Mechanical Characterization of Breast Tissue
Constituents for Cancer Assessment

by
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M.Eng., University of Birmingham, 2011

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Abstract

Breast elastography is a method of cancer detection that uses the response of soft tissue to deformations, leading to discovery of abnormalities. The methods of Clinical Breast Examination and Breast Self-Examination are based primarily on stiffness and, hence, on the mechanics of tissue constituents examined by palpation (Goodson, 1996). However, little is known about the mechanical characteristics of breast tissue under compression and the contribution of tissue mechanics to breast cancer detection. This study focuses therefore on tissue characterization and on identification of the relationship between tissue properties and pathological mechanics via offering an elastography technique based on the Yeoh hyperelastic model. The strength of the Yeoh model has been validated through compression testing of breast phantoms (small and large sizes), animal tissues, and in-vivo human tissues. The proposed method provides thresholds for the mechanical properties of soft tissues, which are useful in medical applications.

Keywords: Breast cancer; hyperelastic characterization; curve fitting; animal tissue; biopsy phantom; soft tissue
To my adorable parents, for all their support, patience, encouragement and passion.
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# Table of Contents

Approval ............................................................................................................................. ii  
Partial Copyright License .................................................................................................. iii  
Ethics Statement ............................................................................................................... iv  
Abstract ............................................................................................................................. v  
Dedication ......................................................................................................................... vi  
Acknowledgements .......................................................................................................... vii  
Table of Contents ............................................................................................................ viii  
List of Tables ..................................................................................................................... x  
List of Figures ................................................................................................................... xii  
List of Acronyms ............................................................................................................... xv  
Nomenclature .................................................................................................................. xvi  

## Chapter 1. Introduction ......................................................................................... 1  
1.1. Anatomy of the Breast .............................................................................................. 2  
1.2. Material Properties of Soft Tissues .......................................................................... 3  
1.3. Breast Cancer and Lesion Classifications ............................................................... 5  
1.4. Clinical Exams for Breast Cancer ............................................................................ 6  
1.5. Objectives and Scope .............................................................................................. 8  

## Chapter 2. Literature Review .................................................................................. 10  
2.1. Introduction ............................................................................................................ 10  
2.2. Mechanical Study of Soft Tissues .......................................................................... 11  

## Chapter 3. Theory of Mathematical Modeling ....................................................... 17  
3.1. Constitutive Theory and Strain Energy Function .................................................... 17  
3.2. Theory of Boussinesq indentation .......................................................................... 21  
3.3. Bio-Statistics .......................................................................................................... 22  
3.4. Conclusions ............................................................................................................ 24  

## Chapter 4. In-Vitro and In-Vivo Experimental Studies ........................................... 25  
4.1. Hyperelastic Characterization of Polyvinyl Alcohol Based Phantoms and In-vitro Animal Tissues ........................................................................................................... 25  
4.1.1. Fabrication of Breast Tissue Mimicking Phantoms from Polyvinyl Alcohol .................................................................................................................. 26  
4.1.2. Sample Dissection and Unconstrained Compression of PVA Phantoms ........................................................................................................... 29  
4.1.3. Sample Dissection and Unconfined Compression of Animal Tissues ........ 32  
4.2. Integration of the Method to Tangible Clinical Examinations ................................. 36  
4.3. Development of Hyperelastic Models ..................................................................... 40  
4.4. Statistical Analysis ................................................................................................... 42
Chapter 4.5. Uncertainty Calculations Approach .......................................................... 43

Chapter 5. Results ............................................................................................................. 45
  5.1. Biopsy Phantom Study ......................................................................................... 45
  5.2. In-vitro Animal Tissue Study .............................................................................. 54
  5.3. Uncertainty Calculations of Biopsy Phantom and Animal Testing Method ......... 63
  5.4. In-Vivo Human Tissue and Breast Phantom Studies ........................................... 64
       5.4.1. In-vivo Human Tissue ............................................................................. 64
       5.4.2. Large Scale Breast Phantom .................................................................... 70
  5.5. Uncertainty Calculations of In-vivo and Large Phantom Method ....................... 72

Chapter 6. Discussion .................................................................................................... 74

Chapter 7. Conclusions and Recommendations .................................................... 80

References .................................................................................................................... 82

Appendix A. Standard Operating Procedures-Preparation of Artificial Tissue Solution .................................................................................................................. 87
Appendix B. Standard Operation Procedures-Preparation of PVA Phantom and Animal Tissue Samples ........................................................................................................ 90
Appendix C. Standard Operating Procedures-Performing Compression Testing on Tissue-Mimicking Phantoms and Animal Tissue Specimens .................... 92
Appendix D. Standard Operating Procedures-Performing Compression Testing on Large-Scale Breast Phantom and in-vivo Tissues ........................................ 95
List of Tables

Table 1. Mean and standard deviation of Polynomial parameters for breast tissues. The units are N/mm-2 ... 13

Table 2. Mean and standard deviation of Yeoh parameters for malignant breast tissues. The units are N/mm-2 ... 14

Table 3. Mean and standard deviation of Yeoh parameters for benign breast tissues. The units are N/mm-2 ... 14

Table 4. Yeoh material parameters of tumor, glandular and adipose PVA phantoms. The units are N/mm-2 ... 14

Table 5. Yeoh material parameters of brain tissues. The units are N/m2 ... 15

Table 6. PVA phantom groups ... 29

Table 7. List of animal tissues ... 34

Table 8. Samples dimensions ... 34

Table 9. Strain level versus type of tissue ... 36

Table 10. Model parameter averages and standard deviations for PVA phantoms. The units are N/m2 ... 50

Table 11. R-squared values of models for PVA phantoms. The units are N/m2 ... 51

Table 12. Kruskal Wallis test results for each hyperelastic parameter of phantom sample ... 54

Table 13. Mann-Whitney U test results for significant hyperelastic parameters ... 54

Table 14. Model parameter averages and standard deviations for animal tissues. The units are N/m2 ... 57

Table 15. R-squared values of models for animal tissues ... 58

Table 16. RMS assessment of each animal model corresponding to each PVA phantom ... 61

Table 17. Uncertainties of variables- in-vitro method ... 63

Table 18. Model parameters averages and standard deviations for in-vivo tissues. The units are N/m2 ... 66
Table 19. R-squared values of models for in-vivo tissues. ...................................... 67

Table 20. Kruskal Wallis test results for each hyperelastic parameter of in-vivo tissues. Null Hypothesis: There is no significant difference between hyperelastic properties of three different in-vivo tissues. .......... 67

Table 21. Kruskal Wallis test results for each hyperelastic parameter of in-vivo tissues. Null Hypothesis: There is no significant difference between hyperelastic properties of palms of 10 people. ......................... 68

Table 22. Kruskal Wallis test results for each hyperelastic parameter of in-vivo tissues. Null Hypothesis: There is no significant difference between hyperelastic properties of forearms of 10 people. ................... 68

Table 23. Kruskal Wallis test results for each hyperelastic parameter of in-vivo tissues. Null Hypothesis: There is no significant difference between hyperelastic properties of biceps of 10 people. ..................... 68

Table 24. Model parameters averages and standard deviations for large breast phantom. The units are N/m². ....................................................... 71

Table 25. R-squared values of models for large breast phantom. ........................... 71

Table 26. Uncertainties of the variables-in-vivo method. ........................................ 72
List of Figures

Figure 1. Structure of the breast tissues ................................................................. 3
Figure 2. Stress-Strain curves for bovine a) muscle, b) liver captured using Instron load cell. ................................................................. 12
Figure 3. PVA phantom fabrication—heating and stirring of PVA solution using magnetic stirrer on hot plate. .............................................. 27
Figure 4. Mixed phantom fabrication—placing cylindrical stiff PVA samples among soft layers of PVA solution .................................................. 28
Figure 5. PVA phantom sample and cutting apparatus ......................................... 30
Figure 6. Electroforce 3200 (Bose Corp, Grand Prairie, MN) equipped with a 50 lbf load cell (Sensotec 31E, Honeywell Corp, Columbus, OH) ....... 31
Figure 7. PVA sample between platens of Electroforce testing machine. .......... 32
Figure 8. Animal samples—from left to right: chicken breast, cow breast fat muscle, veal Kidney ................................................................. 33
Figure 9. Chicken breast sample between platens of Electroforce testing machine ................................................................. 35
Figure 10. Force-displacement sensors set up for breast phantom—finger with force sensor was attached to the rob tip of displacement sensor which was fixed at the edge of wooden box. ....................... 38
Figure 11. Compression testing of breast phantom—as the finger wearing force sensor attached to the rob tip of spring pot went down, force-displacement data was aquired ............................................. 39
Figure 12. Experimental set up for in-vivo compression testing—as the finger wearing force sensor attached to the rob tip of spring pot went down, force-displacement data was acquired ............................................. 40
Figure 13. Optimization algorithm ..................................................................... 42
Figure 14. Hysteresis behavior of phantom specimen ........................................... 45
Figure 15. Stress-Strain raw data of loading stage-PVA biopsy phantoms .......... 46
Figure 16. Raw data vs. fitted curves-biopsy phantoms ........................................ 48
Figure 17. Raw data vs. fitted Yeoh models for four types of phantom ............... 49
Figure 18. Raw data vs. fitted Mooney-Rivlin models for four types of phantom........................................................................................................49
Figure 19. Raw data vs. fitted Neo-Hookean models for four types of phantom........................................................................................................50
Figure 20. Raw data vs. fitted Yeoh models for each sample of 10% PVA phantom.................................................................................................51
Figure 21. Raw data vs. fitted Mooney-Rivlin models for each sample of 10% PVA phantom ..............................................................52
Figure 22. Raw data vs. fitted Neo-Hookean models for each sample of 10% PVA phantom ..............................................................52
Figure 23. Hysteresis behavior of animal tissue specimen...............................55
Figure 24. Raw data vs. fitted curves of loading stage-animal tissues............56
Figure 25. Raw data vs. fitted Yeoh models for each sample of cow muscle....58
Figure 26. Raw data vs. fitted Mooney-Rivlin models for each sample of cow muscle..........................................................59
Figure 27. Raw data vs. fitted Neo-Hookean models for each sample of cow muscle..........................................................59
Figure 28. Yeoh models of PVA phantoms and animal tissues......................60
Figure 29. a) Stress-Strain raw data of loading stage-ten chicken breasts, b) Yeoh models of ten chicken breasts. ....................................................62
Figure 30. Average of 84% of experimental results with error bars. Error bars are ± standard deviation............................................................63
Figure 31. Hysteresis behavior of in-vivo palm..............................................64
Figure 32. Stress-relative indentation raw data of loading stage-in-vivo tissues .........................................................................................65
Figure 33. Raw data vs. fitted curves for tissues of one subject: a) palm, b) forearm, c) biceps, d) palm, forearm and biceps ........................................66
Figure 34. The trend between palm, forearm and biceps of each subject ..........69
Figure 35. The relationship of palm and biceps with respect to forearm ..........69
Figure 36. Breast phantom-tested lump 5 mm spherical dense mass at depth 2.5cm..........................................................................................70
Figure 37. a) Stress-relative indentation raw data of loading stage- large scale breast phantom with and without the dense mass (the dense mass was 5 mm spherical at depth 2.5cm from the side), b) Raw data vs. fitted curves of healthy and cancerous regions of the breast phantom.......................................................... 71

Figure 38. Measured versus actual force and displacement directions over the surface of large phantom and in-vivo tissues. ............................................. 73
### List of Acronyms

<table>
<thead>
<tr>
<th>Term</th>
<th>Initial components of the term</th>
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<tbody>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyrebonucleic Acid</td>
</tr>
<tr>
<td>CBE</td>
<td>Clinical Breast Examination</td>
</tr>
<tr>
<td>BSE</td>
<td>Breast Self-Examination</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>RF</td>
<td>Radio Frequency</td>
</tr>
<tr>
<td>PVA</td>
<td>Polyvinyl Alcohol</td>
</tr>
<tr>
<td>PPSFS</td>
<td>Pressure Profile System Fingertip Sensor</td>
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## Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Unit</th>
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<tbody>
<tr>
<td>( \rho_0 )</td>
<td>Density</td>
<td>[kg/m(^3)]</td>
</tr>
<tr>
<td>( c )</td>
<td>Speed of Sound</td>
<td>[m/s]</td>
</tr>
<tr>
<td>( I_1, I_2, I_3 )</td>
<td>Strain invariants</td>
<td>[-]</td>
</tr>
<tr>
<td>( \lambda_1, \lambda_2, \lambda_3 )</td>
<td>Principle stretches</td>
<td>[-]</td>
</tr>
<tr>
<td>( B, C )</td>
<td>Left, right Cauchy deformation</td>
<td>[-]</td>
</tr>
<tr>
<td>( c_i )</td>
<td>Model coefficient</td>
<td>[N/m(^2)]</td>
</tr>
<tr>
<td>( D )</td>
<td>Drucker stability matrix element</td>
<td>[-]</td>
</tr>
<tr>
<td>( F )</td>
<td>Deformation gradient</td>
<td>[-]</td>
</tr>
<tr>
<td>( L )</td>
<td>Length</td>
<td>[m]</td>
</tr>
<tr>
<td>( M )</td>
<td>Arbitrary function</td>
<td>[-]</td>
</tr>
<tr>
<td>( p )</td>
<td>Lagrange multiplier</td>
<td>[-]</td>
</tr>
<tr>
<td>( P )</td>
<td>First Piola-Kirchhoff stress</td>
<td>[N/m(^2)]</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>R-squared</td>
<td>[-]</td>
</tr>
<tr>
<td>( S )</td>
<td>Second Piola-Kirchhoff stress</td>
<td>[N/m(^2)]</td>
</tr>
<tr>
<td>( \text{SSE}_x )</td>
<td>Error sum of squares</td>
<td>[Dimension of ‘x’(^2)]</td>
</tr>
<tr>
<td>( \text{SST}_x )</td>
<td>Total sum of squares</td>
<td>[Dimension of ‘x’(^2)]</td>
</tr>
<tr>
<td>( t )</td>
<td>Thickness</td>
<td>[m]</td>
</tr>
<tr>
<td>( V )</td>
<td>Volume or Voltage</td>
<td>[m(^3)] or [V]</td>
</tr>
<tr>
<td>( W )</td>
<td>Strain energy or width</td>
<td>[N/m(^2)] or [m]</td>
</tr>
<tr>
<td>( z )</td>
<td>Acoustic Impedance</td>
<td>[kg/sm(^2)]</td>
</tr>
<tr>
<td>( \varepsilon )</td>
<td>Strain</td>
<td>[-]</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>Stress</td>
<td>[N/m(^2)]</td>
</tr>
<tr>
<td>( \omega_x )</td>
<td>Uncertainty</td>
<td>[Dimension of ‘x’]</td>
</tr>
<tr>
<td>( J )</td>
<td>Volume ratio</td>
<td>[-]</td>
</tr>
<tr>
<td>( E )</td>
<td>Elastic Modulus</td>
<td>N/m(^2)</td>
</tr>
<tr>
<td>( \kappa )</td>
<td>Material factor</td>
<td>[-]</td>
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Chapter 1. Introduction

Breast cancer is the most common type of cancer among women worldwide. Overall, 10.9% of all cancer patients, including men and women, belong to the breast cancer community. Breast cancer occurs frequently in women, though, 1% of all breast cancer patients are men (Boyle & Levin, 2008). According to IARC records, a total of 1,677,000 incidents of breast cancer have been reported in both developing and developed countries in 2012 (International Agency for Research on Cancer, 2013). While breast cancer ranks fifth among the causes of mortality due to cancer (522,000 deaths in 2012), it is still the most significant cause of cancer death among women (International Agency for Research on Cancer, 2013).

