstrated that different levels of pre-load compression applied to adipose tissue lead to a different mechanical behaviour. The results obtained by Krouskop et al (1998) most likely occurred because the samples were not confined to a limited volume, as seen

in a normal breast tissue. Wellman et al (1999) studied the stiffness of 26 samples of adipose tissue, 7 of fibroglandular tissue, 1 of ductal carcinoma in situ (DCIS) and 25 of invasive ductal carcinoma (DCI). The authors reported a wide scatter in the mechanical behaviour of the various tissue samples tested. For example, at 1% pre-load compression strain, it was found that the stiffness ratio of tumour tissue to that of the other normal tissues was 10:1, while at 15% pre-load compression strain the ratio increased to approximately 50:1. Comparing the stiffness of the tumour tissue with adipose and normal glandular tissue the study concludes that tumour tissue is 10 times as stiff as normal fat at 1% strain, and more than 70 times as stiff at 15% strain, while the stiffness in the tumour tissue to glandular is more than 2.5 times as stiff at 1% strain and approximately 5 times as stiff at 15% strain.

Samani et al (2007) developed two different methods to measure tissue elasticity. The authors tested 169 *ex vivo* breast tissue samples, including fat, fibroglandular tissue as well as benign and malignant breast tumour types. They reported that fat and fibroglandular tissues exhibit identical mechanical properties, with a Young's modulus of 3.25 kPa under small strains. Tumour tissues data obtained by Samani et al (2007) show a substantially higher Young's modulus than fibroglandular tissue, compared to the data of Sarvazyan et al (1995). Moreover, the authors observed a general increase in the elastic modulus with more invasive cancers, when compared with other type of tumours. Thus, for high-grade invasive ductal carcinomas were the stiffest tumours exhibiting a Young's modulus approximately 13 fold larger than either fat or fibroglandular tissue, with other tumours types demonstrating a 3–6-fold increase in tissue stiffness. In Table 2, it is noted that the values the standard deviation is high in some cases, e.g. high-grade IDC (12.47). This can be attributed to a number of factors including having a small statistical sample (for example in high-grade IDC has only 9), systematic errors associated with the measurement techniques and used FE models, tissue heterogeneity and finally to the variability of tissue stiffness during menstrual phase and for different age groups. Although there is a similarity with the results of Sarvazyan et al (1995), there is no correlation with data from Baki (2000) and Krouskop et al (1998). In general, the authors obtained smaller Young's modulus values compared to the values obtained by Krouskop et al (1998), which makes clear the Young's

modulus variation observed between the studies. These disagreements may arise due to the method of pre-load compression chosen and preparation of samples by these two studies. Examples of these differences can be: using substantially larger compression forces for preloading; and ignoring tissue specimen heterogeneity.

Matsumura et al (2009), measured the elastic moduli, with different pre strain, of 60 *ex vivo* samples of normal and 27 pathological breast tissues. The authors verified non-linearity in tissue elasticity and difference of Young's modulus in all tissues depending on compression level. For example, DCIS revealed larger stiffness than normal fat or gland under a slight stress, but the relation between them changed when stress increased. The IDC and mucinous carcinoma exhibited significantly larger Young's moduli than normal tissues (fat or gland) and the DCIS. The authors also verified that the elasticity of IDC varies over a wide range of compression.

More recently Umemoto et al (2014) measured the elastic moduli of the 87 surgical tissues, including 33 lesions and normal tissues (fat: 29 locations, mammary gland: 24 locations). As seen in Table 2, the Young's moduli of breast tissues differed under conditions of light stress (<1 kPa), and, in ascending order of their elasticity, the tissues were fat, normal gland and ductal carcinoma in situ (DCIS) and invasive ductal carcinoma (IDC). The rates of increase in elasticity of normal breast tissues with respect to a stress axis from 0.0 to 1.2 kPa are significantly larger than those of malignant tissues, especially in IDC; the Young's moduli of normal breast tissues increase to the point where they come close to or exceed those of malignant tissues. The authors also verified significant difference in non-linearity between DCIS and IDC, especially in the stress-elastic modulus relationships under the minimal stress conditions. The authors concluded that the Young's modulus relationship between normal breast tissues and malignant tumours dramatically changes as stress is applied because of the non-linear properties (see Fig. 7).

Table 2 summarizes the results of mechanical properties of the *ex vivo* breast tissue obtained by different authors.

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Table 2 A Summary of the results from mechanical testing of *ex vivo* breast tissue.

Author	Pre-strain (compression)	Young´s Modulus (kPa) Mean (STD)			
		Normal fat tissue	Normal Glandular tissue	Tumour tissue	
				DCIS	IDC
Krouskop et al. (1998) (Loading frequency (Hz) of 0.1 to 4)	**5% pre-load compression**	18 (7) to 22 (12)	28 (14) to 35 (14)	22 (8) to 26 (5)	106 (32) to 112 (43)
	20% pre-load compression	20 (8) to 24 (6)	48 (15) to 66 (17)	291 (67) to 307 (78)	558(180) to 460(178)
Wellman et al. (1999)	**1% strain**	4.8 (2.5)	17.5 (8.6) Fibroglandular sample	71.2 (0.0)	47.1(19.8)
	15% strain	17.4 (8.4)	271.8 (67.7) Fibroglandular sample	2162 (0.0)	1366.5(348.2)
Samani and Plewes (2001a); Samani et al (2007)	**5% Compression**	3.25 (0.9)	3.24 (0.61) Fibroglandular sample	16.38 (1.55)	L:10.4 (2.6) M: 19.99 (4.2) H:42.5(12.47) Data is provided for low, medium and high-grade IDC
Sarvazyan et al. (1995)	**Not given**	5 (0.0)	50 (0.0)	100 (0.0) to 5000 (0.0) for palpable nodule	

Sarvazyan et al. (1994)	**Not Given**	1.0 (0.5) data is given for a combined fatty and fibroglandular sample		3.5 (0.5)	10.0(1.9)
Matsumura et al. (2009)	**0-0.2 Stress**	0.7 (0.2)	0.8 (0.2)	3.4 (1.3)	11.5 (8.4)
	1.0-1.2 Stress	17.3 (4.8)	15.4 (3.9)	15.6 (2.0)	27.0(9.2)
Umemoto et al. (2014)	**0-0.2 Stress**	0.69 (0.19)	0.73 (0.18)	5.25 (0.46)	13.82 (9.60)
	1.0-1.2 Stress	19.08 (4.99)	16.99 (4.92)	16.15 (4.24)	30.5 (11.46)

As can be seen in Sec. 3.1, breast tissue's elastic modulus can be measured *in vivo* using magnetic resonance elastography.

Lawrence et al (1998), were among the first to study *in vivo* breast MRE. A total of nine healthy female volunteers have been evaluated, and demonstrated that MRE is feasible and can adequately illuminate the breast tissues with shear waves and can characterize biomechanical properties of glandular tissue (2.45 ± 0.2 kPa) and fat tissue (0.43 ± 0.07 kPa).

Kruse et al (2000) presented preliminary results from an *in vivo* MRE exam of a patient with a biopsy-proven carcinoma. Showed that a localized area roughly two to three times stiffer than the surrounding fibrous tissues corresponds to a biopsy proven tumour.