Breast cancer has been reported as the second-most common cause of mortality among women in Canada (American Cancer Society, 2012). The Canadian Cancer society predicted that 23,800 Canadian women would contract breast cancer in 2013 and 5000 women would die from it (Canadian cancer society, 2013). According to the American Cancer Society Statistical reports, approximately one in eight women in the US contract breast cancer throughout their life (American Cancer Society, 2012). Despite the many statistical records showing the fast-growing prevalence of breast cancer during the last decade, evidence suggests that the risk of death from breast cancer has been reduced among women aged 40 to 49 who undergo annual mammography (Boyle & Levin, 2008). Although mammography is an effective method of early detection of breast cancer after menopause, it is not very effective for young women, who have dense breasts and are at risk of inherited syndrome. Additionally, mammography has low specificity (80% of false positive cases) in distinguishing malignant from benign tumors (Mehrabian, 2008). Therefore, diverse pathological research regimes have been established to enhance methods of early detection of cancer in women, both young and old.
1.1. Anatomy of the Breast

The human female breast consists mainly of mammary glands, adipose (fat) and connective tissues. As presented in Figure 1, the mammary glands contain a series of milk ducts and some interconnected lobules, forming 15 to 20 lactiferous ducts that are exposed independently to the nipple (Drake, Vogl, & Mitchell, 2009). The overall shape of the mammary gland is a cone attached to the chest wall, with its peak located at the nipple (Drake et al., 2009). The fat surrounds the cone-shaped mammary gland, separating the superficial layer and the skin with a thickness of 0.5-2.5 cm (Gefen & Dilmoney, 2007). The connective tissue within the breast surrounds the mammary glands tissues (lobes and ducts) and forms suspensory ligaments of breast in certain areas in order to support structural stability of the breast (Drake et al., 2009). The percentage of the fat with respect to the glandular and connective tissues within a breast determines the firmness of the breast; the breast of non-lactating women consists mostly of fat, while lactating women usually have firmer breasts due to the high percentage of glandular and supporting tissues within their breast (Drake et al., 2009). The breast is located over the pectoralis facia of the pectoralis major muscle and extends from rib II to rib VI in vertical plan and from sternum to mid-axillary line in transverse (Gefen & Dilmoney, 2007). A layer of connective tissue exists between the breast and the deep fascia, allowing some degree of motion for the structure (Drake et al., 2009).
1.2. Material Properties of Soft Tissues

Orthopedic soft tissue is made primarily of ground substance (hydrophilic gel) and collagen and elastin fibers, forming intercellular elements of extracellular matrix of the tissues. Collagen is a triple helix protein which is produced mainly by fibroblast cells. The key function of collagen is to provide soft tissue constituents with strength, support and connection. Elastin is another type of protein that has long chains. It is less strong than collagen and has elastic properties that allow soft tissues to return to their original shape after a mechanical excitation. Elastin is known as the flexible component of soft tissues. Ground substance is a kind of gel present among the fibers of the extracellular matrix. Ground substance contains water, proteoglycans, glycosaminoglycans and glycoprotein that make soft tissues highly hydrated.

In general, orthopedic soft tissue acts as anisotropic mediums due to their fibers (elastic and collagen) that are oriented in the tissue. Therefore, when a compressive load is
applied in one direction (x), the movements in the other two directions (y and z) would be different (G. a Holzapfel & Ogden, 2003). However, if the compressive force is exerted transversely perpendicular to the matrix of fibres, fibres do not resist compression along their length, so that studies can be performed based on isotropy assumption for soft tissues (Carolyn Jennifer Sparrey, 2008). Soft tissues are considered incompressible during compression, the reason being that more than 70% of the soft tissue is filled with water, and as a result, the volume remains nearly constant when they are compressed (G. a Holzapfel & Ogden, 2003). Another characteristic of orthopedic soft tissue is viscoelasticity, resulting from shear contact between collagen fibres and the proteoglycans component of ground substance. The shear stress causes energy dissipation once the tissue starts to return to its initial position after elongation or contraction, a behavior that creates a hysteresis cycle, including loading and unloading stages of the test (Lemaitre, 2001).

An important characteristic of soft tissue is its nonlinearity at large deformation (Price, Gibson, Tan, & Royle, 2010). Soft tissue illustrates non-linear stress-strain relationship under large strain range and linear stress-strain correlation at low range of deformations. The transition limit from linear to nonlinear attribute depends on the type of tissue and its structure.

Glandular tissue and adipose are the main tissues of the breast that have been studied for their mechanical properties (Mehrabian, 2008; O’Hagan & Samani, 2008; Price et al., 2010). Soft tissue including breast tissue can be assumed to be incompressible material; it has a Poison’s ratio of 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 axial strain (Gefen & Dilmoney, 2007). The elastic modulus stated in the literature for glandular tissue is twice the elastic modulus of fat (Gefen & Dilmoney, 2007). Fatty tissue of the breast consists mainly of lipidic fluid containing triglycerides, free fatty acids, diglycerids, cholesterol phospholipids, cholesterol ester and monglycerides.
1.3. Breast Cancer and Lesion Classifications

Breast cancer is an uncontrolled growth of breast cells, occurring mostly in women but occasionally also in men. Generally, cancer is defined as mutation or abnormal changes in the genes that regulate cell growth. The genes act as manager in the cell’s nucleus, directing all functions of the cell including growing and reproducing. Normally, healthy parent cells divide into daughter cells under the control of genes, doing so whenever a bunch of cells are dead, or to repair an injury when cells are worn out. Healthy new cells take over the tasks of the old ones. In the case of cell abnormalities, the genes are damaged and the cells keep dividing and growing uncontrollably, resulting in the formation of a tumor (Breastcaner.org, 2013).

Breast tumors are divided into two types: benign and malignant. Benign tumors are known as noncancerous tumors, and are not dangerous because they do not invade the local regions or spread to other tissues. They grow slowly compared with malignant lumps and have features of normal cells. Malignant tumors are invasive and cancerous, and may spread to different organs of the body depending on the stage of cancer. The spreading of cancer from its original location to other organs of the body is called metastasis, a phenomenon characterized by the distributing of cancer to all parts of the body through the lymph nodes (Breastcaner.org, 2013).

The term “breast cancer” refers to the presence of a malignant tumor in the breast that has been developed through abnormalities of the breast cells due to DNA damage. Cancer cells normally develop in lobes and ducts of the glandular tissue. The most common type of breast cancer is Ductal Carcinoma, which appears within the ducts, while another type is Lobular Carcinoma, which occurs in the lobules. Additionally, Cribriform Carcinoma is a kind of breast cancer that affects connective tissue, including adipose and supporting ligaments (American Cancer Society, 2012).
1.4. Clinical Exams for Breast Cancer

Various methods of breast examinations are available; they fall into groups that report irregularities in shape, color or stiffness of the breast, and cure if the detected signs are cancer symptoms:

**Screening Tests:** Screening tests are routinely used for the purpose of detecting cancer in the early stages, even if the person being examined appears healthy. Mammography is an example.

**Diagnosis Tests:** Diagnosis exams are recommended for people who are experiencing symptoms of cancer in their breasts or if results of their mammograms show suspicious signs of cancer. The main purpose of diagnostic tests is to identify the presence of cancer and the stage of any cancer. The results of diagnosis tests help in decision making about the method of treatment. MRI is an example.

**Monitoring Tests:** Monitoring tests are given to cancer patients who have been diagnosed, the goal being to monitor the effect of therapies and any re-occurrence of cancer.

Knowing that, the most important categories of breast examination are described in this section. Clinical Breast Examination and Breast Self-Examination are two common approaches to breast screening, which are done by healthcare professionals and by women themselves. In these methods, abnormalities are discovered by visual inspection and palpation of the breasts. Usually, irregularities are related to the stiff lesions felt under the fingers of the examiner due to the changes in mechanical characteristics of tissue constituents (Goodson, 1996; Nover et al., 2009).

The sensitivity and specificity for CBE are recorded as 57.14% and 97.11% (Nover et al., 2009). These two methods do not determine malignancy, however, they help detect suspicious lesions within the breast tissue. In some cases, the examiner may be inexperienced or the signs are not clear, so the result of the examination becomes doubtful. Therefore, CBE and BSE are validated through mammography (Goodson, 1996; Nover et al., 2009). Mammography is recommended for both screening and diagnosis of breast cancer; it uses low energy X-rays to detect cancer or the status of it.
In mammography, X-rays are emitted from a source towards the patient’s breast under compression, and the response of the breast tissue to the emitted X-rays is recorded via a film or an ionization chamber on the reverse side of the body (Nover et al., 2009). The breast is compressed to reduce its thickness and allow the low-energized X-ray beam to pass through the entire thickness of tissue. Mammography has lower sensitivity in scanning dense breasts than in scanning less dense (Mehrabian, 2008; Nover et al., 2009). The sensitivity and specificity values for fatty breast test are 87% and 96.9%. However, the sensitivity and specificity of mammography for dense breast examination was recorded as 62.9% and 89.1% (Nover et al., 2009).

Abnormal results from mammography and dense breast examination are usually referred to sonography (Mehrabian, 2008), an ultrasound imaging technique that can increase the chance of cancer detection by 17% (Nover et al., 2009). Sonography emits ultrasound waves towards the breast, and as the wave propagates through the tissue, part of the ultrasound wave is absorbed by the tissue, part of it passes through the tissue, and the rest is scattered back to the transducer elements (Azhari, 2010). All three kinds of energies – absorbed, passed and echoes – are functions of time and displacement. The response of tissue to sound waves is captured by elements of the transducer as RF lines. Differentiation between tissue structures is achieved from the density and compressibility properties of different sections of the tissue. In other words, the acoustic properties of tissue are characterized using the concept of acoustic impedance in rayls per square meter, which is defined as (Azhari, 2010):

$$z = \rho_0 c,$$  \hspace{1cm} (1)

where, $\rho_0$ is density of the medium and $c$ is speed of sound.

Another method of breast screening is elastography, which uses medical imaging to measure the displacement response of tissue to an external source of force to detect abnormalities. Breast lesions are normally stiffer than their surrounding material and they exhibit less strain in response to an applied load than other parts of the tissue (Ginat et al., 2009). The excitation source could be a compressive load or a vibration, and tissue responses are imaged using ultrasound or MRI or other methods.
Elastography is a new technique, established by J. Ophire et al. (1991) (Ophir, Cespedes, Ponnekanti, Yazdi, & Li, 1991). There are two approaches in elastography: one is focused on the elastic properties of tissue; the other acquires hyperelastic characteristics of the tissue, and is the main focus of this research. During recent years, elastography has been added to the ultrasound repertoire of tests as a new feature, called sonoelastography. Several techniques are used in sonoelastography; compression strain imaging and vibration sonoelastography are the most significant modalities in breast imaging (Ginat et al., 2009).

Magnetic Resonance Imaging is another common diagnosis method of breast cancer that provides 2D and 3D images from cross sections of the breast by changing the alignment of hydrogen nuclei with magnetic field and radio waves. MRI has 66.7% sensitivity and 64.3% specificity. The capability of MRI in cancer detection is high at 93.7%, although it has a high false-positive probability (Nover et al., 2009).

1.5. Objectives and Scope

The goal of this thesis is to identify the effect of soft tissue constituent materials in tissue classification for the application of breast cancer detection. Hyperelastic parameters may be essential in estimating large deformation in tissue for the purpose of surgical procedures such as breast brachytherapy. These properties of human soft tissue play a significant role in diagnosis, screening, and monitoring of cancer (O’Hagan & Samani, 2009). On the other hand, elastography is based mainly on the concept of tissue reactions (linear and nonlinear) to an external source of load such as compression. Moreover, in most breast examination methods, compression is applied to help detect lesions. In CBE and BSE, the examiner applies a compressive load to the breast to investigate stiff regions and must sometimes exert a large deformation to feel lesions in depth. Mammography also uses compression to reduce breast thickness. Additionally, compressive behavior of human breast biopsies obtained through lumpectomy (the surgical procedure to remove a cancer or non-cancer breast lump with some surrounding tissues) is of interest to the cancer research agencies in order to distinguish the mechanical nature of anomalies of the taken biopsy. Despite of the importance of compressive loading and its contribution to hyperelastic characterization of tissue in
applications of cancer detection, few studies have focused on the behavior of tissue components in response to compression. The main objective of this study, therefore, is to conduct a quantitative difference measure of the elements of diverse soft tissues in the presence of large compressive deformation and use the outcomes for conducting breast examinations. Based on the measured responses of various tissues, the most robust model among the most simple and common hyperelastic models should be proposed. The detailed objectives were to: (1) characterize the mechanical response of breast tissue like phantoms in small and large scale to compressive load; (2) fit a variety of constitutive models to the observed behavior; (3) validate the phantom study through compression testing and mathematical modelling of diverse soft tissues in-vitro and in-vivo.

The measurement of hyperelastic properties of human soft tissue biopsies through compression testing has posed a challenge, given the complex structure of soft tissues and non-linear deformation response of the tissue in high strain level. This study therefore brings benefits to pathological and other biological research areas by introducing a strong constitutive model derived from a hyperelastic study of different breast tissue-mimicking models capable of exhibiting responses of most soft tissues. In the case of breast cancer, the data acquired from compression testing of the breast biopsies will be processed and analyzed based on the model, for further diagnosis purposes. Additionally, further studies on in-vivo responses of human soft tissues would provide proof of concept for expanding the method to the real world. As a result, the developed hyperelastic model would be helpful in clinical breast examination in finding the anomalies in-vivo.
Chapter 2. Literature Review

2.1. Introduction

The mechanical properties of soft tissue play a prominent role in the research related to several clinical, pre-clinical, as well as daily applications. These applications include cancer detection, mechanics of injury, surgical simulators, tumor navigation during surgeries, and workout activities (Gasser, Ogden, & Holzapfel, 2006; Ginat et al., 2009; Hemsel, Stroop, Oliva Uribe, & Wallaschek, 2007; Kaster et al., 2011; Righetti, Righetti, Ophir, & Krouskop, 2007; Samani & Plewes, 2007; Carolyn J Sparrey & Keaveny, 2011; Wakeling & Nigg, 2001). Hemsel et al. (2007) proposed a tumor navigation method in brain surgeries that uses a piezoelectric tactile sensory system to define hardness level of the tissue in contact with the sensing area. As the tactile biosensor vibrates in contact with the target tissue, the amount of frequency shift in the resonance determines the quantity of stiffness (Hemsel et al., 2007). The concept of muscle tuning of human lower extremities during running was evaluated through soft tissue vibration measurement of quadriceps. Three-axial accelerometers were placed on four locations of the subject's quadriceps during heel-toe running; the results showed that the peak accelerations and the time to reach the peak acceleration of four lower extremity sections were different (Boyer & Nigg, 2006). Compressive behavior of spinal cord constituents was characterized, and the best constitutive model was introduced in order to improve prevention and treatment methods of spinal cord injuries stemming from an applied compressive load (Carolyn Jennifer Sparrey, 2008). Kaster et al. (2011) revealed the elastic and hyperelastic properties of gray and white matter of the brain by capturing force-displacement data of in-vitro porcine brain samples. They aimed to introduce the most suitable hyperelastic model for the purpose of surgery simulation and evaluate linear response, which is helpful in brain elastography (Kaster et al., 2011). In 2008, the hyperelastic characteristics of pathological in-vitro breast slices were identified via an indentation system intended to discover cancer (O'Hagan & Samani, 2008).
2.2. Mechanical Study of Soft Tissues

The study of soft tissue mechanics includes mainly measurements of elastic, hyperelastic and viscoelastic properties of tissue ingredients. Pathological researchers have focused on the three mentioned fields because the elasticity of malignant tissue is higher than that of benign tumor and normal tissue. Moreover, the stiffness increase rate of malignant lesion is more than that of benign and normal tissue. Additionally, biological soft tissues demonstrate nonlinear elasticity at large strain (Krouskop, Wheeler, & Kallel, 1998; Tsukune, Kobayashi, Hoshi, Miyashita, & Fujie, 2011; Wellman & Howe, 1999). Current studies have put effort on the mechanical classification of both in-vivo tissues (Kim & Srinivasan, 2005; K Miller, Chinzei, Orssengo, & Bednarz, 2000; Nava, Mazza, Furrer, Villiger, & Reinhart, 2008; Pailler-Mattei, Debret, Vargiolu, Sommer, & Zahouani, 2013) and ex-vivo samples, including measurements on samples of tissue-mimicking phantom, animal tissue and human tissue (Kaster et al., 2011; O'Hagan & Samani, 2008, 2009; Samani, Bishop, Luginbuhl, & Plewes, 2003; Samani & Plewes, 2004; Carolyn J Sparrey & Keaveny, 2011; Tsukune et al., 2011).

In 1991, Ophir et al. (1991) developed linear elastography as an imaging modality to screen elastic behavior of tissue (Ophir et al., 1991). Thereafter, the effect of pathological fluctuations on the elastic characteristics of the microscopic and macroscopic structural organization of tissue has been identified (Fung, 1993). Although elastography provides useful information on the elastic features of the tissue, some researchers were looking for the elasticity of a defined geometry of the tissue, an achievement not possible using elastography. Chen et al. found linear stress-strain curves up to 5% strain for cylindrical diverse tissue species and nonlinear behavior – once the strain exceeded 10% (Figure 2)(Chen, Novakofski, Jenkins, & Brien, 1996). Krouskop et al. (1998) studied the mechanical behavior of soft tissues via measuring elasticity of prostate and breast tissues at pre-compression levels of 5% and 20%. They observed approximately constant elastic modulus for fat tissue up to 30% strain; for glandular tissue, elastic modulus was constant for a short initial interval, but after that, it started to increase nonlinearly as strain raised (Krouskop et al., 1998). Wellman and Howe (1999) identified the stress-strain relationship of eight different breast tissues – benign, malignant and normal – at strain levels of 1%, 5%, 10% and 15%. They
concluded that cancerous tissues are stiffer and more nonlinear than normal breast tissues (Wellman & Howe, 1999). Miller (1999) proposed a constitutive polynomial model containing time-dependent coefficients to describe linear, hyperelastic and viscoelastic properties of brain tissues. The material constants of the model were validated through unconfined compression testing of eight swine brains for up to 30% deformation (K Miller, 1999). Miller et al. (2000) expanded the developed hyper-viscoelastic model to in vivo application by conducting indentation experiments on the brain of a swine during an open surgery. They used a strain gauge and a load cell to extract force and displacement data (K Miller et al., 2000). Gasser et al. (2006) introduced a hyperelastic model to the simulation of arterial layers made of collagen fibers (Gasser et al., 2006). Samani et al. (2007) developed a computer-based indentation technique to measure block shape breast tissue samples, and the young’s moduli of the specimens were calculated from the slope of the loading curves. They found that cutting uniform cylinders from the breast tissue poses a challenge (Samani & Plewes, 2007). Another study focused on the elastic properties measurement of synthetic tissue models that may be helpful in developing mechanical models of tissues. The test arrangement included two stainless steel plates to hold the specimen, a linear actuator to apply dynamic compressive load, a load cell to quantify forces, and an optical encoder to obtain displacement data (Mansy, Grahe, & Sandler, 2008).