Similarly, Sinkus et al (2000) reported that carcinoma exhibits an anisotropic elasticity distribution while the surrounding benign tissue appears isotropic. The results obtained *in vivo* revealed increases in stiffness of roughly two to three times between background tissue and lesions. Van Houten et al (2003) separated the properties of the adipose and fibroglandular tissue within the breast by manual segmentation. The authors concluded that the adipose tissue has lower Young's moduli compared to other tissues. Srivastava et al (2011) measured the mechanical properties for a normal, malignant and benign breast tissue. These authors reported that Young´s modulus for malignant breast tissue samples are approximately four times higher than that of the normal tissues, while for benign tissue samples it is about two times higher than that of the normal samples. The data reported is, consistent with previous studies, like Krouskop et al (1998), Wellman et al (1999), and Samani et al (2007).

McKnight et al (2002) studied six healthy volunteers and six patients with biopsy-proven palpable breast malignancies, and concluded that the average shear stiffness of the tumours was 33 kPa (range = 18–94 kPa), which was about four times greater than that of adipose tissue (mean = 8 kPa, range = 4–16 kPa) in breast cancer patients. In the healthy volunteers, the mean value for adipose tissue was 3.3 ± 1.9 kPa, which is less than their fibroglandular tissue (7.5 ± 3.6 kPa).

Xydeas et al (2005) studied viscosity and elasticity of breast tissues in five patients with six malignant lesions, eleven patients with benign lesions, and four patients with no lesions using MRE. The aim of the study was to investigate the potential value of MRE to improve the differentiation between benign and malignant tumours. The mean elasticity parameters were: breast cancer (3.1 ± 0.7 kPa), fibroadenoma (1.4 ± 0.5 kPa), fibrocystic changes (1.7 ± 0.8 kPa) and surrounding tissue (1.2 ± 0.2 kPa). According to the study, malignant tumours documented higher values of elasticity than benign corresponding to signal intensity and morphologic data. Table 3 summarizes some results from *in vivo* MRE elastography.

Sayed et al (2013) used multi-compression 3D ultrasound elastography and demonstrated the ability of the technique to better diagnose stiff masses inside breast tissue. The results obtained *in vivo* revealed the target mass was approximately 6.3 times stiffer than the background soft tissue.

These results were compared with biopsy diagnosis, and showed a good agreement with biopsy outcomes.

It should be noted that normally the stress distribution is not uniform within the body and the tissue elasticity is nonlinear. According with tissue nonlinearity, the Young's modulus tends to increase when the compression is intensified as shown in Fig. 7.

A recent study tested four regions of pre-load compression (Region: A 0-10%; B 10-25%; C 25-40%; D >40%) that explain clinical elastographic results (Barr and Zhang 2012). It was concluded that, when the degree of compression is slight, 10% tissue compression approximately, the difference in the Young's modulus between breast tissue and tumour tissue is large and consequently the tumour tissue is clearly identified on a relatively low-strain region. But for high compression levels (about 40%), the stiffness of the breast tissue will increase, and the difference from the tumour tissue will be smaller. It is recommended that all clinical images are obtained approximately at a level of 10% pre-load compression.

Fig. 7. Behaviour of breast tissue at different levels of pre-load compression. Adapted from (Barr and Zhang 2012; Shiina 2013; Umemoto et al. 2014)

To counter this effect Cheng et al (2013) developed a preliminary study with a novel non-compressive breast MRE setup. This study was performed with seven healthy female volunteers and one female patient with a biopsy-proven invasive ductal carcinoma. For the seven volunteers the stiffness of tissue ranged from 0.25 to 0.41 (mean = 0.33) kPa for adipose tissue, and from 0.46 to 0.9 (mean = 0.64) kPa for glandular tissue. For the other patient the stiffness of adipose tissue was 0.41 ± 0.10 kPa and of glandular tissue was 0.90 ± 0.18 kPa. The invasive ductal carcinoma was stiffer, 1.42 ± 0.17 kPa, as show in table 3. The invasive ductal carcinoma is about 3 times stiffer than the adipose tissue and 1.5 times stiffer than the glandular tissue.

Based on the data collected from the analyzed studies, the following conclusions were achieved:

-The stress–strain curves of the breast tissues, describing the mechanical behaviour of the tissue under different stress levels, follow an exponential behaviour, with malignant masses showing a steeper curve than the benign tissues.

-The moduli of elasticity of the fibrous, glandular and tumour tissue are significantly higher than the adipose tissue, and are not constant along the studied strain variations. The fat tissue behaviour is closer to linear than all other tissues measured.

-There was (Wellman et al. 1999; Krouskop et al. 1998) a dependency of the mechanical properties with the technique used: if an image of the elastic modulus distribution throughout the breast, was obtained at one strain level and then the strain level was doubled, the whole of the tissue compressed would suffer an increase in stiffness. Thus, the Young's modulus of breast tissues is highly dependent on the level of tissue pre-load compression, and the relative stiffness is a good predictor of histological diagnosis.

The results of these studies clearly indicate a wide variation in moduli not only among different types of tissue but also within each tissue type. These differences were most evident in normal fat and fibroglandular tissues.

The research works reviewed, used different techniques for estimating the tissue stiffness distribution within a breast. However there is surprisingly little available information in the literature on the mechanical properties that would allow conclusions about the histological nature of the tissue directly from the estimated stiffness.

Table 3 A summary of results from *in vivo* magnetic resonance elastography for breast tissue.

	Elastic Modulus (kPa) Mean (STD)		
Author	**Normal fat tissue**	**Normal Glandular tissue**	**Tumour tissue**
Kruse et al. (2000) (Frequency of 100Hz)	15 to 25	30 to 45	50 to 75 for carcinoma
Sinkus et al. (2000) (Frequency of 60Hz)	0.5 to 1	2 to 2.5	3.5 to 4 for carcinoma

McKnight et al. (2002) (Frequency of 100Hz)	3.3	7.5	25
Van Houten et al. (2003)	23.5 (4.03)	26.6 (4.49)	----------
Lawrence et al. (1998) (Frequency of 50-100 Hz)	0.43 (0.07)	2.45 (0.2)	----------
Cheng et al. (2013) (Without compression)	0.41 (0.10)	0.90 (0.18)	1.42 (0.17) for ductal carcinoma
Xydeas et al. (2005) (Frequency of 65 Hz)	1.2 (0.2)	1.2 (0.2)	3.1 (0.7) for breast
Srivastava et al. (2011)	4.17 (0.074)		16.45 (1.103) for invasive ductal carcinoma

5. Discussion and Conclusions

595 One of the main motivations for evaluating the mechanical properties of breast tissue is its potential for disease assessment applications. Normally, the tissue tends to stiffen with the disease. These modifications result in a restructuring of the normal tissue components, which manifests itself as a change in the elastic modulus of tissue - the common mechanical property used for evaluation (Buckley et al. 1988). Thus, the studies performed have been focused on the 600 measurement of breast tissue stiffness through the elasticity moduli. On the other hand, a

comprehensive knowledge of the mechanical properties for glandular and adipose tissue is not yet available in the literature, which explains the lack of recent articles in this review. Although all these studies were made to visualize the distribution of stiffness within the breast, there are few studies on the mechanical properties aimed at understanding the histological nature of the tissue directly from the estimated stiffness.

Table 2 and 3 show the mechanical properties range reported by different authors. As can be seen in the tables, there is a significant variability which makes it difficult to use statistical data to model the individual properties of the breast. This variability is highly dependent on several types of pre-load compression. In summary, Young's moduli of normal breast tissue increased dramatically with increasing compression. As for the DCIS (ductal carcinoma in situ) specimens, their elastic moduli becomes close to those of normal breast tissues, as the stress applied increases under higher compression. Moreover, these studies showed a general increase in the elastic modulus associated with more invasive carcinoma. As a consequence, Young's moduli measured for invasive carcinoma specimens exhibited greater variation than those for normal tissues. Variation must have its roots on the complex pathologic structure of the tissue i.e., the heterogeneous mixture of cellular and fibro stromal components. Thus, the elasticity measurements clearly indicate that each tissue in the breast exhibits different non-linear characteristics in stress versus elastic modulus relationships under light compression conditions. Characterization of the mechanical behaviour of the breast requires a combination of experimental techniques, specialized software particularly regarding the compression levels used. This approach is of capital importance to predict deformations accurately using biomechanical simulation models such as FEM models.

By analyzing table 2, the results of Krouskop et al (1998) were clear relatively to: Young's moduli variation between different tissues of the breast, and Young's moduli increase with the initial condition of strain applied (i.e., percentage of pre-load compression). In comparison, the Young's moduli measured for adipose, normal gland, DCIS and IDC in several authors, such as, Samani et al (2007), Matsumura et al (2009) and Umemoto et al (2014) tended to be smaller than those reported by Krouskop et al. (1998). The observed disagreements in this case are attributed to the

fact that, in their measurement, Krouskop et al (1998) applied substantial pre-load compression of 5% and 20%, and consequently the authors did not describe their initial stress conditions fully. However, it can be speculated that the differences in Young's moduli were originated from the different stresses applied to the specimen. It was also presumed that the stress used in studies described by Matsumura et al (2009) and Umemoto et al (2014) was lower than the stress used by Krouskop et al (1998).

Similar results to Krouskop et al (1998) have been shown by Wellman et al (1999). Both studies obtained higher Young's when compared with the other studies. Matsumura et al (2009) and Umemoto et al (2014) used a similar protocol testing (same stress), which found very similar results for the different breast and tumour tissues. They concluded that the results revealed a reduction or inversion in the difference of Young's moduli between normal and tumour tissues with increasing stress.

By comparing the results by Samani et al (2007) with those reported by Sarvazyan et al (1994), it was verified that some of the results were in accordance while others show Young's modulus generally smaller. For example, Samani et al (2007) reported Young's modulus values of approximately 3.25 kPa and 19.99 kPa for normal tissues and IDC, respectively, which are fairly well compared with the 2.0 kPa and 15.0 kPa that obtained by Sarvazyan et al (1994). However, the results described by the other authors in Table 2 are different when comparing with Sarvazyan et al (1994) and Sarvazyan et al (1995). So, it is important to refer that Sarvazyan et al (1994) and Sarvazyan et al (1995) did not reported details of their measurement system, thus it is hard to speculate the source of the observed disagreements.

The data reported in Table 3 is, consistent with previous studies, like Krouskop et al (1998), Wellman et al (1999), Samani et al (2007), Matsumura et al (2009) and Umemoto et al (2014). The results of these studies indicate a wide variation in elastic moduli not only among different types of tissue but also within each tissue type.

Although the studies from Kruse et al (2000) and McKnight et al (2002) used a similar elasticity imaging technique (frequency at 100Hz), the results for the various tissues were different. For example, Kruse et al (2000) reported values of approximately 15 kPa and 50 kPa for fat tissues

and tumour, respectively, which are different compared with the 3.0 kPa and 25.0 kPa obtained by McKnight et al (2002). Xydeas et al. (2005), Sinkus et al. (2000) and Lawrence et al. (1998) shown a similar results for normal tissue (fat and glandular tissues). It is important to note that the variability of the results reported by MRE elastography may be associated with the variability in the test procedure (such as the different shear wave frequencies applied).

Until now researchers have used different approaches to estimate the mechanical properties of soft biological tissue. The differences in stiffness between normal and abnormal breast tissue have been recognized for a long time (Ophir et al. 1991). To analyze large deformations (ex. pre-strains up to 20%) the *ex vivo* tests are the most suitable. However, *in vivo* data is only collected under small pre-strain conditions and often the pre-strain used is not recorded. Considering this limitation, *in vivo* data is of limited usefulness for modelling large deformations of the breast. Often, the force information is discarded during the test to estimate the mechanical properties of the tissue because it is applied as an adjunct to existing imaging modalities. Thus, it is necessary to establish a method to measure large deformations of tumour tissue and its nonlinear elastic behaviour with accuracy. Typically these techniques make images of the tissue at two different applied loads and compute a displacement field from them. This displacement field is then used to infer the stiffness of the tissue, from assumptions made about the stress field. Basically the pre-load compression has a considerable effect on the quality and results of elastography. For example, the breast elastography is very susceptible to pre-load compression because the chest wall acts as a hard posterior surface, allowing for substantial pre-load compression when scanning. The effects of pre-load compression are significant in the breast and can easily affect test outcomes (benign versus malignant). A clear example is referred by Matsumura et al (2009), which showed that DCIS cannot be sometimes easily detected (i.e. false negatives) at excessive breast compression in clinical exam on elastography. This limitation highlights the need to quantify the preload for compression magnitude (strain or stress) in various breast and tumour tissues. Umemoto et al (2014) understood this need and measured the compression magnitude through loaded stress on the tissue sample in compression test. Thereby showing quantitatively the relationship between the magnitude of compression and tissue elasticity (Young's modulus)

in the target lesion. Therefore, the importance of nonlinear responses of soft tissue to compressive loads in clinical breast examination highlights the need for launching a comprehensive study on the hyperelastic characterization of *ex vivo* and *in vivo* soft tissues, to enhance clinical approaches including the detection of breast cancer.

Despite all available results from compression experiments, until now there is no data available about the material properties of the breast under uniaxial or biaxial tensile loading conditions (because of its fragile constitution). Recently, Sommer et al. (2013) performed tests in human abdominal adipose tissue by biaxial tensile and triaxial shear tests. This experimental attempt to understand the anisotropy in the properties of the adipose tissue produced promising results. Adipose tissue was characterized as a nonlinear, anisotropic and viscoelastic soft biological material. These tests are a new approach to study the breast adipose tissue. None of the experiments reported takes into account the pre-strain caused by gravity, hydration and tissue fibers. It is a limitation of *ex vivo* tests, contrary to *in vivo* tests where the measurements are performed in the natural state, i.e., the blood supply and interstitial fluids are present. When compared to the *ex vivo* tests, *in vivo* tests are used as a diagnostic tool that help assessing the changes in the mechanical properties of the tissues under compression in a simple and non-invasive way. As they can separate tumours from adjacent healthy tissues and distinguish if the tumour is malignant or benign according to their stiffness, these tests are valuable for characterizing the mechanical properties of the different breast tissues due to their high degree of specificity and sensitivity (McKnight et al. 2002; Sinkus et al. 2000; Srivastava et al. 2011; Van Houten et al. 2003; Mariappan et al. 2010; Kruse et al. 2000; Korte and Steen 2002; Manduca et al. 1997).