Figure 2. Stress-Strain curves for bovine a) muscle, b) liver captured using Instron load cell.
Note. (Chen et al., 1996).

Because of the importance of nonlinear behavior of soft tissue in diverse fields of medicine, several studies have attempted to classify nonlinearity of tissues using well-known constitutive models. In 2004, Samani and Plewes identified the hyperelastic
stress-strain properties of healthy human breast tissues (fat and fibroglandular) using the results obtained from unconfined compression testing in combination with finite element modelling of the samples. They developed and applied an optimization method on the second order polynomial model. Table 1 indicates the values for polynomial material parameters (Samani & Plewes, 2004). Later, O’Hagen and Samani (2008) extended the work to the measurement of hyperelastic material constant of tissue slices with tumors and Polyvinyl Alcohol samples. In this study, force-displacement responses of breast tissue-mimicking phantoms and human breast tissue slices with tumor were captured, and the results were exhibited by Arruda-Boyce, Ogden, Yeoh and polynomial models. In the phantom study, the Yeoh model was recognized as the most accurate one, with less than 15.5% error (O’Hagan & Samani, 2008). They followed up the research by characterizing a further 44 pathological breast-tissue slices using Yeoh, Ogden, Polynomial, Arruda-Boyce and Veronda-Westmann constitutive equations. Table 2 and 3 illustrate parameter values corresponding to each pathological tissue. To distinguish various benign and malignant breast tissues, they performed Kruskal-Wallis and Mann-Whitney U tests. According to their statistical analysis, the second parameter of Yeoh model and two of the Polynomial parameters had the strength of characterizing tissues (O’Hagan & Samani, 2009).

### Table 1. Mean and standard deviation of Polynomial parameters for breast tissues. The units are N/mm-2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$C_{10}(\times 10^{-4})$</th>
<th>$C_{01}(\times 10^{-4})$</th>
<th>$C_{11}(\times 10^{-4})$</th>
<th>$C_{20}(\times 10^{-4})$</th>
<th>$C_{02}(\times 10^{-4})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>3.1 ± 0.3</td>
<td>3.0 ± 0.2</td>
<td>22.5 ± 3</td>
<td>38.0 ± 6</td>
<td>47.2 ± 7</td>
</tr>
<tr>
<td>Fibro glandular</td>
<td>3.3 ± 0.4</td>
<td>2.8 ± 0.3</td>
<td>44.9 ± 8</td>
<td>77.2 ± 11</td>
<td>94.5 ± 13</td>
</tr>
</tbody>
</table>

Note. (Samani & Plewes, 2004).
Table 2. Mean and standard deviation of Yeoh parameters for malignant breast tissues. The units are N/mm^2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DCIS</th>
<th>IMC</th>
<th>IDC 1</th>
<th>IDC 2</th>
<th>IDC 3</th>
<th>ILC</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{10}</td>
<td>2.09×10^{-3}</td>
<td>2.66×10^{-3}</td>
<td>(3.48 ± 2.14) ×10^{-3}</td>
<td>(2.77 ± 1.38) ×10^{-3}</td>
<td>(3.42 ± 2.06×10^{-3})</td>
<td>(2.49±6.34) ×10^{-3}</td>
</tr>
<tr>
<td>C_{20}</td>
<td>3.32×10^{-2}</td>
<td>3.17×10^{-2}</td>
<td>(7.90 ± 5.43) ×10^{-1}</td>
<td>(1.78 ± 2.63) ×10^{-1}</td>
<td>(1.10 ± 1.52) ×10^{-1}</td>
<td>(8.07 ± 10.4) ×10^{-3}</td>
</tr>
<tr>
<td>C_{30}</td>
<td>0.2×10^{-5}</td>
<td>0.3×10^{-5}</td>
<td>(6.09 ± 8.36) ×10^{-5}</td>
<td>(2.49 ± 3.17) ×10^{-5}</td>
<td>(7.88 ± 16.5) ×10^{-5}</td>
<td>(1.06 ± 0.78) ×10^{-3}</td>
</tr>
</tbody>
</table>

Note. (O’Hagan & Samani, 2009).

Table 3. Mean and standard deviation of Yeoh parameters for benign breast tissues. The units are N/mm^2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FCD</th>
<th>Fibrodenoma</th>
<th>Fat necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{10}</td>
<td>(2.33 ± 1.04) ×10^{-3}</td>
<td>(4.39 ± 4.53) ×10^{-3}</td>
<td>0.717×10^{-3}</td>
</tr>
<tr>
<td>C_{20}</td>
<td>(1.01 ± 1.43) ×10^{-1}</td>
<td>(1.36 ± 1.63) ×10^{-1}</td>
<td>0.071×10^{-1}</td>
</tr>
<tr>
<td>C_{30}</td>
<td>(2.03 ± 3.72) ×10^{-4}</td>
<td>(6.20 ± 8.77) ×10^{-4}</td>
<td>0</td>
</tr>
</tbody>
</table>

Note. (O’Hagan & Samani, 2009).

Although testing of actual tissue offers a real benefit in modelling, it is not always possible to access fresh tissue; hence, developing tissue mimicking phantoms from appropriate materials and performing research using these appears to be the most convenient choice for tissue characterization. Polyvinyl Alcohol breast tissue mimicking phantoms were characterized along with numerical analysis by Mehrabian (2008). He modelled tissue behavior using Yeoh, Polynomial and Veronda-Westmann strain energy equations. In this study, the Yeoh model was recognized as the most accurate model (Mehrabian, 2008). Table 4 shows Yeoh material parameters for different types of tissue model.

Table 4. Yeoh material parameters of tumor, glandular and adipose PVA phantoms. The units are N/mm^2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tumor</th>
<th>Fibro glandular</th>
<th>Adipose</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10</td>
<td>0.0206</td>
<td>0.0079</td>
<td>0.0046</td>
</tr>
<tr>
<td>C20</td>
<td>0.0062</td>
<td>0.0029</td>
<td>0.023</td>
</tr>
<tr>
<td>C30</td>
<td>0.0448</td>
<td>0.023</td>
<td>0.0054</td>
</tr>
</tbody>
</table>

Note. (Mehrabian, 2008).
Carolyn and Keaveny (2011) characterized hyperelastic responses of spinal cord white matter under compression, and identified the best model to fit the acquired data. They concluded that a first order Ogden model was capable of best fitting spinal cord behavior, with \( R^2 = 0.99 \). However, Mooney-Rivlin and Neo-Hookean models exhibited lower performance in mimicking nonlinear characteristic of spinal cord by having \( R^2 = 0.96 \) and \( R^2 = 0.88 \), respectively (Carolyn J Sparrey & Keaveny, 2011). Kaster et al. (2011) identified elastic and hyperelastic responses of ex-vivo porcine brain specimens using Ogden, Yeoh, Polynomial and Arruda-Boyce models in order to integrate the achievements in brain mechanical models. They realized that parameters of Yeoh model and second order polynomial showed significant difference between white and gray matter. They also determined the Yeoh model as the best model in capturing hyperelastic characteristics of brain tissue (Kaster et al., 2011). The Yeoh model parameters of tested brain tissue are listed in table 5. Tsukune et al. (2011) conducted a research on the nonlinear elastic characterization of fat, fibroglandular and muscle of a hog using a robotic palpation system that executed creep tests. They developed and used a model of three parameters to identify the structure of each tissue (Tsukune et al., 2011).

Table 5. Yeoh material parameters of brain tissues. The units are N/m2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gray tissue</th>
<th>White tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>C10</td>
<td>185 ± 40</td>
<td>287 ± 69</td>
</tr>
<tr>
<td>C20</td>
<td>601 ± 251</td>
<td>1002 ± 441</td>
</tr>
<tr>
<td>C30</td>
<td>0.010 ± 0.004</td>
<td>0.012 ± 0.006</td>
</tr>
</tbody>
</table>

Note: (Kaster et al., 2011).

In addition to including linear and nonlinear elastic properties of soft tissues, some studies also included viscoelastic characterization of the tissues into their work (Mansy et al., 2008; K Miller, 1999; Carolyn J Sparrey & Keaveny, 2011). Sinkus et al. (2006) used elastography to evaluate the effect of changes in the structural cellular network of the tissue on viscoelastic characteristics of soft tissues. They measured the responses of an ultrasound breast phantom and ex-vivo beef tissue to an external low frequency mechanical excitation, using a vibrator as an excitation source, to acquire the B-mode ultrasound scans of the specimens (Sinkus et al., 2006). Liu et al. (2007) developed the
Dual Maxwell Model with nonlinear stress-strain functions in order to describe nonlinear viscoelastic behavior of soft tissues. The model was derived using experimental results obtained through static indentation tests on ovine liver (Liu, Noonan, Zweiri, Althoefer, & Seneviratne, 2007). In 2008, the Ogden model was used to identify the hyperelastic properties of tensor tympani tendons, and stress relaxation function was applied to define viscoelasticity of the tendon. The experimental data was gathered through uniaxial tensile testing and stress relaxation testing on 10 tendon samples (Cheng & Gan, 2008). Sparrey and Keavey (2011) combined the fitted model representing the hyperelastic behavior of spinal cord species with a 3-term Prony series to include the effect of tissue viscoelasticity into their model (Carolyn J Sparrey & Keaveny, 2011).

Despite of all these studies that focused on just one specific type of tissue such as breast phantoms, breast tissue slices or other soft tissue, doubt still persists about which of the mathematical models is the most suitable for describing the hyperelasticity of breast tissues in large and small scales under palpation exam; there is also a need to realize the performance of the model for other soft tissue to prove that the model has the potential to be integrated into diverse medical applications. The model should be accurate and simple, with the capability of describing the behavior of most of soft tissue. This work characterized the tradeoff between accuracy and complexity of the final model in tissue characterization. The study focused on the field of cancer from the indication that researches have proved cancer tissues exhibit highly nonlinear stress-strain behavior with great amount of stiffness (Tsukune et al., 2011). Moreover, using the final model and required apparatus, pathological tissue slices can be assessed, and the method can be applied to the current palpation techniques. At last, this research was established to fill the gaps in the experimental data base of mechanical characteristics of soft tissues related to cancer studies. Although the study applies to cancer, it also benefits other medical applications by proving the model based on a quantitative measure of tissue mechanics.
Chapter 3. Theory of Mathematical Modeling

Development of mathematical representation of soft tissues requires a comprehensive knowledge of related constitutive theories and principles. In this chapter, the concept of constitutive theories and equations is introduced. Thereafter, the main ideas contributing to material characterization of soft tissues are presented by defining appropriate constraints and assumptions. In the next section, the theory of Boussinesq indentation is explained and integrated to the case of large deformation. The importance of choice of statistical analysis is also discussed.

3.1. Constitutive Theory and Strain Energy Function

Constitutive theories focus mainly on the development of mathematical equations to describe actual behavior of mediums. The physical behavior of a material is approximated through a functional relationship as a constitutive equation that relates stress elements to the strain. A constitutive equation identifies the amount of stress at each point of a continuum medium at each moment, which is different for various kinds of continuous bodies. In this research, the phenomenological approach in solid mechanics is obeyed, which is concerned with fitting mathematical constitutive equations to experimental data. The constitutive theory related to hyperelastic material is called finite hyperelasticity theory, based on continuum mechanics. The constitutive equations representing hyperelastic material behavior use the concept of strain energy function, which is a function of deformation gradient \( F \) for homogeneous materials – that is, material with uniform distribution of internal elements on a continuum scale, Eq. (2). Strain energy function is the energy absorbed by the material as the result of deformation. The derivative of strain energy function with respect to the deformation gradient is defined as first Piola-Kirchhoff stress \( P \) (G. A. Holzapfel, 2000):

\[
W = W(F),
\]  

(2)
Deformation gradient is stated as:

\[
F = \begin{bmatrix}
\frac{\partial x}{\partial X} & \frac{\partial x}{\partial Y} & \frac{\partial x}{\partial Z} \\
\frac{\partial y}{\partial X} & \frac{\partial y}{\partial Y} & \frac{\partial y}{\partial Z} \\
\frac{\partial z}{\partial X} & \frac{\partial z}{\partial Y} & \frac{\partial z}{\partial Z}
\end{bmatrix},
\]

(3)

where for uniaxial deformation having \(\lambda_1, \lambda_2, \lambda_3\) as principle stretches in \(X, Y, Z\) directions, deformation gradient becomes:

\[
F = \begin{bmatrix}
\lambda_1 & 0 & 0 \\
0 & \lambda_2 & 0 \\
0 & 0 & \lambda_3
\end{bmatrix},
\]

(4)

and left (\(B\)) and right (\(C\)) Cauchy deformations are,

\[
C = F^T F
\]

(5)

\[
B = FF^T.
\]

(6)

As described in Section 1.2, orthopedic soft tissues are assumed to be isotropic when compressed perpendicular to the matrix of fibers and incompressible due to their high content of water. In this study, therefore, it was assumed that soft tissues are isotropic and incompressible. Isotropy properties are defined as those observing uniform stress-strain material responses in all directions. For isotropic materials, strain energy is a function of strain invariants \((I_1, I_2, I_3)\), in which \(\lambda_2 = \lambda_3\). An incompressible material is one that keeps the volume constant during deformation \((V = cte)\). One incompressibility constraint is that volume ratio \(J = \text{det} F = 1\). The third strain invariant of incompressible material is \(I_3 = \text{det} C = \text{det} B = 1\). Assuming isotropic and incompressible conditions, strain energy function for soft tissues is stated as (G. A. Holzapfel, 2000):

\[
W = W(I_1, I_2) - p(J - 1) = W(I_1, I_2) - \frac{1}{2} p(I_3 - 1),
\]

(7)
where $p$ is a Lagrange multiplier, known as hydrostatic pressure, and is found from equilibrium equations and boundary conditions.

Strain invariants are expressed as

$$I_1 = \text{tr}C, \quad I_2 = \frac{1}{2}((\text{tr}C)^2 - \text{tr}C^2), \quad I_3 = |C|,$$  \hspace{1cm} (8)

For uniaxial deformation, strain invariants are defined in terms of stretches as

$$I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2$$ \hspace{1cm} (9)

$$I_2 = \lambda_1^2 \lambda_2^2 + \lambda_2^2 \lambda_3^2 + \lambda_1^2 \lambda_3^2$$ \hspace{1cm} (10)

$$I_3 = \lambda_1^2 \lambda_2 \lambda_3^2$$ \hspace{1cm} (11)

There are several constitutive strain energy functions for hyperelastic material; among them, the following three are considered to be studies:

$$W = c_0 (I_1 - 3) \quad \text{Neo-Hookean Model},$$ \hspace{1cm} (12)

$$W = c_{20} (I_1 - 3) + c_{02} (I_2 - 3) \quad \text{Mooney-Rivlin Model},$$ \hspace{1cm} (13)

$$W = \sum_{i=1}^{3} c_i (I_1 - 3)^i \quad \text{Yeoh Model},$$ \hspace{1cm} (14)

cs represent material parameters that vary according to the physical constituents of the systems. The reason for choosing those three models is that the ultimate goal was to come up with the simplest, though still accurate, model for describing behavior of tested specimens. For this purpose, one variable (Neo-Hookean), two variable (Mooney-Rivling), and three variable (Yeoh) models were examined. Moreover, the effects of first and second strain invariants were evaluated by assessing the Mooney-Rivlin model versus the Yeoh model. Additionally, the Yeoh model has 2nd and 3rd orders of stretches that will represent nonlinear responses of the tissue more accurate than the Mooney-Rivlin and Neo-Hookean models (Kaster et al., 2011; O’Hagan & Samani, 2008, 2009).
Optimally curve fitting of experimental observations requires First Piola-Kirchhoff stress $P$ in terms of principle stretches, which can be acquired from second Piola-Kirchhoff stress $S$ (G. A. Holzapfel, 2000):

$$ S = 2 \frac{\partial W}{\partial C}, \quad (15) $$

$$ P = F^{-1}S. \quad (16) $$

Hyperelastic responses of the material should be curve fitted to the models, considering Drucker stability criteria. The Drucker stability principle states that the rate of change of Kirchhoff stress as the result of infinitesimal change in the strain conforms to the following inequality (DASSAULT SYSTEMS, 2009):

$$ d\tau : d\varepsilon > 0, \quad (17) $$

The relationship between the rate of change of Kirchhoff stress and strain for uniaxial deformation can be attained in the form of a matrix equation (DASSAULT SYSTEMS, 2009):

$$ \begin{bmatrix} d\tau_1 \\ d\tau_2 \\ d\tau_3 \end{bmatrix} = \begin{bmatrix} D_{11} & D_{12} & D_{13} \\ D_{21} & D_{22} & D_{23} \\ D_{31} & D_{32} & D_{33} \end{bmatrix} \begin{bmatrix} d\varepsilon_1 \\ d\varepsilon_2 \\ d\varepsilon_3 \end{bmatrix}, \quad (18) $$

To satisfy equation (17) for the above relationship, matrix $D$ should be positive definite. Therefore, it is necessary that

$$ D_{11} + D_{22} + D_{33} > 0, \quad (19) $$

$$ D_{11}D_{22} + D_{22}D_{33} + D_{33}D_{11} - D_{23}^2 - D_{13}^2 - D_{12}^2 > 0, \quad (20) $$

$$ \det(D) > 0, \quad (21) $$

The elements of the stiffness matrix are calculated using the following formulas (DASSAULT SYSTEMS, 2009):
3.2. Theory of Boussinesq indentation

Indentation is a method of capturing mechanical properties of articular cartilage and other soft tissue with skin and subcutaneous tissues. In the present work, the indentation theories were applied considering soft tissue as an elastic layer bounded to a rigid body which is deformed by flat-ended cylindrical punch in normal direction. Hayes et al. (1972) proposed a theoretical solution to calculate the elastic modulus of the soft tissue using load-displacement data of axisymmetric indentation test (Hayes, Keer, Herrmann, & Mockros, 1972):

\[ E = \frac{P(1-\nu^2)}{2D\kappa(h/\nu)} \]  

where \( P \) is the indentation load, \( D \) is the indentation displacement, \( \nu \) is Poisson’s ratio, \( a \) is the radius of the indenter, \( h \) is the thickness of the tissue and \( \kappa \) is a factor which is a function of material properties and geometry of the tissue.