The high Young´s modulus variability reported by several studies is directly correlated with the use of different mechanical tests, experimental conditions (*in vivo* or *ex vivo*), different pre-load compression, tissue heterogeneity and systematic errors associated with the measurement techniques. Some of these errors may be introduced due to the blood supply and interstitial fluids absence during the tests, although these are efforts to keep the samples hydrated. The other source

of error could be associated to the location where the tissue samples were removed. In addition, it is expected to see different measures of firmness in the same tissue.

Regarding the different pre-load compressions, the Young's modulus between breast tissue and tumour tissue is large when the degree of compression is slight. However, when the compression is too strong, the stiffness of the breast tissue will increase, and the difference from the tumour tissue will be smaller (Barr and Zhang 2012; Shiina 2013). This compression effects partly explains the inconsistencies in the reported stiffness values of breast tissues from different methods in the literature (Abdullah et al. 2009; Barr and Zhang 2012; McKnight et al. 2002; Sadigh et al. 2012; Shiina 2013; Sudhakar et al. 2014).

Another feature is that the mechanical properties of breast tissues differ between individuals and over time due to the variability in breast morphology, hormonal status, age, and physiological condition (Srivastava et al. 2011). Despite of in the majority of the studies the authors refer a range of age of the samples, there was a lack of information regarding some important factors such as pre or post menopause, menstrual cycle and so on, which could have influence in the experimental results. For example, Lorenzen et al (2003) found that fibroglandular tissue roughly doubled in stiffness during the menstrual cycle. Therefore, future studies should include these factors in order to understand the variations of breast tissue in the several stages of the women's life.

Glandular, adipose and fibrous tissues are the main tissues of the breast that have been studied to estimate their mechanical properties. There are no studies to date of the suspensory cooper's ligaments (they provide support and hold the breasts in place). So, it is necessary to develop techniques to test the suspensory cooper´s ligaments. This effort can contribute to establish a methodology based on the finite element method to simulate a realistic 3D model of the breast. Thus, all knowledge on the mechanical properties of the breast tissue is important for studying the effect of plastic and oncoplastic surgery techniques in breast reconstruction, as well as for design of cosmetic breast implants.

In conclusion, it was possible to verify that the difference in mechanical behaviour between tissues, provides useful information with potential impact on clinical diagnosis. The development

in experimental protocols led to an improvement of clinical diagnosis. In order words, better experimental protocols led to a refinement of the compression magnitude to apply in clinical examination. This procedure is fundamental to avoid false-negatives.

The mechanical tests of soft biological tissues require a test system suitable to the specificity of these materials. The determination of mechanical properties can be used to: correlate the mechanical behaviour with pathology (eg cancer) or with population characteristics (age, menopause, lactation, etc...) and to simulate the biomechanics of the breast tissue. Further research is therefore needed to: (1) integrate the etiological factors influencing the biomechanical proprieties of breast tissues, such as age, body mass index or hormonal status (menopause); (2) characterize all tissues, including the suspensory cooper's ligaments; (3) build experimental set-ups that includes *in vivo* and *ex vivo* testing in order to validate the results; (4) standardizing the experimental protocol, in order to analyze samples from the same breast location; (5) controlling the amount of pre-load compression (for instance, test two levels of pre strain, a proper and a higher level used in clinical breast examination). Because the pre-load compression is a substantial factor in obtaining accurate results.

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Declaration of Conflicting Interests

None declared.

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Captions

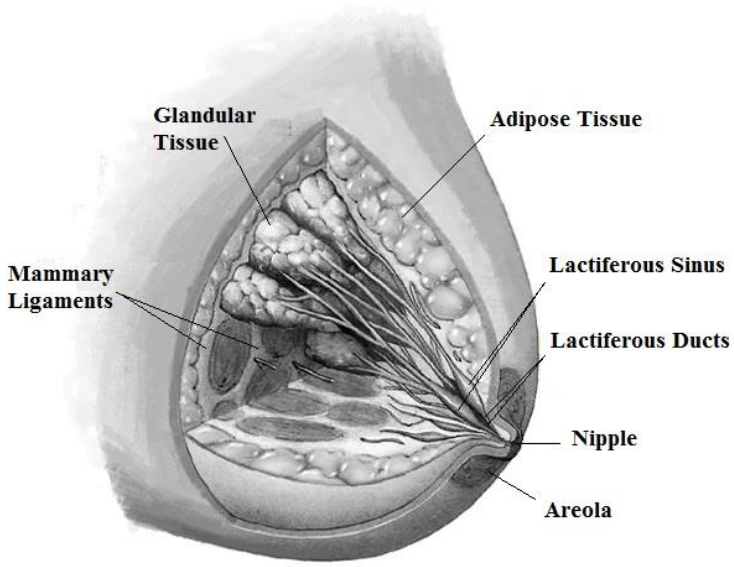

Fig.1. Anatomy of Breast.

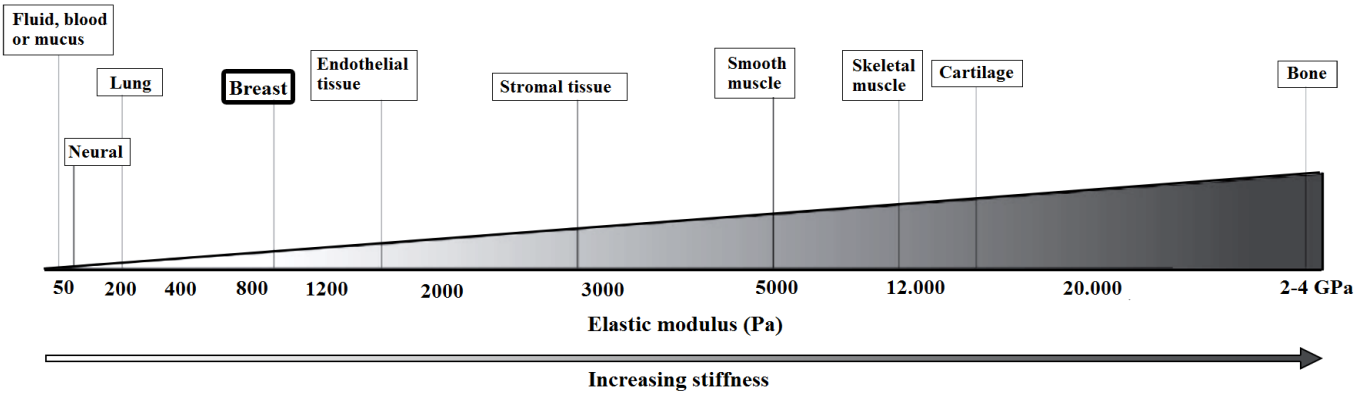

Fig. 2. Stiffness in different soft tissue**.** Adapted from (Cox and Erler 2011)**.**

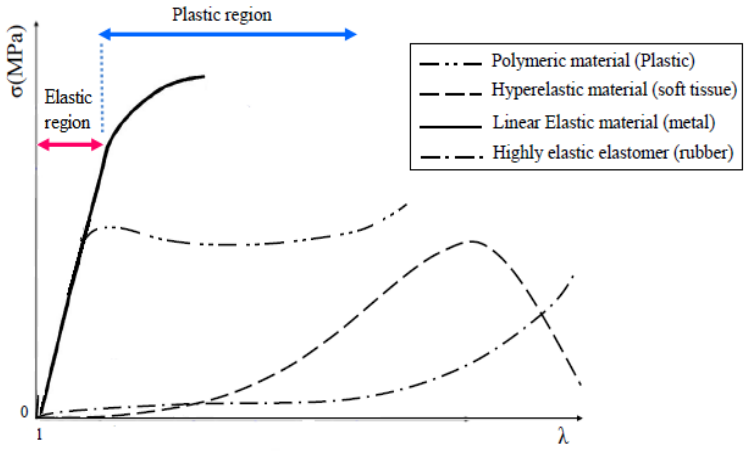

Fig.3. Mechanical behaviour of linear elastic and hyperelastic materials

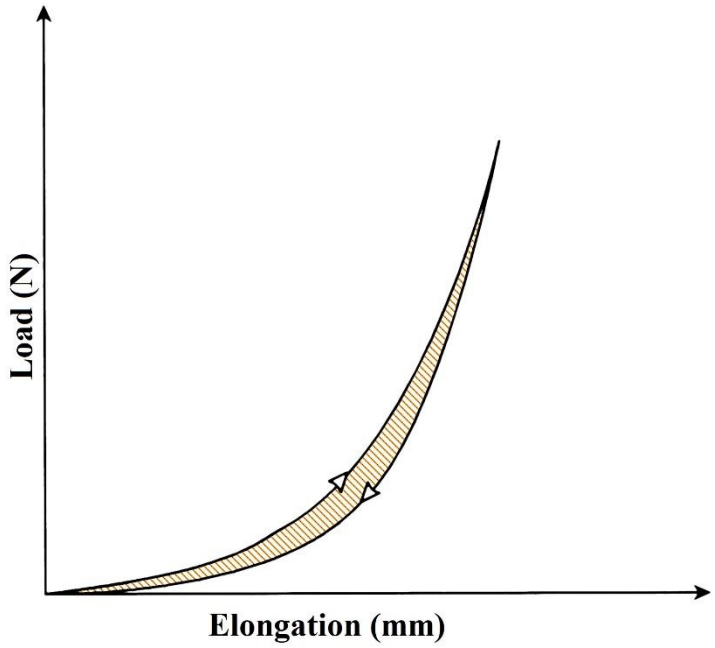

Fig. 4. The dashed is a hyteresis loop and shows the amount of energy lost (as heat) in a loading and unloading cycle. Adapted from (Fung 1993).

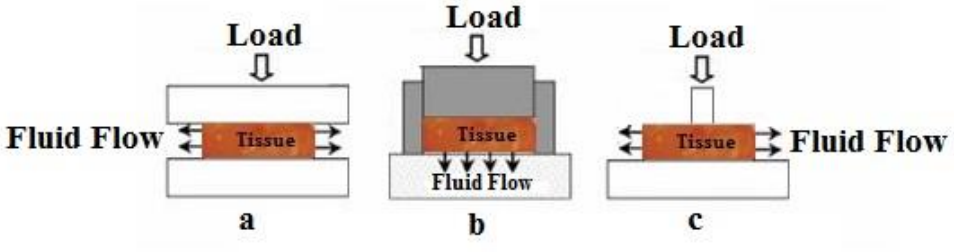

Fig. 5. (a) Unconfined compression, (b) Confined compression and (c) Indentation test

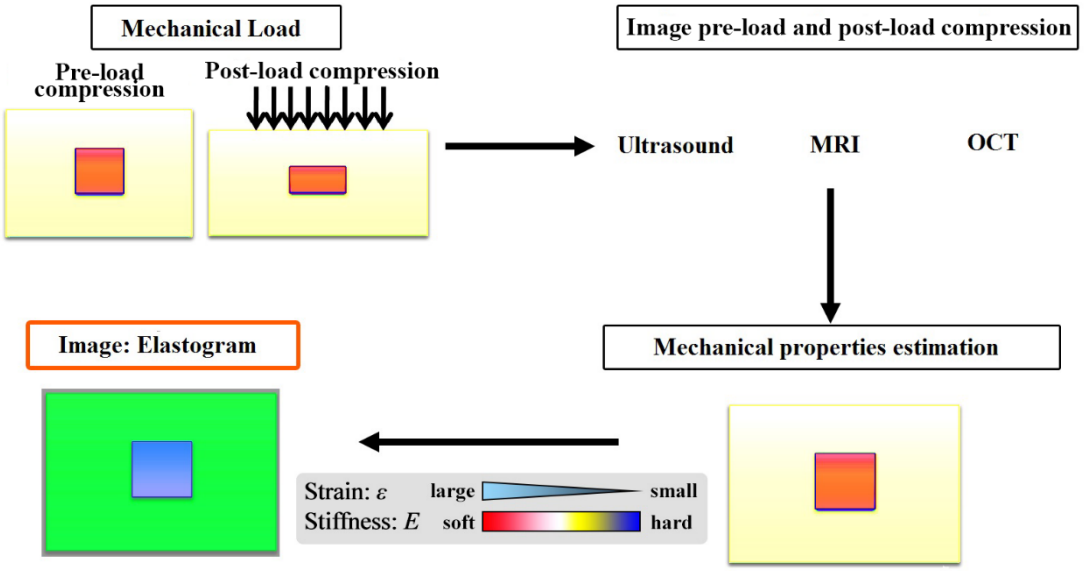

Fig.6. An overview of elasticity imaging methods. Adapted from (Shiina 2013; Sudhakar et al. 2014).