The stress distribution beneath the punch is governed from the following equation (Sneddon, 1965):

\[ \sigma = \frac{ED}{\pi(1-\nu^2)} \left(\frac{a^2 - \rho^2}{\rho^2}\right)^{\frac{1}{2}}, \]  

\[ D_{11} = 4(\lambda_3^2 + \lambda_3^2)(\frac{\partial W}{\partial l_1} + \lambda_3^2 \frac{\partial W}{\partial l_2}) + 4(\lambda_1^2 - \lambda_3^2)^2(\frac{\partial^2 W}{\partial l_1^2} + 2\lambda_2 \frac{\partial^2 W}{\partial l_1 \partial l_2} + \lambda_2^4 \frac{\partial^2 W}{\partial l_2^2}), \]  

\[ D_{22} = 4(\lambda_2^2 + \lambda_3^2)(\frac{\partial W}{\partial l_1} + \lambda_3^2 \frac{\partial W}{\partial l_2}) + 4(\lambda_2^2 - \lambda_3^2)^2(\frac{\partial^2 W}{\partial l_1^2} + 2\lambda_2 \frac{\partial^2 W}{\partial l_1 \partial l_2} + \lambda_2^4 \frac{\partial^2 W}{\partial l_2^2}), \]  

\[ D_{12} = D_{21} = 4\lambda_3^2 \frac{\partial W}{\partial l_1} + 4\lambda_3^2 \frac{\partial W}{\partial l_2} + 4(\lambda_1^2 - \lambda_3^2)(\lambda_2^2 - \lambda_3^2)(\frac{\partial^2 W}{\partial l_1^2} + (\lambda_1^2 + \lambda_2^2) \frac{\partial^2 W}{\partial l_1 \partial l_2} + \lambda_2^2 \lambda_3^2 \frac{\partial^2 W}{\partial l_2^2}). \]
where $\rho$ is the loaded location under the punch and the above equation is for $0 \leq \rho \leq a$. For $\rho > a$, $\sigma = 0$. The average integral of pressure distribution below the indenter is calculated using

$$
\bar{\sigma} = \frac{1}{\pi a^2} \int_0^a \frac{P}{2\pi Ak} (a^2 - \rho^2)^{\frac{1}{2}} 2\pi \rho \, d\rho,
$$

(27)

Solving the above integral would result in $\bar{\sigma} = \frac{P}{Ak}$.

The indentation solution offered by Hayes was expanded to the application of different levels of deformation by Zhang et al. (1997). They provided the researchers with a table representing $\kappa$ values in terms of Poisson’s ratios and aspect ratios for soft tissue being indented to a large relative indentation by a cylindrical flat-ended punch. They found that as the relative indentation ($\frac{D}{h}$) increases for a test, the factor $\kappa$ increases linearly (Zhang, Zheng, & Mak, 1997). The $\kappa$ values corresponding to relative indentation and aspect ratios of the current study were calculated by doing some interpolations on the provided values.

### 3.3. Bio-Statistics

To establish statistical analysis, the first step is to realize the nature of the population. Populations that are normally distributed can be described by mean and standard deviation parameters. In a normally distributed population, the actual raw data is used to calculate standard deviation and mean of the population, and further studies are based on the values of these two parameters; it is therefore called parametric statistical analysis. On the other hand, if the population is not normally distributed, then the mean and standard deviation of raw data are no longer proper representations of the population, and hence, they are not considered valid as the basis of assessment. In this case, the analysis is founded on the ranks of observations rather than on the actual experimental observations in which the ranks of observations are used to compute test
statistics and further calculations. These populations are known as nonparametric populations, and their method of assessment is called nonparametric (Glantz, 2002).

The statistical technique suitable for distinguishing differences in a nonparametric population with more than two groups of observation is the Kruskal-Wallis statistic test. Kruskal-Wallis identifies whether at least one of the groups is different from other groups. The steps followed in this method are as follows (Glantz, 2002)

1. Define the hypothesis, evaluating the effect of the treatment on different groups of observations.
2. Rank each observation considering Rank 1 for the smallest observation without paying attention to which group the observation belongs to.
3. Compute test statistics in order to have a normalized amount of deviation of each group average rank with respect to the total average rank of all data.
4. Comparing p-value corresponding to the degree of freedom with significance level.
5. If the P-value is smaller than significance level (5%), rejecting the null-hypothesis and concluding that the treatment has a significant effect on the observations (Glantz, 2002).

If the Kruskal-Wallis method detects any difference between the observations, a Mann-Whitney U analysis is performed for paired groups of observations in order to identify the place where the alteration has occurred. Mann-Whitney U is a non-parametric method of distinguishing a difference between two groups based on the ranks of observations, not the actual data (Glantz, 2002) This method is summarized as follows:

1. Identifying the hypothesis, detecting where the difference occurs among the observations.
2. Ranking each observation by considering Rank 1 for the smallest observation without paying attention to which group the observation belongs.
3. Summing up the ranks corresponding to both groups of observations.
4. Computing U statistic using larger rank sum and number of observations for each group.
5. Comparing p-value with significance level.
7. If the p-value is smaller than significance level (5%), rejecting the null-hypothesis and concluding that the treatment has a significant effect on the observations (Glantz, 2002).

3.4. Conclusions

In conclusion, mathematical relations between stress and deformation were demonstrated through strain energy functions, assuming compressibility and isotropy characters of soft tissues. Stability criterion was defined, which is a needed parameter in the material modeling of soft tissues. The theory of indentation was explained and finally, according to the type of population, a bio-statistical method relevant to this study was clarified.
Chapter 4. In-Vitro and In-Vivo Experimental Studies

The overall objective of this study is to characterize the material response of human breast tissue to large compressive deformation and use the resulting data to develop the most appropriate constitutive model representing the hyperelastic behavior of breast tissue ingredients that would help in tissue characterization. To achieve the goal, this chapter concerns the following: 1) fabrication and force-displacement measurement of phantoms mimicking breast tissue biopsies; 2) determination of an accurate hyperelastic model using the observed experiments; 3) compressive testing of different animal tissue slices in order to validate the strength of the model to characterize the hyperelastic behavior of different tissues and realize similar animal tissues to the breast models under large deformation; 4) compression loading of 10 chicken breasts within five hours post-mortem to observe behavior of chicken breasts with different identities; 5) force-displacement measurements of a breast-tissue-mimicking phantom with tumors, using an indentation test to validate the outcomes of animal and phantom biopsies studies in the real application of breast cancer clinical examination; and 6) in-vivo indentation testing of different human tissues in order to identify the strength of the derived model in the case of differentiating in-vivo tissues.

4.1. Hyperelastic Characterization of Polyvinyl Alcohol Based Phantoms and In-vitro Animal Tissues

This section describes the materials and methods used in fabricating tissue-mimicking phantoms along with sample dissection techniques for animals and phantoms. It also describes the procedures used to execute compression testing of in-vitro samples and in-vivo subjects. The detailed explanation of each procedure is attached in appendices.
4.1.1. **Fabrication of Breast Tissue Mimicking Phantoms from Polyvinyl Alcohol**

The material used to build a breast-mimicking phantom should not only mimic mechanical linear behavior of the tissue at small strain but also demonstrate non-linearity of the tissue at large deformation. The nonlinear character of soft tissues is more essential for tumors that are stiffer than normal tissues and that therefore require a high force in order to be deformed sufficiently. Additionally, the difference between cancerous and healthy tissue is more distinguishable at high strain level due to the high non-linear stress-strain relationship of the cancerous tissues (Tsukune et al., 2011). Based on the work of Wellman and Howe (1999), cancerous tissue exhibits more noticeable stiffness difference compared to normal tissue at strain level 15% (5 times) than at level 1% (2.5 times). It was also concluded that the stiffness of cancerous tissue increases nonlinearly by a factor of 10 at 1% strain and a factor of 50 at 15% strain (Wellman & Howe, 1999). As a result, it was recommended to apply strain higher than 15% to differentiate cancerous from noncancerous tissues.

Polyvinyl Alcohol (Sigma-Aldrich-Cat No.363146) is a type of polymer frequently used to create breast phantoms mimicking nonlinear stress-strain behavior of the tissue under large deformation. (Mehrabian, 2008; O'Hagan & Samani, 2008; Price et al., 2010). Different percentages (5%, 10%, 15 %, mix of 5% and 10%) of PVA were mixed with 50:50 water-ethanol solutions – ethanol was added as an anti-freeze. The phantoms were made using the following apparatus: beakers to hold the solution, hot plates to heat the solutions, magnetic stirrers to stir the solutions continuously, and a manual stirrer to stir the water and ethanol solution prior to adding PVA. Also used were a thermometer to monitor the solution temperature, and corks to seal the beakers inlet in order to prevent the ethanol from evaporating.

First, water and ethanol were added to the beaker and the solution stirred with the manual stirrer. The beaker containing the magnetic stirrer was then placed on the hot plate. As the hot plate was warming the solution and when magnetic stirring had started, PVA was added slowly to the solution from the flask inlet. The PVA solution was heated until it reached the boiling point and PVA had dissolved completely (Figure 3).
Experimental observations are listed as follows:

- Depending on the level of PVA concentration in the solution, it took a long time for PVA crystals to dissolve in water-ethanol solution.
- The relative percentages of PVA and water-ethanol should be chosen correctly in order to avoid saturating the solution.
- Adding a high amount of PVA and increasing the heat at some point ended in PVA crystals being attached to the wall of the flask. As a result, the rate of heat transfer into the vessel should be constant and the temperature should be kept at about 80°C in order to prevent fusion of PVA to the wall of the container (Price et al., 2010).

After the PVA crystals had melted, the solution was poured into a cylindrical vessel and allowed to cool to room temperature. The vessel was then placed in a fridge (700 Series, Thermo Fisher Scientific Inc.) at -50°C for freeze-thawing to solidify the phantom.
Freeze-thawing is the process of increasing sample stiffness by gradually reducing the temperature from room temperature to the temperature range -20°C to -50°C and then increasing the sample temperature to the room temperature. In the case of polyvinyl alcohol-based phantoms, the phantom solution stood from 30 minutes to 1 hour in the fridge at -50°C and from 30 minutes to 1 hour at room temperature 24°C (the fridge time and environment time depended on the volume of the solution; the greater the volume, the longer the freeze-thawing cycle). The procedure for making the phantoms is described in Appendix A.

There was a slight difference in the construction method of mixed phantoms. To obtain a tissue biopsy containing a tumor, 10% PVA phantoms were cut into small cylindrical pieces (2 mm-3 mm diameter, 2 mm-3 mm height) using biopsy punches (Figure 4) and the resulting samples were placed in a container. Next, 5% PVA solution was poured into another container layer by layer. The first thin layer was poured and was left for 3 to 5 minutes to cool and solidify. When the first layer was slightly solidified, the 10% samples were placed over the layer. Then the second layer was poured and more layers were added until the system reached the desired thickness (Figure 4).

Figure 4. Mixed phantom fabrication—placing cylindrical stiff PVA samples among soft layers of PVA solution.
The stiffness of the fabricated phantoms depended on the concentration of PVA in the solution and the number of freeze-thaw cycles. The phantoms were divided into four groups according to their mechanical characteristics (PVA concentration and number of freeze-thaw cycling): low stiffness, medium stiffness, high stiffness and mixed-phantom (Table 6).

The purpose was to simulate different tissues of a breast, cancerous tumor and anomaly surrounded by normal fat tissue. In order to prove the concept of difference between trends of tissue responses, the phantoms were fabricated through ensuring reasonable relations amongst them.

Table 6. PVA phantom groups

<table>
<thead>
<tr>
<th>Phantom Name</th>
<th>Mimicked Tissue</th>
<th>PVA Concentration (%)</th>
<th>No. of Freeze-Thaw Cycling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Stiffness-Phantom</td>
<td>Fat</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Medium Stiffness-Phantom</td>
<td>Glandular(duct)</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>High Stiffness-Phantom</td>
<td>Ductal Carcinoma Cancer Tumor</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Mixed-Phantom</td>
<td>Fat + Anomaly</td>
<td>5+10</td>
<td>2</td>
</tr>
</tbody>
</table>

4.1.2. Sample Dissection and Unconstrained Compression of PVA Phantoms

The final fabricated phantoms should be cut into small slices in order to be compressed. At first, the phantoms were sliced into thin, transverse pieces (1 mm-4 mm) using a cutter. Thereafter, small cylindrical shape samples (3 mm-6 mm diameter) were taken from the transverse pieces using a biopsy punch. For soft artificial tissues, the samples were cut in rectangles (length: 6 mm-9 mm, width: 5 mm-7 mm). The thickness range for all the samples was 1 mm to 4 mm. The uniformity of parallel faces of the samples was visually inspected and samples with obvious deformity were excluded from testing. The dimensions of each of the specimens were measured with a digital caliper three times
and the averages were recorded. Figure 5 shows an example of samples and cutting apparatus. Sample preparation and compression testing of the artificial tissues should be performed within 24 hours of their fabrication because PVA-phantoms become dehydrated over time even if they are kept in a sealed container at room temperature or at 0°C. The standard operating procedure of phantom sample preparation is explained in Appendix B.

![Figure 5. PVA phantom sample and cutting apparatus.](image)

The hyperelastic characteristics of the samples were identified using an Electroforce 3200 (Bose Corp, Grand Prairie, MN) equipped with a 50 lbf load cell (Sensotec 31E, Honeywell Corp, Columbus, OH). The samples were compressed with polished titanium platens at 0.1 N preload (Figures 6 and 7). Before applying the preload, drops of water were added to keep the samples hydrated and reduce friction between samples and platens. While preloading the samples, the surface contacts between the samples and platens were also checked visually. When the specimens were in contacting well with the platens, the loading and unloading cycle was started. All the phantom samples were compressed to 30% strain at a strain rate of 0.002 S⁻¹. The strain rate was kept constant when compressing the samples in order to exclude the effect of strain rate that would bring out the viscoelastic behavior of soft tissues (Devi, Bharat Chandran, Vasu, & Sood, 2007). The radial expansion of each specimen was visually monitored during the loading stage in order to detect the occurrence of elastic-buckling or other irregular deformation. The samples that had undergone irregular deformation or bucking were removed from the study. The raw force and displacement data was recorded and processed further to
obtain engineering stresses and strains from the original areas and thicknesses. The methods to prepare the testing machine and perform compression testing are summarized in a standard operating procedure (Appendix C).

Figure 6. Electroforce 3200 (Bose Corp, Grand Prairie, MN) equipped with a 50 lbf load cell (Sensotec 31E, Honeywell Corp, Columbus, OH).
4.1.3. Sample Dissection and Unconfined Compression of Animal Tissues

The results obtained from the biopsy phantom study were validated by testing different animal tissues in order to accomplish a unique model that fitted most of the soft tissues. Compressive loading was applied on the eight types of animal tissues listed in Table 7. The tissues were taken from a meat shop and tested within twelve hours post-mortem, consistence with other studies (Darvish & Crandall, 2001; Maclean, 2010; Sinkus et al., 2006). Tissue collection from the meat shop was arranged and scheduled with the manager prior to pick up to minimize post-mortem time. Tissues were kept in a zipped clear bag and placed in a cooler during transportation.
Depending on the texture and stiffness of the tissues, the test samples were cut in cylindrical or rectangular shapes from transverse slices of the tissues because stress-strain response of the sample theoretically does not depend on the surface area; however, it was experimentally assumed that the stress-strain distribution is uniform all over the sample shape. Veal kidney and liver were sliced into cylindrical samples using a biopsy punch. Cow breast fat and muscle, and pig rib end centre fat and muscle were cut into rectangular samples with a cutter, because they are too stiff to be cut with a biopsy punch. The specimens from chicken breast and sheep brain were also prepared in rectangular cross section with a cutter; the reason for this is that cylinder sampling from chicken breast and sheep brain yielded irregular-shape samples because sheep brain is made mostly of fat and chicken breast contains parallel fibres. To exclude the effect of fibers and, hence, anisotropic character of soft tissues in the study, samples were cut from transverse slices of the tissues and compression testing was performed transversely perpendicular to the matrix of fibers (Kaster et al., 2011; O’Hagan & Samani, 2008, 2009; Carolyn Jennifer Sparrey, 2008). Figure 8 illustrates some animal tissue samples. At least five samples were prepared from each tissue at a specific dimension range. The regularity in the ultimate shape and parallel faces of the specimens were visually checked. Some cylindrical samples had an hourglass shape and were removed from the study. The dimensions of specimens were measured three times using a digital caliper, and the averages were calculated and documented (Table 8).