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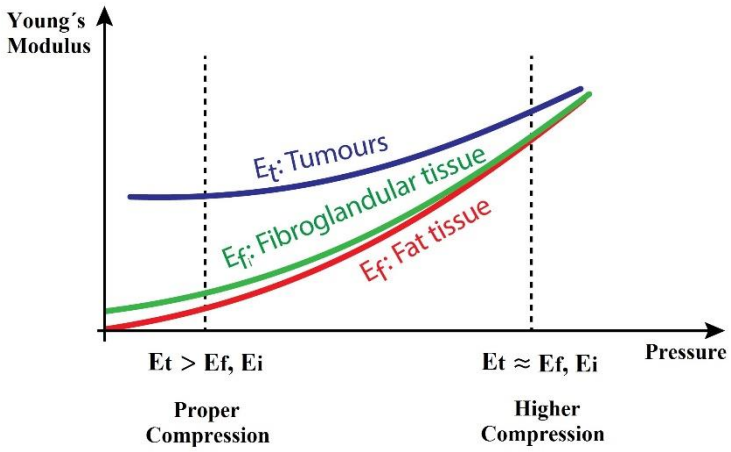

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Fig.7. Behaviour of breast tissue at different levels of pre-load compression. Adapted from (Barr and Zhang 2012; Shiina 2013; Umemoto et al. 2014)

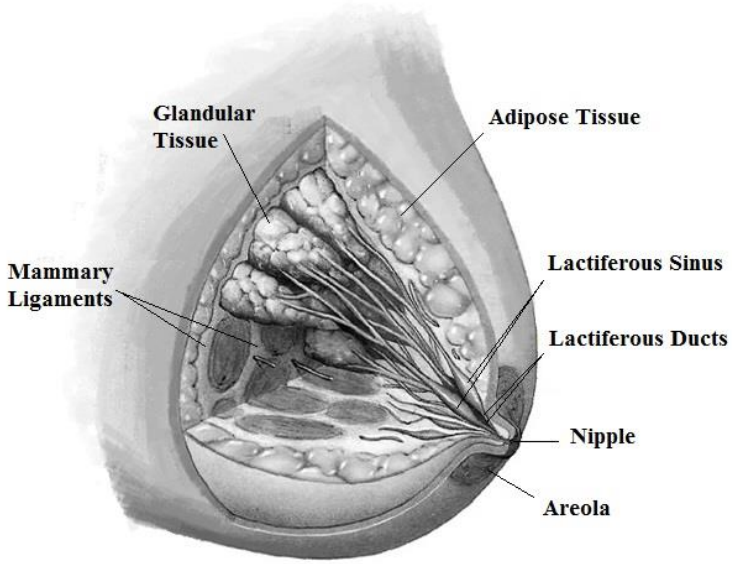

Fig.1. Anatomy of Breast.

Figure 2

Click here to download Figure Fig2.docx

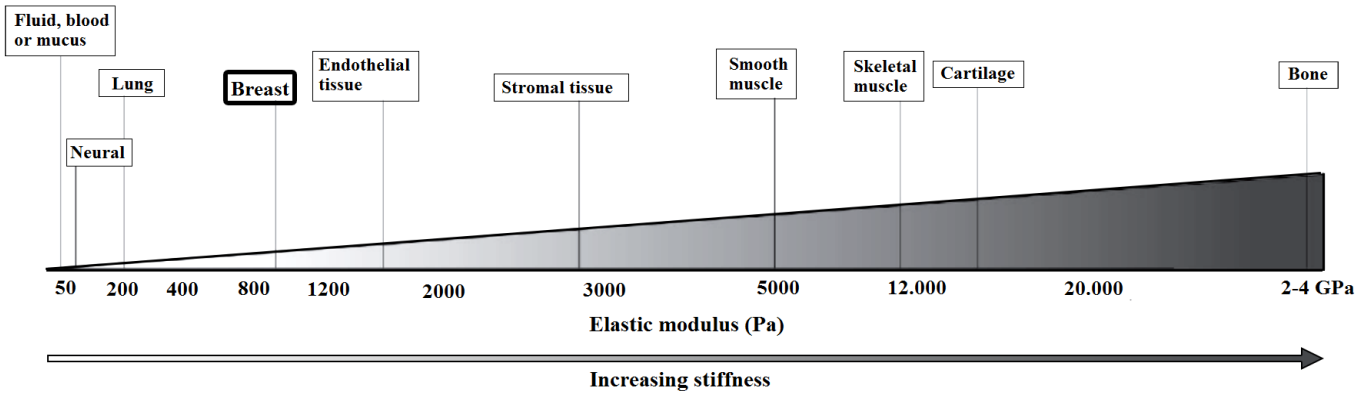

Fig. 2. Stiffness in different soft tissue**.** Adapted from (Cox and Erler 2011)**.**

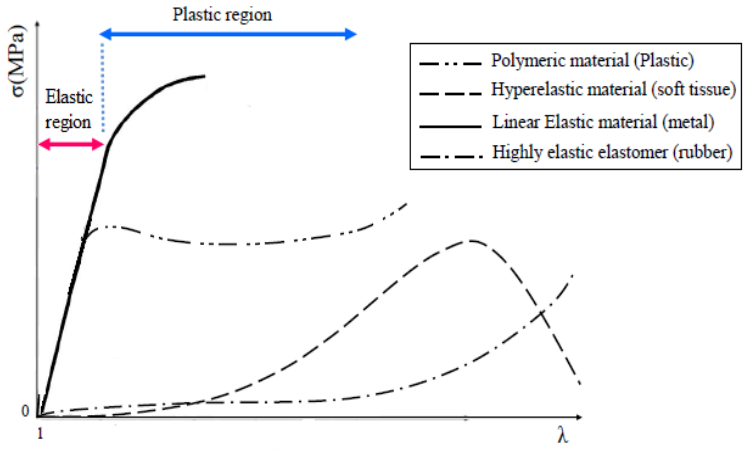

Fig.3. Mechanical behaviour of linear elastic and hyperelastic materials

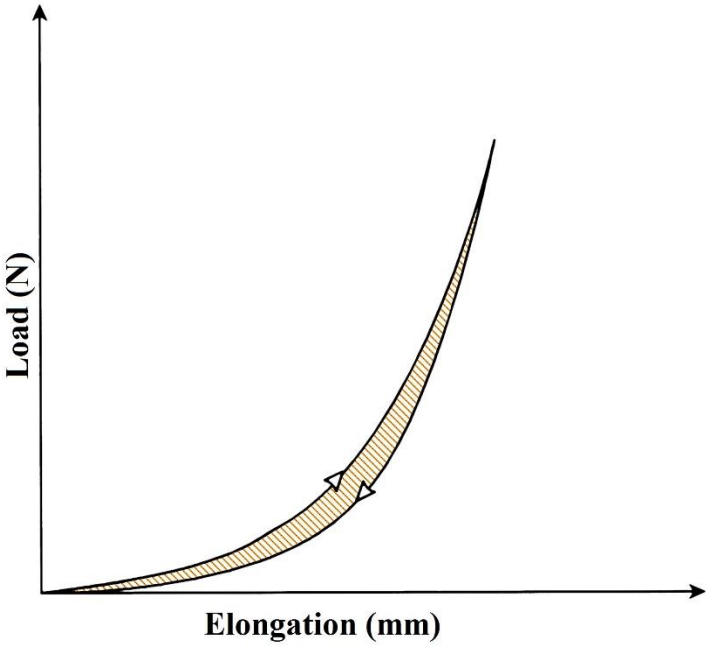

Fig. 4. The dashed is a hyteresis loop and shows the amount of energy lost (as heat) in a loading and unloading cycle. Adapted from (Fung 1993).

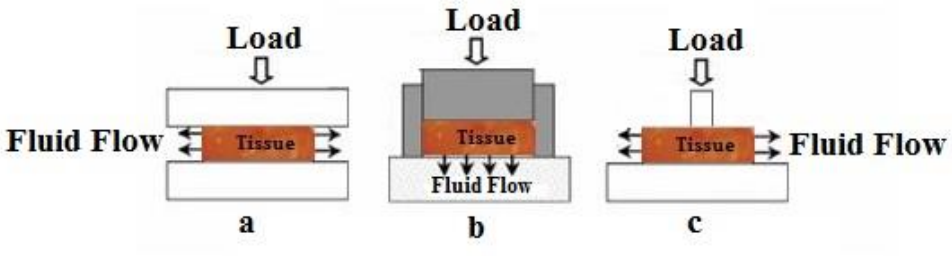

Fig. 5. (a) Unconfined compression, (b) Confined compression and (c) Indentation test

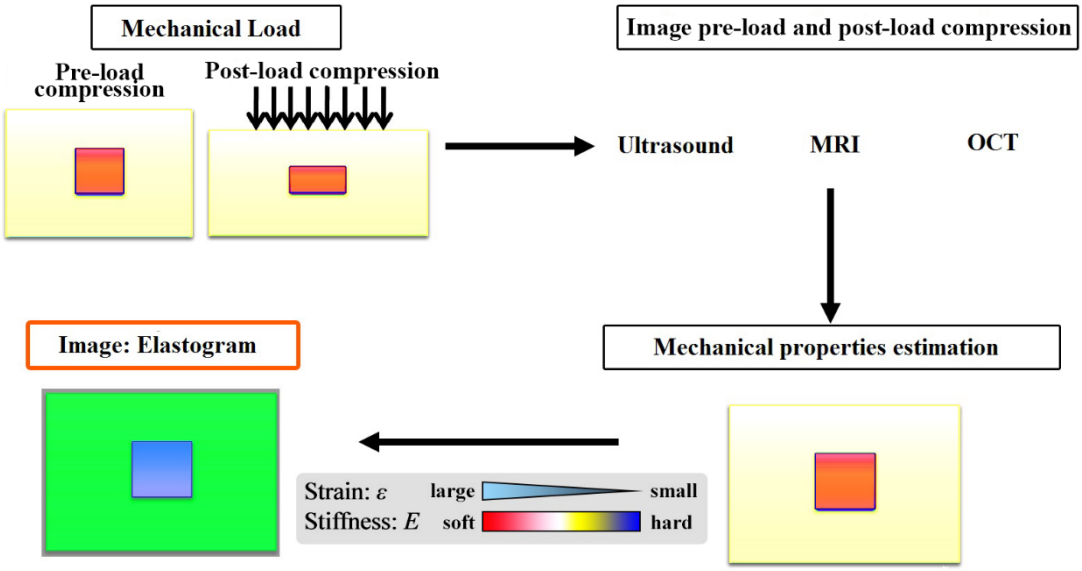

Fig.6. An overview of elasticity imaging methods. Adapted from (Shiina 2013; Sudhakar et al. 2014).

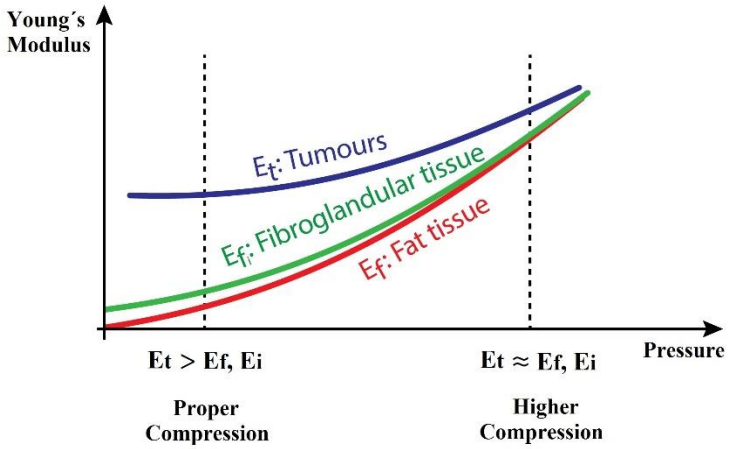

Fig.7. Behaviour of breast tissue at different levels of pre-load compression. Adapted from (Barr and Zhang 2012; Shiina 2013; Umemoto et al. 2014)

Table 1 Mechanical tests for the breast tissue reported in literature, grouped according with vital state of the subject (*in vivo* / *ex vivo*) and testing techniques.

Mechanical tests	Experimental Condition	Author
Compression/ultrasound Elastography	*In vivo*	J. Ophir et al. (1991) Garra et al. (1997) Hiltawsky et al. (2001) Thomas et al. (2006)
Magnetic resonance elastography	*In vivo*	Sinkus et al. (2000a) (2000) (2005) Plewes et al. (2000) McKnight et al. (2002) Van Houten et al. (2003) Manduca et al. (1997) Kruse et al. (2000) Lorenzen et al. (2001) Siegmann et al. (2010) Lawrence et al. (1998) Xydeas et al. (2005) Cheng et al. (2011) (2013)
Optical coherence tomographic elastography	*In vivo*	Srivastava et al. (2011)
Uniaxial compression and punch indentation	*Ex vivo*	Krouskop et al. (1998) Sarvazyan et al. (1994) (1995) Wellman et al. (1999) Samani and Plewes (2001a) (2004) Samani et al. (2007) Umemoto et al. (2014) Matsumura et al. (2009)

Table 2 A Summary of the results from mechanical testing of *ex vivo* breast tissue.

Author	Pre-strain (compression)	Young´s Modulus (kPa) Mean (STD)			
		Normal fat tissue	Normal Glandular tissue	Tumour tissue	
				DCIS	IDC
Krouskop et al. (1998)	**5% pre-load compression**	18 (7) to 22 (12)	28 (14) to 35 (14)	22 (8) to 26 (5)	106 (32) to 112 (43)
(Loading frequency (Hz) of 0.1 to 4)	**20% pre-load compression**	20 (8) to 24 (6)	48 (15) to 66 (17)	291 (67) to 307 (78)	558(180) to 460(178)
Wellman et al. (1999)	**1% strain**	4.8 (2.5)	17.5 (8.6) Fibroglandular sample	71.2 (0.0)	47.1(19.8)
	15% strain	17.4 (8.4)	271.8 (67.7) Fibroglandular sample	2162 (0.0)	1366.5(348.2)
Samani and Plewes (2001a); Samani et al (2007)	**5% Compression**	3.25 (0.9)	3.24 (0.61) Fibroglandular sample	16.38 (1.55)	L:10.4 (2.6) M: 19.99 (4.2) H:42.5(12.47) Data is provided for low, medium and high-grade IDC
Sarvazyan et al. (1995)	**Not given**	5 (0.0)	50 (0.0)	100 (0.0) to 5000 (0.0) for palpable nodule	

Sarvazyan et al. (1994)	**Not Given**	1.0 (0.5) data is given for a combined fatty and fibroglandular sample		3.5 (0.5)	10.0(1.9)
Matsumura et al. (2009)	**0-0.2 Stress**	0.7 (0.2)	0.8 (0.2)	3.4 (1.3)	11.5 (8.4)
	1.0-1.2 Stress	17.3 (4.8)	15.4 (3.9)	15.6 (2.0)	27.0(9.2)
Umemoto et al. (2014)	**0-0.2 Stress**	0.69 (0.19)	0.73 (0.18)	5.25 (0.46)	13.82 (9.60)
	1.0-1.2 Stress	19.08 (4.99)	16.99 (4.92)	16.15 (4.24)	30.5 (11.46)

Table 3 A summary of results from *in vivo* magnetic resonance elastography for breast tissue.