![Animal samples](image.png)

**Figure 8.** Animal samples—from left to right: chicken breast, cow breast fat muscle, veal Kidney.
### Table 7. List of animal tissues

<table>
<thead>
<tr>
<th>Number</th>
<th>Quantity</th>
<th>Animal Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Chicken Breast</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>Sheep Brain</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>Cow breast Fat</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>Cow breast Muscle</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>Pig Rib End Centre Fat</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>Pig Rib End Centre Muscle</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>Veal Liver</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>Veal Kidney</td>
</tr>
</tbody>
</table>

### Table 8. Samples dimensions

<table>
<thead>
<tr>
<th>Type of Tissue</th>
<th>Rectangular Samples</th>
<th>Range of Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range of Length( mm )</td>
<td>Range of Width( mm )</td>
</tr>
<tr>
<td>Sheep Brain</td>
<td>8-12</td>
<td>11-15</td>
</tr>
<tr>
<td>Chicken Breast</td>
<td>13-18</td>
<td>7-9</td>
</tr>
<tr>
<td>Cow Breast Fat</td>
<td>11-14.5</td>
<td>9-12</td>
</tr>
<tr>
<td>Cow Breast Muscle</td>
<td>6-9</td>
<td>7-9</td>
</tr>
<tr>
<td>Cow Breast Fat + Muscle</td>
<td>9-12</td>
<td>7-9.5</td>
</tr>
<tr>
<td>Pig Rib End Centre Fat</td>
<td>8.5-12.5</td>
<td>6-9.5</td>
</tr>
<tr>
<td>Pig Rib End Centre Muscle</td>
<td>8-15</td>
<td>6-8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of Tissue</th>
<th>Cylindrical Samples</th>
<th>Diameter( mm )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow Kidney</td>
<td>3-7</td>
<td>2-5</td>
</tr>
<tr>
<td>Cow Liver</td>
<td>6-6.5</td>
<td>4-6.5</td>
</tr>
</tbody>
</table>
All the specimens were compressed between the platens of an Electro-Force testing machine using 50 lbf load cell at 0.1 N preload to provide a good surface contact. Chicken breast sample under compression is shown in Figure 9. As done with biopsy phantoms, drops of water were applied in order to diminish the friction between the platens and the specimens and keep the samples hydrated during testing. Animal samples compression testing was performed at constant strain rate of 0.002 S$^{-1}$. Each of the animal groups underwent compressive loading at a certain strain level depending on the type of tissue and displacement limitation of the testing machine (20% or 30%). Table 9 shows the amount of strain limits for each group of tissue. The elastic buckled testing samples were removed from further analysis. Preparation and compression testing of animal tissue samples are described in Appendices B and C.

![Chicken breast sample between platens of Electroforce testing machine.](image)
Table 9. Strain level versus type of tissue

<table>
<thead>
<tr>
<th>Tissue Group</th>
<th>Strain Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Breast</td>
<td>0.3</td>
</tr>
<tr>
<td>Cow Kidney</td>
<td>0.2</td>
</tr>
<tr>
<td>Sheep Brain</td>
<td>0.3</td>
</tr>
<tr>
<td>Cow Breast Muscle + Fat</td>
<td>0.3</td>
</tr>
<tr>
<td>Cow Breast Muscle</td>
<td>0.2</td>
</tr>
<tr>
<td>Cow Breast Fat</td>
<td>0.3</td>
</tr>
<tr>
<td>Cow Liver</td>
<td>0.2</td>
</tr>
<tr>
<td>Pig Rib End Centre Fat</td>
<td>0.3</td>
</tr>
<tr>
<td>Pig Rib End Centre Muscle</td>
<td>0.3</td>
</tr>
</tbody>
</table>

In addition to the study on eight animal tissues, ten chicken breasts were purchased from a local slaughter house and tested within five hours post-mortem interval. Chicken breast is a type of homogenous muscle which is primarily made of white fibres that are known as type I and type II fibres. There are small red regions near the location where both type I and type II fibers are present. To exclude the effect of fibers and, hence, anisotropic character of soft tissues, test specimens were cut from transverse pieces of chicken breasts in rectangular shape (5 samples from each) and compressed the same way as other tissues were. The compressive load was applied on 50 samples up to 0.3 strain at 0.002 S$^{-1}$ strain rate (Appendices B and C). The force-displacement data from all animal studies were acquired and stored for further hyperelastic investigations based on the original sample dimensions.

4.2. Integration of the Method to Tangible Clinical Examinations

The application of hyperelastic characterization of tissue constituents in cancer detection was evaluated by capturing responses of a breast phantom and three soft tissues of healthy subjects to a compressive loading. A Pressure Profile System Fingertip Sensor (Pressure Profile Systems Inc., LA, USA) was used as an indenter to obtain force values. PPSFS is a wearable tactile pressure sensor that detects forces of up to 10 lbs applied on the surface of the phantom or in-vivo tissues by human fingers. Data
acquisition and analog to digital conversion of the data were performed using the Chameleon software (Chameleon TVR v.1.3.16.3, Pressure Profile Systems Inc., LA, USA) provided by the company, a software that provides real-time force data acquisition and recalibration of the sensors as needed.

The displacement data was acquired via Celesco Cable Extension Transducers (SP2 Series, Celesco Transducer Products Inc., Chatsworth, CA). Each of these spring pots senses linear displacement using a flexible cable with 317 mm /635 mm long, a spring-loaded spool, and a potentiometer. The spring pots were calibrated by quantifying the output voltages at different positions using a voltmeter. The calibration formulas related to both spring pots were extracted from those measurements as follow:

\[ X_{\text{output}} = 0.0648 \times V_{\text{output}} - 6.3058, \]  
\[ X_{\text{output}} = 0.1361 \times V_{\text{output}} - 5.5211, \]

where \( X_{\text{output}} \) is in millimeter and \( V_{\text{output}} \) is in millivolt. These equations show that there is a linear relationship between spring pot voltage outputs and displacement outputs. The calibration procedure is shown in Appendix D.

The phantom used for characterization was a Stereotactic Needle Biopsy Training Phantom (Model 013, Computerized Imaging Reference Systems Inc., Virginia, USA). The phantom is made of polyurethane and is similar to human breast in physical consistency. It weighs 0.4 kg and its volume is \( 1 \times 1.65 \times 0.5 \) m\(^3\). This phantom contains eleven dense masses, in black and of diverse sizes (1 mm-6 mm). Indentation testing was performed on both healthy and unhealthy regions (regions with and without a dense mass) of the phantom via fingertip force sensor and Celesco spring pot. The experimental arrangement is shown in Figure 10. The spring pot was fixed over a piece of wood, and the所属 end was fixed over the fingertip force sensor worn by the tester. As the index finger was moving towards the phantom, force and displacement data were measured simultaneously in real time (Figure 11). The operator performed several loading and unloading trials to make sure that the strain rate was constant and the position sensor was in vertical direction. The vertical position of the sensor was inspected visually, and uncertainty errors related to the equipment and vertical position.
of displacement sensor measurements are calculated in the result section. The index finger pressed the phantom by more than 10% thickness of the testing section. The experiment was repeated in different regions of the phantom to compare and differentiate between normal and abnormal areas. The force-displacement data was acquired and analyzed based on the Boussinesq indentation approach explained in the theory chapter.

Figure 10. Force-displacement sensors set up for breast phantom—finger with force sensor was attached to the rob tip of displacement sensor which was fixed at the edge of wooden box.
Figure 11. Compression testing of breast phantom—as the finger wearing force sensor attached to the rob tip of spring pot went down, force-displacement data was acquired.

In-vivo indentation testing involves using a fingertip force sensor and Celesco position sensor to capture force-displacement data on soft tissue of healthy human subjects (the experimental set up was the same as for CIRS phantom testing). Palm, forearm, and bicep of ten healthy human subjects were tested over a rigid surface and compared—because those tissues contain soft tissue with specific thickness. The raw data of in-vivo testing were converted to relative indentation and load distribution obeying Boussinesq theory (chapter 3). The subjects were three females and seven males age 24 to 45. Figure 12 shows the experimental configuration for in-vivo testing. The standard operating procedure of in-vivo and large-scale phantom is explained in Appendix D.
4.3. Development of Hyperelastic Models

Considering the assumptions of compressibility and isotropic that are validated in Chapter 1, Neo-Hookean, Mooney-Rivlin and Yeoh models were optimally fitted with each set of experimental force-displacement data of loading stages. As described in Chapter 3, the aim in modeling was to achieve simple models with one, two, and three variables and to evaluate the effect of strain invariants in the accuracy of curve fitting. The goal was to propose the most accurate model among those three simple models. The strain energy functions of all three models (described in the theory chapter, Eqs.(12), (13),(14)) were converted to the stress-stretch formulas for further curve fitting:

\[
\text{Neo Hookean: } \sigma = 2c_0(\lambda - \lambda^{-2}), \tag{30}
\]

\[
\text{Mooney-Rivlin: } \sigma = 2(c_{20} + c_{02}\lambda^{-1})(\lambda - \lambda^{-2}), \tag{31}
\]

\[
\text{Yeoh: } \sigma = 2(\lambda - \lambda^{-2})(c_1 + 2c_2(\lambda^2 + 2\lambda^{-1} - 3) + 3c_3(\lambda^2 + 2\lambda^{-1} - 3)^2, \tag{32}
\]

where \( \sigma \) is the elastic response in the compression direction, \( \lambda \) is the principle tensile stretch in the compression direction, \( c_0 \) is Neo-Hookean model material properties, \( c_{20} \) and \( c_{02} \) are Mooney-Rivlin model material properties, and \( c_1, c_2, c_3 \) are Yeoh model material properties.
Using Eqs (30), (31) and (32), the curve fitting of raw datum was performed by means of a constrained nonlinear optimization algorithm in Matlab (Matlab 7.1, The MathWorks Inc Natick, MA). This algorithm discovered the best fit by minimizing R-squared value between raw data and model data under the stability constraint for each of the models. As described in the theory Chapter, the stiffness matrix D should be positive definite in order for the material to be stable. Referring to the original formulas for calculating elements of D matrix (Chapter 3), the following calculations were made.

Stiffness matrices belonging to each of the models were calculated as follow:

\[
D = \begin{bmatrix}
D_{11} & D_{12} \\
D_{21} & D_{22}
\end{bmatrix},
\]  
(33)

Stiffness matrix elements for the Neo-Hookean model:

\[
D_{11} = 4(\lambda^2 + \lambda^{-1})(c_0),
\]  
(34)

\[
D_{12} = D_{21} = 4c_0\lambda^{-1},
\]  
(35)

\[
D_{22} = 8c_0\lambda^{-1},
\]  
(36)

Stiffness matrix elements for the Mooney-Rivlin model:

\[
D_{11} = 4(\lambda^2 + \lambda^{-1})(c_{20} + \lambda^{-1}c_{02}),
\]  
(37)

\[
D_{12} = D_{21} = 4\lambda^{-1}c_{20} + 4\lambda c_{02},
\]  
(38)

\[
D_{22} = 8\lambda^{-1}(c_{20} + \lambda^2 c_{02}),
\]  
(39)

Stiffness matrix elements for the Yeoh model:

\[
D_{11} = 4(\lambda^2 + \lambda^{-1})(c_1 + 2c_2(\lambda^2 + 2\lambda^{-1} - 3) + 3c_3(\lambda^2 + 2\lambda^{-1} - 3)^2 + 4(\lambda^2 - \lambda^{-1})^2(2c_2 + 6c_3(\lambda^2 + 2\lambda^{-1} - 3)),
\]  
(40)

\[
D_{12} = D_{21} = 4\lambda^{-1}(c_1 + 2c_2(\lambda^2 + 2\lambda^{-1} - 3) + 3c_3(\lambda^2 + 2\lambda^{-1} - 3)^2),
\]  
(41)
\[ D_{22} = 8 \lambda^{-1}(c_1 + 2c_2(\lambda^2 + 2\lambda^{-1} - 3) + 3c_3(\lambda^2 + 2\lambda^{-1} - 3)^2). \] (42)

The optimization algorithm cycle initiated by receiving \( N \) different initial hyperelastic parameters from the user. Then the optimization process searched for the maximum R-Squared value between raw data and fitted data under the defined stability constraint until it reached the tolerance limit. In each sample group, the optimum hyperelastic parameters were extracted and averaged. The final averaged optimal parameters were the representatives of the hyperelastic material constants of the corresponding group in the three models. The optimization algorithm for stress-strain data of one sample is summarized in a flowchart (Figure 13).

![Figure 13. Optimization algorithm.](image)

### 4.4. Statistical Analysis

Kruskal-Wallis statistical analyses were applied on the derived optimized hyperelastic coefficients of all studies in order to identify the differences between material properties of various artificial tissues, animal tissues and in-vivo tissues, and to realize the most significant coefficients in tissue characterization. The null hypothesis for each case was defined. Chi-Squares and P-values were calculated for each material coefficient and the values were evaluated and compared. The ultimate goal was to reject or accept the null
hypotheses and identify the strongest model constants in classifying the hyperelastic behavior of different types of artificial, animal and human tissues. If the P-value was smaller than the significance level, then there was a great difference in that specific parameter among the evaluated groups and, hence, the null hypothesis was rejected. The most significant parameter can be discovered by comparing chi-squares of the parameters and introducing the parameter with the largest chi-square as the most dominant coefficient in tissue classification. Once the difference was recognized, Mann-Whitney U paired test was applied for groups of two to ascertain which groups were dissimilar.

4.5. Uncertainty Calculations Approach

Experimental uncertainties are always associated with the method of measurement. The uncertainties related to the in-vitro and in-vivo measurement methods were calculated using the following procedure. Let $M$ be a given function in terms of independent variables $x_1, x_2… x_n$. The uncertainty of $M$, i.e., $\omega_m$ (Holman, 2004) is,

$$\omega_m = \left[ \left( \frac{\partial M}{\partial x_1} \omega_{x_1} \right)^2 + \left( \frac{\partial M}{\partial x_2} \omega_{x_2} \right)^2 + \cdots + \left( \frac{\partial M}{\partial x_n} \omega_{x_n} \right)^2 \right]^{1/2}, \quad (43)$$

where $\omega_{x_i}$ is the uncertainty related to variable $x_i$.

In the compression testing process, there are two main functions that have independent variables. The first is stress, which has either three or two independent variables depending on the cross section of the sample: force (N), length (m), and width (m) or diameter (m). The second is strain, with two independent variables: thickness (m) and displacement (m). The relationship between function and variables for a rectangular sample is stated as follows:

$$\varepsilon = \frac{\Delta x}{t}, \quad (44)$$

$$\sigma = \frac{F}{WL}, \quad (45)$$
where $\Delta x$ and $t$ are displacement and thickness in meters; $F$, $W$ and $L$ are force in newton; and width and length are in meters.

According to eq.(43), the uncertainties related to main functions are as follows:

$$\omega_\varepsilon = \left[ \left( \frac{\omega_{\Delta x}}{t} \right)^2 + \left( \frac{\omega_t}{t} \right)^2 \right]^{\frac{1}{2}},$$

(46)

$$\omega_\sigma = \left[ \left( \frac{\omega_F}{WL} \right)^2 + \left( \frac{\omega_L}{L} \right)^2 + \left( \frac{\omega_W}{W} \right)^2 \right]^{\frac{1}{2}},$$

(47)

Referring to Eqs. (46) and (47), one can conclude that the maximum uncertainty for strain occurs for the sample with the smallest thickness at the maximum strain. The maximum uncertainty for stress also occurs for the smallest sample area at the maximum amount of stress.
Chapter 5. Results

According to the experimental procedures, modelling and statistical analysis approaches explained in Chapter 4, this chapter presents the results of experiments and further analysis on the outcomes. At the end of each section, the maximum uncertainty corresponding to the method of measurement is computed.

5.1. Biopsy Phantom Study

Force-displacement raw data of polyvinyl alcohol phantom biopsies were captured and the hyperelastic behavior of all four types of phantoms was identified using Neo-Hookean, Mooney-Rivlin and Yeoh models. Figure 14 shows raw data obtained from compression testing of one sample. As illustrated in Figure 14, the loading and unloading stages formed a hysteresis due to viscoelasticity of the phantoms. Additionally, the material exhibited nonlinear feature in both stages.

Figure 14. Hysteresis behavior of phantom specimen.
The stress-strain loading curves corresponding to each of the phantoms are revealed in Figure 15. Photographs of three samples from each of 5%, 10% and 15% phantom types and two samples from mixed phantom are shown in the figure, with each group falling within a specific maximum stress range. As the concentration of polyvinyl alcohol increased from 5% to 15%, the stiffness of the phantom also increased, and 15% PVA samples established the maximum stress. The relationship observed between mechanical responses of three types of phantoms proved the concept of modelling the mechanical behavior of different types of breast tissue (fat, glandular and cancer). The data gathered from mixed phantom (5% PVA+10% PVA) exhibited stress values between 5% PVA and 10% PVA that are reasonable responses.

Figure 15. Stress-Strain raw data of loading stage-PVA biopsy phantoms.

Three different hyperelastic models were optimally fitted to the raw data of each phantom sample using fminunc function in MATLAB and importing 30 different initial values of material parameters. Among the output fitted values of the coefficients, those showing the lowest deviation errors were acquired. The deviation error was obtained by subtracting the value of R-squared from 1. The R-squared specifies the capability of the model in fitting raw data curves, and is calculated as in Eq. (48).
\[ R^2 = 1 - \frac{SSE}{SST}, \]  
\[ (48) \]

where SST is total sum of squares:

\[ SST = \sum_i (\sigma_i - \bar{\sigma}) \]  
\[ (49) \]

and SSE is error sum of squares:

\[ SSE = \sum_i (\sigma_i - \sigma_M)^2. \]  
\[ (50) \]

In the above three formulas, \( \sigma_i \) and \( \sigma_M \) are observed and modelled values of stress. The material parameters obtained from curve fitting of samples were averaged, and the average was considered the material constitutive parameters of that group of samples. Averaged curve fitting results for each type of phantom are demonstrated in Figure 16 showing that Yeoh model exhibited nonlinear pattern of phantoms more accurately than Mooney-Rivling and Neo-Hookean models. The graphs representing Neo-Hookean, Mooney-Rivlin and Yeoh models versus raw data of all phantoms are also added through Figures 17, 18 and 19. These figures show the performance of each model in determining the response of material elements to compression. Mooney-Riving and Neo-Hookean performed proper mimicking of material behavior up to strain level of 15%; but they were unable to fully capture the characteristics of PVA phantoms above 15% strain, which is entirely nonlinear. The Yeoh model (Figure 17) identified the whole pattern of stress-strain relationship observed for all types of phantoms and it could accurately distinguish the difference between cancerous fat tissue model and healthy fat tissue model.
Figure 16. Raw data vs. fitted curves-biopsy phantoms.
Figure 17. Raw data vs. fitted Yeoh models for four types of phantom.

Figure 18. Raw data vs. fitted Mooney-Rivlin models for four types of phantom.
Table 10 shows average and standard deviation of each of the constitutive model coefficients for different PVA phantoms.

<table>
<thead>
<tr>
<th>Model Type</th>
<th>C1_Yeoh ($\times 10^3$)</th>
<th>C2_Yeoh ($\times 10^3$)</th>
<th>C3_Yeoh ($\times 10^3$)</th>
<th>C1_Mooney ($\times 10^3$)</th>
<th>C2_Mooney ($\times 10^3$)</th>
<th>C_Neo ($\times 10^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% PVA</td>
<td>1.58±0.18</td>
<td>1.27±0.43</td>
<td>0.85±0.44</td>
<td>0.43±2.54</td>
<td>3.06±1.93</td>
<td>2.34±0.38</td>
</tr>
<tr>
<td>15% PVA</td>
<td>17.55±3.76</td>
<td>64.24±7.25</td>
<td>-13.1±32.78</td>
<td>-22.35±38.01</td>
<td>74.8±35.39</td>
<td>33.22±0.93</td>
</tr>
<tr>
<td>Mix</td>
<td>4.44±0.34</td>
<td>2.37±0.09</td>
<td>2.71±2.44</td>
<td>2.79±7.17</td>
<td>7.1±5.62</td>
<td>6.46±0.83</td>
</tr>
</tbody>
</table>

The strengths of the models in capturing loading behavior of PVA phantoms were evaluated, and compared by calculating the average of R-squared values belonging to each model. The average and standard deviation of R-squared corresponding to each model for each type of phantom are summarized in Table 11.
Table 11. R-squared values of models for PVA phantoms. The units are N/m².

<table>
<thead>
<tr>
<th></th>
<th>Yeoh</th>
<th>Mooney</th>
<th>Neo</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% PVA</td>
<td>0.992±0.013</td>
<td>0.967±0.026</td>
<td>0.920±0.042</td>
</tr>
<tr>
<td>10% PVA</td>
<td>0.983±0.033</td>
<td>0.978±0.022</td>
<td>0.963±0.033</td>
</tr>
<tr>
<td>15% PVA</td>
<td>0.982±0.031</td>
<td>0.971±0.033</td>
<td>0.860±0.052</td>
</tr>
<tr>
<td>Mixed</td>
<td>0.997±0.003</td>
<td>0.979±0.018</td>
<td>0.956±0.013</td>
</tr>
</tbody>
</table>

Table 11 illustrates that the Yeoh model captures best the hyperelastic behavior of all PVA phantoms with $R^2_{avg} > 0.98$. Although the Mooney-Rivlin and Neo-Hookean models generated reasonable $R^2$ values, 0.973 and 0.924 on average, they were unable to define fully the hyperelastic characteristics of the samples. To demonstrate the values reported in Table 11, the individual fitted curves corresponding to each sample of 10% PVA phantom are shown in Figures 20, 21 and 22. As displayed in Figure 20, the biggest R-squared values related to Yeoh curve fits.

![Figure 20](image_url)

Figure 20. Raw data vs. fitted Yeoh models for each sample of 10% PVA phantom.
Figure 21. Raw data vs. fitted Mooney-Rivlin models for each sample of 10% PVA phantom.

Figure 22. Raw data vs. fitted Neo-Hookean models for each sample of 10% PVA phantom.
Once the models’ constants for each tested sample were obtained, statistical analysis was employed to evaluate the correlation between healthy and unhealthy breast tissue models (5%, 10% and 15% PVA). Referring to Table 10, the standard deviations for material constants vary among different phantoms; this results in a non-parametric population that cannot be interpreted by two parameters of mean and standard deviation values. Therefore, Kruskal-Wallis analysis, a non-parametric test, was applied in the phantom study, which is defined for more than two groups of observations (Glantz, 2002). For this purpose, the null hypothesis was defined as: *there is no significant difference between hyperelastic properties of three different types of phantoms (5% PVA, 10% PVA, and 15% PVA).* Table 12 illustrates the outcomes of the Kruskal-Wallis test, showing chi-squares and P-values for each hyperelastic parameter among three groups of 5%, 10% and 15% PVA phantom observations. Among model parameters, C2_Yeoh, C2_Mooney and C_Neo had P-values smaller than the significance level of 0.05. This statement means that the probability of being wrong that there was a significant difference among the parameters, is quite low for those coefficients. Therefore, the null-hypothesis is rejected in terms of C2_Yeoh, C2_Mooney and C_Neo, and they were able to realize that there is at least a difference in the population. The P-values related to the other parameters were greater than 0.05, showing that those parameters could not detect a difference between phantoms. Additionally, Table 12 indicates that C2_Yeoh and C_Neo are the most critical parameters in distinguishing the difference between breast tissue models due their high chi-square values; however, C_Neo is excluded because of low R-squared values for Neo-Hookean model.

When it was realized using Kruskal-Wallis that phantom groups were different at some point, the next stage was to perform a Mann-Whitney U test to identify where the difference occurred. Table 13 displays the results acquired from Mann-Whitney U analysis on three significant model parameters of pair of phantom groups. The P-values for all paired tests were smaller than error (0.05), which means that all pairs are different.
Table 12. Kruskal Wallis test results for each hyperelastic parameter of phantom sample.

<table>
<thead>
<tr>
<th>parameter</th>
<th>Yeoh</th>
<th>Mooney-Rivlin</th>
<th>Neo-Hookean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1_Yeoh</td>
<td>C2_Yeoh</td>
<td>C3_Yeoh</td>
</tr>
<tr>
<td>chi-square</td>
<td>5.42</td>
<td>7.2</td>
<td>1.16</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0665</td>
<td>0.0273</td>
<td>0.5611</td>
</tr>
</tbody>
</table>

Table 13. Mann-Whitney U test results for significant hyperelastic parameters.

<table>
<thead>
<tr>
<th>p-value</th>
<th>C2_Yeoh</th>
<th>C2_Mooney</th>
<th>C_Neo</th>
</tr>
</thead>
<tbody>
<tr>
<td>phantom</td>
<td>15%</td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td>15%</td>
<td>0.0079</td>
<td>0.0079</td>
<td>0.0159</td>
</tr>
<tr>
<td>10%</td>
<td>0.0079</td>
<td>0.0079</td>
<td>0.0079</td>
</tr>
</tbody>
</table>

5.2. In-vitro Animal Tissue Study

The hyperelastic characteristics of eight animal tissues were recorded; the hysteresis obtained from compressive loading and unloading of one animal sample is shown in Figure 23. The loading portion exhibited nonlinear behavior of animal tissue; however, there were a period of time for the unloading portion when the stress approached zero. The reason is that the platen was not in full contact with the specimens in that period because of dehydration of the samples after being compressed in the loading stage (O'Hagan & Samani, 2008).
Figure 23. **Hysteresis behavior of animal tissue specimen.**

Raw data of in-vitro animal tissue testing were modelled using Yeoh, Neo-Hookean and Mooney-Rivling hyperelastic, models obeying the same procedure as in the phantom study (applying fmincon and diverse range of initial parameters). The same criteria were applied in animal testing to obtain the least deviation errors. Some of data sets never converged with any of the models, or they deviated from other samples of the same group due to the challenges in experimental procedure such as sample dissection and uniformity of the samples, a point argued in the discussion chapter; those data were excluded from the study. The graphs showing raw data and three averaged curve fitted models for each type of tissue are presented in Figure 24. All the samples deformed up to 30% strain, except some types of animal tissue (veal liver and kidney) that was torn at 30% strain level, and cow muscle that was too stiff for the force limit of the testing machine. All the tests done at 30% strain were excluded from further study; instead, cow muscle, veal kidney and liver that still showed nonlinearity compressed up to 20% strain rate.

Figure 24 indicates the capability of the three models in determining mechanical features of nine diverse animal tissues. All the models approximately acquired the material behavior up to about 10% strain rate; but, when the strain exceeded 10%, the
performance of Mooney-Rivlin and Neo-Hookean models was reduced while Yeoh model still accomplished an appropriate fitting. Compared with phantom study, Mooney-Rivlin model and Neo-Hookean model could not properly define the pattern because of the highly hyperplastic nature of in-vitro animal samples.

Figure 24. Raw data vs. fitted curves of loading stage-animal tissues.
The average and standard deviation of each material constant for each animal tissue are presented in Table 14.

**Table 14. Model parameter averages and standard deviations for animal tissues. The units are N/m².**

<table>
<thead>
<tr>
<th></th>
<th>C1_Yeoh ($\times 10^3$)</th>
<th>C2_Yeoh ($\times 10^3$)</th>
<th>C3_Yeoh ($\times 10^3$)</th>
<th>C1_Mooney ($\times 10^3$)</th>
<th>C2_Mooney ($\times 10^3$)</th>
<th>C_Neo ($\times 10^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chicken Breast</strong></td>
<td>4.29±0.67</td>
<td>10.83±3.08</td>
<td>7.32±8.09</td>
<td>-13.63±1.73</td>
<td>26.7±3.39</td>
<td>10.52±1.37</td>
</tr>
<tr>
<td><strong>Cow fat</strong></td>
<td>5.66±3.6</td>
<td>14.25±13.68</td>
<td>47.15±31.99</td>
<td>-24.81±3.24</td>
<td>48.54±6.31</td>
<td>18.57±2.12</td>
</tr>
<tr>
<td><strong>Cow muscle</strong></td>
<td>45.3±2.24</td>
<td>101.76±30.02</td>
<td>81.07±123.41</td>
<td>-34.65±3.61</td>
<td>67.68±7.06</td>
<td>22.26±2.34</td>
</tr>
<tr>
<td><strong>Cow muscle + fat</strong></td>
<td>9.8±2.52</td>
<td>11.96±28.94</td>
<td>137.91±90.18</td>
<td>-46.74±4.29</td>
<td>91.25±8.4</td>
<td>34.8±2.69</td>
</tr>
<tr>
<td><strong>Veal kidney</strong></td>
<td>9.46±2.8</td>
<td>197.48±16.67</td>
<td>73.17±114</td>
<td>-77.65±15.53</td>
<td>151.52±30.29</td>
<td>49.91±10.15</td>
</tr>
<tr>
<td><strong>Veal liver</strong></td>
<td>19.1±1.4</td>
<td>116.8±41.14</td>
<td>4.22±31.94</td>
<td>-71.29±14.26</td>
<td>139.17±17.8</td>
<td>44.78±8.26</td>
</tr>
<tr>
<td><strong>Pig fat</strong></td>
<td>7.48±1.58</td>
<td>62.65±1.69</td>
<td>33.73±12.45</td>
<td>-54.24±4.85</td>
<td>105.75±9.46</td>
<td>40.73±2.95</td>
</tr>
<tr>
<td><strong>Pig muscle</strong></td>
<td>9.26±1</td>
<td>57.38±8.4</td>
<td>-14.23±20</td>
<td>-25.16±21.8</td>
<td>67.9±13.7</td>
<td>31.42±2.01</td>
</tr>
<tr>
<td><strong>Sheep brain</strong></td>
<td>2.6±0.26</td>
<td>-0.120±1.13</td>
<td>0.22±1.6</td>
<td>3.49±3.92</td>
<td>1.45±2.95</td>
<td>2.7±0.24</td>
</tr>
</tbody>
</table>

As in the phantom study, R-squared evaluation was implemented on the models in order to validate their strength through animal tissue characterization. Table 15 illustrates R-squared averages and standard deviations of models relevant to types of animal tissue. This assessment confirmed the Yeoh model ($R^2_{Avg} = 0.98$) as the best model in capturing nonlinearity of soft tissues. Mooney-Rivlin and Neo-Hookean models fitted the raw data with lower R-squared values ($R^2_{Avg} = 0.89$ and $R^2_{Avg} = 0.82$). As an example, the curve fitting results of cow muscle samples are presented in Figures 25, 26 and 27. The R-squared values obtained from modelling of each individual sample of cow muscle confirms the exceptional strength of the Yeoh model in capturing the pattern of specimens.
Table 15. R-squared values of models for animal tissues.

<table>
<thead>
<tr>
<th></th>
<th>Yeoh</th>
<th>Mooney</th>
<th>Neo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Breast</td>
<td>0.991±0.020</td>
<td>0.940±0.022</td>
<td>0.850±0.023</td>
</tr>
<tr>
<td>Cow fat</td>
<td>0.992±0.017</td>
<td>0.833±0.021</td>
<td>0.721±0.022</td>
</tr>
<tr>
<td>Cow muscle</td>
<td>0.993±0.016</td>
<td>0.847±0.046</td>
<td>0.767±0.051</td>
</tr>
<tr>
<td>Cow muscle + fat</td>
<td>0.993±0.010</td>
<td>0.822±0.051</td>
<td>0.710±0.055</td>
</tr>
<tr>
<td>Veal kidney</td>
<td>0.980±0.062</td>
<td>0.870±0.044</td>
<td>0.792±0.050</td>
</tr>
<tr>
<td>Veal liver</td>
<td>0.981±0.028</td>
<td>0.963±0.017</td>
<td>0.914±0.028</td>
</tr>
<tr>
<td>Pig fat</td>
<td>0.988±0.032</td>
<td>0.891±0.020</td>
<td>0.786±0.022</td>
</tr>
<tr>
<td>Pig muscle</td>
<td>0.992±0.020</td>
<td>0.935±0.024</td>
<td>0.856±0.011</td>
</tr>
<tr>
<td>Sheep brain</td>
<td>0.982±0.028</td>
<td>0.983±0.023</td>
<td>0.987±0.011</td>
</tr>
</tbody>
</table>

Figure 25. Raw data vs. fitted Yeoh models for each sample of cow muscle.
Figure 26. Raw data vs. fitted Mooney-Rivlin models for each sample of cow muscle.

Figure 27. Raw data vs. fitted Neo-Hookean models for each sample of cow muscle.
The results in Table 14 show that animal tissue study is a non-parametric study with more than two groups of observations (Varying standard deviations). Therefore, the Kruskal Wallis test was also applied to identify hyperelastic differences between animal tissues and the best parameter to describe such a difference. The null-hypothesis was explained as: There is no significant difference between hyperelastic properties of nine different types of animal tissues. However, the outcomes of the statistical analysis were not valid because of the limitation of having just one tissue of a type.