	Elastic Modulus (kPa) Mean (STD)		
Author	**Normal fat tissue**	**Normal Glandular tissue**	**Tumor tissue**
Kruse et al. (2000) (Frequency of 100Hz)	15 to 25	30 to 45	50 to 75 for carcinoma
Sinkus et al. (2000) (Frequency of 60Hz)	0.5 to 1	2 to 2.5	3.5 to 4 for carcinoma
McKnight et al. (2002) (Frequency of 100Hz)	3.3	7.5	25
Van Houten et al. (2003)	23.5 (4.03)	26.6 (4.49)	----------
Lawrence et al. (1998) (Frequency of 50-100 Hz)	0.43 (0.07)	2.45 (0.2)	----------
Cheng et al. (2013) (Without compression)	0.41 (0.10)	0.90 (0.18)	1.42 (0.17) for ductal carcinoma
Xydeas et al. (2005) (Frequency of 65 Hz)	1.2 (0.2)	1.2 (0.2)	3.1 (0.7) for breast

Srivastava et al. (2011)	4.17 (0.074)	16.45 (1.103) for invasive ductal carcinoma

Revision of the Manuscript *"Biomechanical properties of breast tissue, a state of the art review"*

(BMMBD1 5001 46R1)

Dear Editor,

We are grateful again to the Reviewer for his comments and suggestions that hopefully will help to improve the quality of our paper.

We changed the Ackowledgments section of the manuscript by adding the following text,

"The authors would like to acknowledge the outstanding revision work carried out by the reviewers of the paper. Their constructive criticism was a fundamental contribution to elevate the overall quality of the manuscript."

We tried our best to answer all the comments accordingly. We made revisions based on the comments/suggestions of the Reviewer. They are listed below, followed by our response (clarifications and changes).

The changes performed in the revised manuscript are written in red.

Best regards,

Pedro Martins

Nilza Ramião

Reviewer comments

Reviewer: 1

General comments:

The authors made substantial changes to the manuscript and addressed most issues raised by the reviewers adequately. In addition to minor issues which are listed below, the manuscript still requires substantial editing to correct several stylistic errors.

Authors Reply: The authors are, once more, grateful for the reviewer's comments and suggestions. The manuscript was reviewed and several stylistic errors were corrected.

Comment 1: Therefore, according to the experimental protocol for measuring the mechanical properties of breast tissues mentioned by several authors (Krouskop et al. 1998; Samani et al. 2007; Matsumura et al. 2009; Umemoto et al. 2014) Young' s modulus, E, is analyzed using equation (1) (Krouskop et al. 1998)

.

E=(2(1-ν^2) qa) /w (1)

The above equation is valid for semi-infinite medium only. A fundamental equation of the Young' s modulus, e. g. E = \Delta \sigma / \Delta \epslion, should be used instead. The reviewer recommends that this be followed by: In indentation test where part of the specimen' s surface is indented while forces corresponding to applied indentation displacements are recorded, the slope of force vs. indentation displacement (S) is calculated to estimate the Young' s modulus using the following equation:

E - \kappa S (*)

where \kappa is a conversion factor that depends on the indenter' s geometry, specimen' s geometry and boundary conditions.

Authors Reply: The authors decided to maintain the text and equation (1) since this is the most conventional way of presenting the Young's modulus for indentation tests. However we agree with the Reviewer's comment and added the following sentences,

" … The above equation is valid for semi-infinite medium only. Since the sample's thickness is finite, Samani et al. 2007 developed an interactive inversion Finite Element algorithm used to calculate Yong's modulus according to equation E=kS, where k is a conversion factor that depends on the indenter's geometry, specimen's geometry and boundary conditions and S is the slope of force vs. indentation displacement."

Comment 2: In "The most common mechanical analysis performed is the indentation test discussed ahead in this section. " change ahead to next

Authors Reply: The authors agree with the reviewer's correction of the word, but used the word further instead of next.

“The most common mechanical analysis performed is the indentation test discussed ahead in this section” → The most common mechanical analysis performed is the indentation test further discussed in this section.

Comment 3: In "Thus, according to the main structure of the breast tissues and the main objectives of each study, several authors opted by the compression (Sarvazyan et al. 1994) (unconfined or confined) and indentation tests (Krouskop et al. 1998; Samani et al. 2007; Matsumura et al. 2009; Umemoto et al. 2014; Wellman et al. 1999) . "

a. main structure is ambiguous.

b. opted by should be opted for.

Authors Reply:

a. The authors agree with the reviewer’s comment. Therefore, we change the expression “main structure” to different structures.

b. The authors added “for”, following the reviewer’s suggestions.

Thus, according to the main structure of the breast tissues and the main objectives of each study, several authors opted by the compression (Sarvazyan et al. 1994) (unconfined or confined) and indentation tests (Krouskop et al. 1998; Samani et al. 2007; Matsumura et al. 2009; Umemoto et al. 2014; Wellman et al. 1999). →Thus, according to the different structures of the breast tissues and the main objectives of each study, several authors opted for the compression (Sarvazyan et al. 1994) (unconfined or confined) and indentation tests (Krouskop et al. 1998; Samani et al. 2007; Matsumura et al. 2009; Umemoto et al. 2014; Wellman et al. 1999).

Comment 4: In "The resulting deformation of the external surface is recorded. The slope relating stress with strain (force-displacement) represents the compressive Young' s modulus (E) shown in equation (1) . " should be revised to:

"The resulting deformation of the external surface is recorded. The slope relating force with indentation displacement represents the compressive Young' s modulus (E) according to Equation (*) . " Note that Equation (*) is suggested to be added (see item 1) .

Authors Reply: The authors included the Reviewer’s sugestion in the manuscript,

The resulting deformation of the external surface is recorded. In this case, the fluid flow outside the indenter-tissue contact point is possible in both lateral and axial directions. The slope relating stress with strain (force-displacement) represents the compressive Young's modulus (E) shown in equation (1). Another approach as suggested by Samani et al. 2007 corrects equation (1) using $E = kS$.

Comment 5: Change "There are different methods of elastography depending on the tissue response measurement, namely ultrasonography/compression, MR and optical (Fig. 6) . " to "There are different methods of elastography depending on tissue stimulation method and imaging modality used to measure generated displacement field (e. g. quasi-static or harmonic ultrasonography elastography, MR elastography and Optical coherence elastography (Fig. 6)) .

Authors Reply: The authors agree with the reviewer and accepted the suggestion. Therefore, the sentence was changed according to the proposal of the reviewer.

“There are different methods of elastography depending on the tissue response measurement, namely ultrasonography/compression, MR and optical (Fig. 6)” → There are different methods of elastography depending on tissue stimulation method and imaging modality used to measure generated displacement field (e. g. quasi-static or harmonic ultrasonography elastography, MR elastography and Optical coherence elastography (Fig. 6))