In the next step, the material behaviors of tissue mimicking phantoms and diverse animal tissues were compared in order to find the relationship between hyperelastic characteristics of breast models and those of animal tissues. Figure 28 contains averaged Yeoh curve fitted models for both phantom and animal samples. To achieve animal models which are closer to breast models, RMS values of animal models with respect to each of the PVA phantom models were extracted from the curves, and the results are demonstrated in Table 16. The closer to 1 the RMS value, the more similar the curves; this means that sheep brain model is the animal tissue most like breast fat model, and chicken breast model is the closest model to a mix of cancer and fat lesion model. The model of cow fat exhibited behavior similar to that of glandular tissue model, and some other kinds of tissue models (cow muscle, cow muscle plus fat, pig fat, and pig muscle) showed the same behavioral characteristics as cancer tumor model.

Figure 28. Yeoh models of PVA phantoms and animal tissues.
Table 16. RMS assessment of each animal model corresponding to each PVA phantom.

<table>
<thead>
<tr>
<th></th>
<th>Chicken Breast</th>
<th>Cow fat</th>
<th>Cow muscle</th>
<th>Cow muscle + fat</th>
<th>Veal kidney</th>
<th>Veal liver</th>
<th>Pig fat</th>
<th>Pig muscle</th>
<th>Sheep brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% PVA</td>
<td>-33.8</td>
<td>-122.5</td>
<td>-556</td>
<td>-803</td>
<td>-2052</td>
<td>-1378</td>
<td>-837</td>
<td>-737</td>
<td>-469</td>
</tr>
<tr>
<td>10% PVA</td>
<td>0.09</td>
<td>0.76</td>
<td>0.51</td>
<td>-0.83</td>
<td>-4.55</td>
<td>-1.56</td>
<td>-0.76</td>
<td>0.60</td>
<td>-1.12</td>
</tr>
<tr>
<td>15% PVA</td>
<td>-0.19</td>
<td>0.42</td>
<td>0.90</td>
<td>0.91</td>
<td>-0.37</td>
<td>0.56</td>
<td>0.97</td>
<td>0.86</td>
<td>-0.83</td>
</tr>
<tr>
<td>Mixed</td>
<td>0.81</td>
<td>-0.21</td>
<td>-4.47</td>
<td>-11.8</td>
<td>-31.2</td>
<td>-17.9</td>
<td>-12.1</td>
<td>-4.5</td>
<td>-0.73</td>
</tr>
</tbody>
</table>

Given the animal-phantom comparison, chicken breast was chosen for further study in order to identify hyperelastic variability among different tissues of one type that is close to the breast-mimicking phantoms. For this purpose, ten chicken breasts (five samples from each chicken breast) were compressed up to 30% strain; the raw data of this study is presented in Figure 29.a. Figure 29.a shows that 84% of all stress-strain curves fitted into the middle region (approximately between [-25000, -50000] N/m² maximum stress), 4% fell into the upper region (approximately between [-10000, -25000] N/m² maximum stress), and 12% placed the lower section (approximately between [-50000, -120000] N/m² maximum stress). Therefore, the majority of chicken breasts behaved within a specific range, which is marked in the graph and can be considered the threshold of chicken breast stress values under compressive load at 30% strain level. The 16% of the data was out of the region because of experimental limitations detailed in the discussion section. Since the Yeoh model was the best in capturing mechanical behavior of animal tissue, the data from each chicken breast sample was modeled using the Yeoh model and the averaged Yeoh models of ten chicken breasts are displayed in Figure 29.b. Most of the models fitted into the same certain region as experimental data (Figure 29.b).
To clarify that variability occurred within 84% interval, the average and standard deviation error bars of experimental results are demonstrated in Figure 30. As shown in the figure, the coefficient of variation at each stage was approximately 25%, due to experimental uncertainties and limitations. The uncertainty associated with the method of measurement is computed in the next section. There were also some limitations along with the experimental procedure, such as cutting samples and post mortem time, and these will be elaborated in the discussion chapter. The maximum deviation in the data was recorded as 33.33%, which the discussion explains as experimental restrictions.
5.3. Uncertainty Calculations of Biopsy Phantom and Animal Testing Method

The maximum measurement uncertainty associated with phantom and animal testing was computed based on the dimensions of the smallest sample among phantom and animal specimens and maximum values of stress and strain (Eqs. (46) and (47)). Uncertainties in variables are stated in Table 17.

Table 17. Uncertainties of variables- in-vitro method.

<table>
<thead>
<tr>
<th>Uncertainty</th>
<th>Load Cell</th>
<th>Displacement Sensor</th>
<th>Caliper</th>
</tr>
</thead>
<tbody>
<tr>
<td>ωε</td>
<td>ωF = 5mN</td>
<td>ωAx = 2μm</td>
<td>ωL,W1 = 10μm</td>
</tr>
</tbody>
</table>

ωε = 0.8% , ωσ = 0.4%.

ωε and ωσ are maximum uncertainties in the measurements of strain and stress.
5.4. In-Vivo Human Tissue and Breast Phantom Studies

5.4.1. In-vivo Human Tissue

Compression testing was performed on in-vivo bicep, palm, and forearm of ten subjects, with each subject’s tissue tested twice to confirm the results. As an example, hysteresis cycle of palm of one subject is displayed in Figure 31. Compared with the hysteresis behavior of in-vitro tissue, the area within loading and unloading curves is small because of low energy dissipation in unloading stage. Figure 32 displays stress-relative indentation raw data of palms, forearms and biceps of ten subjects. The pattern that emerged for each type of tissue was approximately the same qualitatively for all the subjects; however, patterns differed quantitatively depending on the stiffness characteristics of the tissue. In palm results, subjects 2, 11 and 5 were women who had softer tissues that required less stress when compressed to a certain indentation level. Subjects 9 and 4 were men with stiffest palms that needed higher amount of force. With respect to forearm graphs, subjects 4 and 8 were men who exhibited stiffest behavior, while subjects 2 (woman) and 10 (man) showed the softest comportment. Regarding biceps outcomes, subjects 1 and 4 (men) required the highest amount of load, and subjects 10 (man) and 5 (woman) played the softest role among others.

![Figure 31. Hysteresis behavior of in-vivo palm.](image)
As in the in-vitro study, Yeoh, Neo-Hookean and Mooney-Rivlin models were used for curve fitting of in-vivo data. Figure 33 shows optimum fits of the three models for palm, forearm and bicep of one subject. All the models executed reasonable performance before 10% strain was applied; however, the capability of Neo-Hookean and Mooney-Rivlin models diminished after 10% strain. These graphs indicate that the best optimum curve for this purpose corresponded to the Yeoh model – especially for forearm and biceps, which exhibited highly nonlinear stress-strain relationship. Neo-Hookean was the worse model in the behavior of three types of tissue. A certain correlation was evident among hyperelastic behavior of tissues of the subject, as stated in Figure 33.d. The average and standard deviation of each model coefficient are indicated in Table 18.
Figure 33. Raw data vs. fitted curves for tissues of one subject: a) palm, b) forearm, c) biceps, d) palm, forearm and biceps.

Table 18. Model parameters averages and standard deviations for in-vivo tissues. The units are N/m².

<table>
<thead>
<tr>
<th></th>
<th>C1_Yeoh (×10³)</th>
<th>C2_Yeoh (×10³)</th>
<th>C3_Yeoh (×10³)</th>
<th>C1_Mooney (×10³)</th>
<th>C2_Mooney (×10³)</th>
<th>C_Neo (×10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm</td>
<td>10.5±3.4</td>
<td>1.96±1.90</td>
<td>1.15±1.8</td>
<td>3.05±11.7</td>
<td>9.45±13.3</td>
<td>13.8±6.2</td>
</tr>
<tr>
<td>Forearm</td>
<td>16.1±6.24</td>
<td>2.01±11.3</td>
<td>8.33±15.9</td>
<td>-20.3±20.5</td>
<td>7.6±3.45</td>
<td>27.5±8.25</td>
</tr>
<tr>
<td>Biceps</td>
<td>17.7±7.8</td>
<td>-3.3±2.5</td>
<td>3.50±10.8</td>
<td>-21.2±13</td>
<td>13.8±20.0</td>
<td>24.3±697</td>
</tr>
</tbody>
</table>

The strength of three models was assessed using R-squared values, as stated in Table 19. The Yeoh model accomplished the highest R-squared values and smallest deviation ($R^2_{\text{Avg}} = 0.99$). Despite the in-vitro animal testing, Mooney-Rivlin and Neo-Hookean models had reasonable capability in identifying hyperelasticity of in-vivo human tissues with $R^2_{\text{Avg}} = 0.95$ and $R^2_{\text{Avg}} = 0.878$, respectively. Figure 34 exhibits a graphical
representation of the capability of different models in determining the behavior of tissues of one subject.

Table 19. R-squared values of models for in-vivo tissues.

<table>
<thead>
<tr>
<th></th>
<th>Yeoh model</th>
<th>Mooney model</th>
<th>Neo model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm</td>
<td>0.991±0.005</td>
<td>0.968±0.056</td>
<td>0.923±0.056</td>
</tr>
<tr>
<td>Forearm</td>
<td>0.993±0.007</td>
<td>0.923±0.064</td>
<td>0.851±0.040</td>
</tr>
<tr>
<td>Biceps</td>
<td>0.994±0.014</td>
<td>0.972±0.022</td>
<td>0.860±0.064</td>
</tr>
</tbody>
</table>

The Kruskal-Wallis analysis was performed in various forms for in-vivo study. Table 20, 21, 22 and 23 show null-hypotheses and the outcomes for each type of analysis. Table 20 presents P-values when comparing three kinds of tissue for ten subjects. All the P-values were too large compared with significance level (0.05), the reason being that there were variable trends between palm, forearm and bicep of each subject that was not distinguishable.

Table 20. Kruskal Wallis test results for each hyperelastic parameter of in-vivo tissues. Null Hypothesis: There is no significant difference between hyperelastic properties of three different in-vivo tissues.

<table>
<thead>
<tr>
<th>parameter</th>
<th>Yeoh</th>
<th>Mooney-Rivlin</th>
<th>Neo-Hookean</th>
</tr>
</thead>
<tbody>
<tr>
<td>parameter</td>
<td>C1_Yeoh</td>
<td>C2_Yeoh</td>
<td>C3_Yeoh</td>
</tr>
<tr>
<td>chi-square</td>
<td>4.27</td>
<td>4.43</td>
<td>5.96</td>
</tr>
<tr>
<td>P-value</td>
<td>0.1184</td>
<td>0.1032</td>
<td>0.0509</td>
</tr>
</tbody>
</table>

In the next step, hyperelastic parameters of each tissue of ten subjects were evaluated. As shown in Table 21, C3_Yeoh and C_Neo rejected the null-hypothesis for subjects’ palms, and distinguished the difference. The null-hypotheses for forearm and bicep of subjects were rejected only by C_Neo (Tables 22 and 23).
Table 21.  Kruskal Wallis test results for each hyperelastic parameter of in-vivo tissues. Null Hypothesis: There is no significant difference between hyperelastic properties of palms of 10 people.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Yeoh</th>
<th>Mooney-Rivlin</th>
<th>Neo-Hookean</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>C1_Yeoh</code></td>
<td>13.17</td>
<td>8.31</td>
<td>14.34</td>
</tr>
<tr>
<td><code>C2_Yeoh</code></td>
<td>11.74</td>
<td>14.34</td>
<td>16.99</td>
</tr>
<tr>
<td><code>C3_Yeoh</code></td>
<td>16.46</td>
<td>0.534</td>
<td>0.0433</td>
</tr>
<tr>
<td><code>C1_Mooney</code></td>
<td>16.46</td>
<td>0.0433</td>
<td></td>
</tr>
<tr>
<td><code>C2_Mooney</code></td>
<td>8.31</td>
<td>0.0433</td>
<td></td>
</tr>
<tr>
<td><code>C_Neo</code></td>
<td>14.34</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 22.  Kruskal Wallis test results for each hyperelastic parameter of in-vivo tissues. Null Hypothesis: There is no significant difference between hyperelastic properties of forearms of 10 people.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Yeoh</th>
<th>Mooney-Rivlin</th>
<th>Neo-Hookean</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>C1_Yeoh</code></td>
<td>12.54</td>
<td>13.69</td>
<td>17.54</td>
</tr>
<tr>
<td><code>C2_Yeoh</code></td>
<td>11.63</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td><code>C3_Yeoh</code></td>
<td>13.4</td>
<td>0.1108</td>
<td></td>
</tr>
<tr>
<td><code>C1_Mooney</code></td>
<td></td>
<td>0.1374</td>
<td></td>
</tr>
<tr>
<td><code>C2_Mooney</code></td>
<td></td>
<td>0.1108</td>
<td></td>
</tr>
<tr>
<td><code>C_Neo</code></td>
<td></td>
<td>0.0314</td>
<td></td>
</tr>
</tbody>
</table>

Table 23.  Kruskal Wallis test results for each hyperelastic parameter of in-vivo tissues. Null Hypothesis: There is no significant difference between hyperelastic properties of biceps of 10 people.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Yeoh</th>
<th>Mooney-Rivlin</th>
<th>Neo-Hookean</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>C1_Yeoh</code></td>
<td>14.31</td>
<td>10.03</td>
<td>17.17</td>
</tr>
<tr>
<td><code>C2_Yeoh</code></td>
<td>14.2</td>
<td>16.43</td>
<td></td>
</tr>
<tr>
<td><code>C3_Yeoh</code></td>
<td>13.31</td>
<td>0.2138</td>
<td></td>
</tr>
<tr>
<td><code>C1_Mooney</code></td>
<td></td>
<td>0.0585</td>
<td></td>
</tr>
<tr>
<td><code>C2_Mooney</code></td>
<td></td>
<td>0.0392</td>
<td></td>
</tr>
<tr>
<td><code>C_Neo</code></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Additionally, the trend among three tissues of each subject is presented through Figures 38 and 39. Figure 34 shows that in 70% of the subjects, forearm was stiffer than palm and bicep, because the testing area in the forearm contained bones that brought the maximum stress to the highest level. Figure 35 shows the maximum stress values of palm and biceps normalized with respect to forearm. Bicep of 50% of the subjects exhibited 60-65% stiffness of the forearm; 60% of the subjects had less than 60% stiff palm than forearm.
Figure 34. The trend between palm, forearm and biceps of each subject.

Figure 35. The relationship of palm and biceps with respect to forearm.
5.4.2. Large Scale Breast Phantom

The measuring equipment used for in-vivo testing was also used to capture compressive responses of breast phantom in large scale. Two regions of the phantom were tested: one healthy region and one region containing a 5-mm spherical dense mass at depth 2.5 cm from the side (the tested cancerous lump is shown in Figure 36). Figure 37.a illustrates stress-relative indentation relationship of two regions of the phantom. It is clear that cancerous area was loaded with a higher amount of stress due, no doubt, to its stiff material constituents.

Yeoh, Neo-Hookean and Mooney-Rivling hyperelastic models were fitted to the observed behavior of two portions in order to get the optimized values of material parameters. In the case of large breast phantom, Neo-Hookean could not meet the minimum criteria, so it was excluded from the study. The fitted curves using the other two models are presented in Figure 37.b. The Yeoh model fitted the investigated nonlinearity with less deviation than the Mooney-Rivlin model. Table 24 states the averages and standard deviations of constitutive coefficients. Based on R-squared analysis results (Table 25) Yeoh model ($R_{Avg}^2 = 0.96$) was found more appropriate in hyperelastic mimicking of large scale phantom than Moonet-Rivlin model ($R_{Avg}^2 = 0.94$).

Figure 36. Breast phantom-tested lump 5 mm spherical dense mass at depth 2.5cm.
Figure 37.  a) Stress-relative indentation raw data of loading stage- large scale breast phantom with and without the dense mass (the dense mass was 5 mm spherical at depth 2.5cm from the side), b) Raw data vs. fitted curves of healthy and cancerous regions of the breast phantom

Table 24. Model parameters averages and standard deviations for large breast phantom. The units are N/m².

<table>
<thead>
<tr>
<th></th>
<th>C1_Yeoh ($\times 10^3$)</th>
<th>C2_Yeoh ($\times 10^3$)</th>
<th>C3_Yeoh ($\times 10^3$)</th>
<th>C1_Mooney ($\times 10^3$)</th>
<th>C2_Mooney ($\times 10^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy phantom</td>
<td>2.04±0.28</td>
<td>-0.19±0.03</td>
<td>0.003±0.001</td>
<td>3.52±0.71</td>
<td>-0.3±0.08</td>
</tr>
<tr>
<td>Cancerous phantom</td>
<td>6.25±1.08</td>
<td>-0.46±0.37</td>
<td>0.015±0.024</td>
<td>14.7±3.42</td>
<td>-2.35±1.01</td>
</tr>
</tbody>
</table>

Table 25. R-squared values of models for large breast phantom.

<table>
<thead>
<tr>
<th></th>
<th>Yeoh model</th>
<th>Mooney model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy phantom</td>
<td>0.954±0.011</td>
<td>0.930±0.019</td>
</tr>
<tr>
<td>Cancerous phantom</td>
<td>0.977±0.066</td>
<td>0.961±0.037</td>
</tr>
</tbody>
</table>
5.5. Uncertainty Calculations of In-vivo and Large Phantom Method

According to the calibration data of Celesco displacement sensor, the displacement is a linear function of voltage change. To calculate the uncertainty of displacement measurements, first we assume the following linear relationship between displacement and voltage change:

\[ \Delta x = \alpha \Delta V, \]  

(51)

where \( \alpha \) is the slope of calibration curve. Due to the linear form of the curve, the value of \( \alpha \) is constant, so

\[ \omega_{\Delta x} = \alpha \omega_{\Delta V}, \]  

(52)

Hence, using the uncertainty equations for stress and strain (Eq. (46) and (47)) along with the above relationship for \( \omega_{\Delta x} \) and uncertainty of each measurement tool presented in Table 26, the uncertainties of measurements for the experimental data of this section are calculated.

| Table 26. Uncertainties of the variables-in-vivo method. |
|---------------------------------|----------------|-----------------|
|                                | Voltmeter       | Force sensor    | Ruler           |
| Uncertainty                    | \( \omega_{\Delta V} = 1mV \) | \( \omega_{F} = 0.05N \) | \( \omega_{LW,t} = 0.5mm \) |

\( \omega_{\epsilon} = 1.7\% \), \( \omega_{\sigma} = 7\% \).

Based on the maximum values of strain and stress and minimum values of sample dimension, the uncertainty for stress and strain functions (\( \omega_{\epsilon} \) and \( \omega_{\sigma} \)) were calculated. The maximum uncertainties were higher than those of the in-vitro.

In addition to the equipment uncertainty measurements, the error resulting from positioning of the displacement sensor was also calculated. If we assume that the measured values of force and displacement are \( F_m \) and \( \Delta x_m \) (Figure 38), the real values of load and position (\( F_r \) and \( \Delta x_r \)) were found by the following formulas:
Figure 38. Measured versus actual force and displacement directions over the surface of large phantom and in-vivo tissues.

\[ F_r = F_m \cos \theta \]  \hspace{1cm} (53)

\[ \Delta x_r = \Delta x_m \cos \theta \]  \hspace{1cm} (54)

where \( \theta \) is the angle between real and measured position of the sensor. The error is found from the formula

\[ \text{error} = \left( \frac{1}{\cos \theta} - 1 \right) \times 100 \]  \hspace{1cm} (55)

The maximum tilt angle experienced during experiments was measured 15°. Therefore, the maximum error for location of position sensor is 3.5%.
Chapter 6. Discussion

The mechanical characteristics of soft tissue are the basis for several clinical and nonclinical research studies, such as brain surgical simulation or breast needle biopsy (Kaster et al., 2011; O’Hagan & Samani, 2009). Usually, anomalies found within the breast are investigated by palpation techniques, in which responses of soft tissue to large deformation are utilized (Goodson, 1996). Recent research on the mechanical properties of soft tissue under compressive load has revealed that tissues exhibit a linear stress-strain relationship at low strain and nonlinear behavior at high strain (Fung, 1993; Kaster et al., 2011; O’Hagan & Samani, 2009; Tsukune et al., 2011). Some researchers have recommended mathematical models to describe the nonlinearity of soft tissue (Kaster et al., 2011; K Miller, 1999; O’Hagan & Samani, 2009; Carolyn J Sparrey & Keaveny, 2011). The importance of nonlinear responses of soft tissue to compressive load in clinical breast examination highlighted the need for launching a comprehensive study on the hyperelastic characterization of in-vitro and in-vivo soft tissues, to enhance clinical approaches including the detection of breast cancer.

The observed mechanical behavior of different breast biopsy phantoms validated the statement proposed in several breast examination methods that breast fat, and glandular and cancerous tissues behave differently mechanically (O’Hagan & Samani, 2008; Price et al., 2010). Moreover, there was a consistent correlation between their nonlinear patterns. The patterns seen in the PVA models for fat and glandular samples match the trends established in the literature (Krouskop et al., 1998; Wellman & Howe, 1999). Krouskop et al. (1998) and Wellman and Howe (1999) found that human fat tissue exhibits less nonlinearity than glandular tissue and pathological samples. This research has shown similar behavior for breast tissue models made of PVA. In this work, PVA models of glandular and cancerous tissues became highly nonlinear after 15% strain was applied, a conclusion affirmed by Krouskop et al. (1998) on actual human samples.
Furthermore, a study showed that animal tissues reveal their nonlinear characteristics after 10% strain, the same as the outcome noted by Chen et al. (1996) on bovine muscle and liver. In addition, muscle tissue was shown to be more nonlinear than liver tissue (Chen et al., 1996).

The presented work primarily focused on the pattern analysis and relative stiffness difference among different tissue model; however, the maximum stress values required to compress pva samples up to 30% deformation were compared with the maximum amount of stresses needed to reach the same level of compressive deformation on actual breast samples and pva samples in the literature. The result of analysis showed that the pva samples with 5% concentration required twice higher maximum stress at 0.3 strain limit than that of breast fat tissue samples. The amount of extreme load for 10% pva samples was approximately 2.5 times higher than that of breast glandular tissue specimens (Krouskop et al., 1998). PVA tumor model represented by Mehrabanian (2008) was almost 2.5 times less stiff than our 15% pva samples. The reported discrepancies among the presented pva maximum stresses and those published in the literature are mainly due to the effect of pva concentration within the solution that should be chosen accurately and with prior knowledge. Additionally, number of FTC would affect the desired level of stiffness of the final gels.

Samples from all studies exhibited hysteresis behavior during loading and unloading, with highest energy dissipation observed in animal testing and lowest in in-vivo experiments. Since the amount of energy dissipated as heat (area inside the hysteresis loop) depends on the viscoelastic properties of the tissue, in-vitro animal specimens acted as the most viscous material, with higher amount of internal friction, which resulted in more heart dissipation. Such a difference in hysteresis behavior of in-vitro and in-vivo samples was also observed by Brown et al. (2003) (Brown et al., 2003). The hysteresis obtained from animal testing returned to zero after a while in unloading part of the cycle due to the dehydration of the samples, an outcome not seen in phantom and in-vivo studies. Maclean (2010) observed the same behavior in fresh monkey brain during indentation experiments with high strain and strain rate (Maclean, 2010).

In general, there is a trade-off between accuracy and complexity. The accuracies of the hyperelastic models were determined, considering less complexity as a desired feature;
the main purpose was to achieve higher accuracy at a lower level of complexity. The present work has characterized these two opposite trends, depending on the amount of required accuracy. To achieve the goal and based on the assessments made, Yeoh was the most accurate and simplest model in capturing the hyperelastic behavior of breast biopsy phantoms, and this accomplishment was validated through in-vitro animal testing, in-vivo human experiments, and large-scale breast phantom measurements. The Neo-Hookean model was excluded from further study in large-scale phantom, because of its high deviation error. O’Hagan et al. (2008) confirmed the weakness of the Neo-Hookean model (O’Hagan & Samani, 2008). Therefore, the Yeoh model is proven the most precise hyperelastic model, with more than 0.98% R-squared values. Other researchers also recognize this agreement about Yeoh model in soft tissue characterization (Kaster et al., 2011; O’Hagan & Samani, 2008, 2009). The findings confirm that the hyperelastic characteristics of breast and other soft tissues do not depend on second strain invariant; therefore, utilizing the Yeoh model, containing only first strain invariant, in tissue classification would provide better convergence, simpler interpretation, and easier simulation of raw data.

Material parameters of the Yeoh model were compared with the reported Yeoh parameters by others (Kaster et al., 2011; Mehrabian, 2008; O’Hagan & Samani, 2009). Material parameters of 5%, 10%, 15% pva models are bigger than the reported values for fat, glandular and tumor pva models by Mehrabian (Mehrabian, 2008). Comparative study among 15%, mix pva phantom samples and malignant breast tissue specimens (DCIS, IMC, IDC 1 and etc) proved that 15% pva models are consistently stiffer than actual malignant samples with respect to C1_Yeoh. Reported C1 material parameters of benign tumors (FCD and Fibrodenoma) indicate that benign tumors are approximately 5 times less stiff than 15% pva model (O’Hagan & Samani, 2009). Such differences occurred due to the high amount of pva within the solution and number of FTC. Using relatively smaller pva concentration would give actual material parameters of tissue. The brain tissue samples were an order of magnitude stiffer than the samples tested by Kaster et al. (2011) because they applied 15%-20% strain that resulted in smaller material parameters (Kaster et al., 2011) All in all, it was observed that the first parameter of Yeoh is the most dominant material parameter in determining the stiffness of the medium because it appears as the first order in the model equation; however,
second and third coefficients are mainly responsible for defining the nonlinear curvature of the material due to having higher orders.

According to phantom statistical analysis, C2_Yeoh was the most significant constitutive constant in determining the hyperelastic difference between biopsy phantoms. The same outcome was also established by O’Hagan and Samani (2009) for pathological samples (O’Hagan & Samani, 2009). This study accomplished that the second parameter of Yeoh model is still valid for characterizing pva models of breast tissues by doing comparative analysis among material coefficients of the Yeoh model and the Mooney-Rivlin and Neo-Hookean models. This achievement would benefit the cases where actual breast tissue is not available and researches are based on the tissue-mimicking phantoms. A specific trend was recognized among palms, forearms and biceps of ten subjects, demonstrating that more force is required to compress forearms to a certain strain than that required for palms and biceps.

The variability of material properties among different tissues of chicken breast (as the closest one to breast fat with inclusion phantom) was identified, and the maximum stress threshold for the majority of them was defined. This study proved the concept of variability among several tissues of one type due to difference in size, weight, age, being from left side or right specifically for breast, range of stiffness, and relative proportion of material constituents (fat, muscle etc). A 25% variation error was related to the majority range (84%). Such a variability was expected for the following reasons: First, ten different chicken tissues with diverse characteristics (age, size) were tested; second, the post-mortem time for the last tissues being tested was much more than for those tested at the beginning, because only the same equipment was used to test all ten tissues (although all tissues were kept in zipped bags, this phenomenon made the last tissues more dehydrated); third, cutting samples with certain shapes from the tissues was a difficult procedure; and fourth, there were some limitations (explained below) related to the overall method of measurement. Despite all those limitations, all the tissues were examined with the same method and procedure, resulting in valuable and unique study.

The main strength of this research lay in evaluating a broad variety of soft tissues, including small and large-scale breast phantoms, animal tissues, and in vivo tissues, using the same strategy. Despite the challenges associated with dissecting and testing
extremely small in-vitro samples within a limited time, a large number of specimens were
dissected and compressed for each in-vitro study – 85 animal specimens and 11
phantom samples. All the samples were compressed and characterized by the same
experimental procedure and modelling approach, making the studies comparable.
Furthermore, preparing the experimental set up to execute in-vivo experiments, and
having 10 healthy human subjects, delivered more experimental information than is
statistically needed. Having a high number of data sets allowed detailed investigation of
biomechanics of diverse soft tissues and a defining of thresholds for hyperelasticity of
chicken breast. The shortage of experimental data for the mechanics of soft tissue
impelled researchers to use linear elastography in anomaly detection (Hall, Bilgen,
Insana, & Krouskop, 1997; Mansy et al., 2008; Risholm, Ross, Washko, & Wells, 2011;
Samani & Plewes, 2007). The lack of experimental evidence was compensated in this
study. Executing compression testing with high strain revealed useful material about the
mechanical characteristics of soft tissues, which is critical in simulations. Evaluating
three choices of constitutive hyperelastic models provided the opportunity to assess and
compare the capability of the choices and explore the most suitable and accurate
candidate model. Validating in-vitro results with in-vivo studies has been a rare
accomplishment in biomaterial research of soft tissues, given the complexity in and
barriers to in-vivo testing (Brown et al., 2003). Finally, calculating uncertainty
percentages of the two methods used for in-vitro and in-vivo testing would provide useful
guidance for future studies based on these techniques.

The effect of indentation on the modelling perspective was assumed to be negligible
because the purpose was to represent mechanical structure of the in-vivo tissues and
large phantom and mathematical derivation of the constitutive models were out of scope
of this work. The complex nature of PVA phantoms and animal tissues imposed some
limitations on the study. Due to the dehydration of PVA phantoms, sample dissection
and compression testing had to be performed within 24 hours of fabricating the
phantoms, resulting in loss of some of the samples prior to testing. As a trial, a portion of
the phantom was placed in distilled water; however, the phantom dissolved in the water
and disappeared. Additionally, in the animal studies, some of the data diverged from the
range of their group mate samples – perhaps because of inaccurate cutting of small
samples, inaccuracy in sample dimension measurement, environmental noises, or
surface uniformity of sample. To overcome those limitations, the dimensions of the samples were measured three times and the average of the three was used; the samples with an hourglass shape or irregularities were removed from testing; environmental noises were reduced by placing a sign on the laboratory door asking people not to enter the testing area. The strain levels used for some types of animal tissues were less than for the others (20% instead of 30%), because either they were too soft to tolerate 30% strain level, or they were damaged during the test, or they were too stiff and needed high amount of load to be compressed to 0.3 of their thickness, which was beyond Electro-Force maximum load limit. Performing tests for some specific types of tissue at 20% while keeping strain rate constant (0.002 S\(^{-1}\)), could still bring out hyperelastic properties of the samples. Visually inspecting for proper contact between platen and sample as well as detecting zero strain at the initial contact point was also an issue due to the sensitivity of displacement measurement system to sample surface imperfections. Miller and Chinzei (1997) used laser tracking to solve this problem (Karol Miller & Chinzei, 1997). Isotropic assumption of soft tissue may be considered as a limitation; however, soft tissue fibers do not carry load during compression. As a result, this research measured the compressive reactions of the matrix of fibers which is isotropic. Another limitation of this study is that the effect of temperature was neglected (Karol Miller & Chinzei, 1997). The effect of temperature on unconfined compression testing of in-vitro porcine samples was evaluated and stated as negligible between room temperature (22ºC) and body temperature (37ºC) (Rashid, Destrade, & Gilchrist, 2012). Additionally, maintaining vertical position of the Celesco spring pot during the experiment might cause an error in the measurements which was calculated and it was negligible. Finally, having a frictionless contact between sample and platens posed a challenge, but it was solved by adding drops of water over the platen surface (Carolyn Jennifer Sparrey, 2008).
Chapter 7. Conclusions and Recommendations

The aim of this research is to identify, through a widespread experimental investigation, useful material and methods for classifying breast tissue undergoing clinical examination. To achieve that goal, hyperelastic parameters of soft tissues of diverse type (artificial, in-vitro and in vivo) were characterized. The study that was performed on biopsy-size breast tissue mimicking phantoms was pushed towards large scale commercial breast phantom by validating the strength of the model in characterizing cancer versus healthy tissue in the case of a phantom in actual size of a breast containing inclusions. The proposed method is therefore helpful for both small- and large-scale measurements, depending on the region of the breast of interest to the physician. Moreover, this research can be expanded to include nonclinical research on biomaterials of the breast tissue and the link of breast tissue biomaterial properties to cancer diagnosis. Since real clinical examinations are executed on in-vivo tissues, the in-vivo study was designed to provide a primary sense of in-vivo tissue responses to compressive load.

During studies of human subjects and large-scale phantom, it was realized that displacement measurement becomes an issue in the application of clinical breast examinations. Other researchers have come to a similar conclusion (Mehrabian, 2008). Some researches added an imaging modality to capture displacement responses of the tissue to an external excitation (Feng, Lotz, Chase, & Hann, 2010; Sinkus et al., 2006). Despite the useful evidence obtained using imaging systems, integrating and applying them into the palpation methods in clinical examinations would prove too expensive. The use of a motion controller device could help in navigating the position of the finger or a probe when palpating the breast. Examples of motion control systems include infrared motion controllers and optical navigation systems (Leap motion, 2014; NDI, 2014). Further research is therefore needed to integrate a technique of displacement measurement into the proposed clinical method for detecting breast cancer.
In conclusion, this study marks the first thorough research on exploring the biomechanics of various soft tissues utilizing one single method. The final constitutive model in soft tissue classification was introduced to enhance future soft tissue models for cancer detection or surgery simulators. Using the proposed model and experimental validations, material responses of most soft tissues can be identified.
References


84
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Appendix A.

Standard Operating Procedures-Preparation of Artificial Tissue Solution

Simon Fraser University
Standard Operating Procedure
Preparation of Artificial Tissue Solution
Date: June 2013
Author: Shima Zaeimdar
Principle Investigator: Dr. Farid Golnaraghi

Summary: The following SOP explains how to make artificial tissue mimicking phantoms from polyvinyl alcohol as a mechanical model of human breast tissue. Phantom solutions were made in the Chemistry Lab of Simon Fraser University.

Key Words: Polyvinyl Alcohol, Phantom

Materials:

Beakers (500 mL, 250 mL), hot plate, magnetic stir bar, manual stirrer, polyvinyl alcohol (30 gr), distilled water (135mL), thermometer, cork, latex gloves, lab coat, protective goggles, ethanol (135mL), balance, spatula, chemical plate
Preparing workplace:

1. Plug in a suitable hot plate.
2. Place a suitable size beaker, 250 mL or 500 mL, on the hot plate.
3. Plug in balance and leave it running for an hour before use.
4. Ensure beaker and magnetic stir bars are adequate size to mix solution at edges of beaker.
5. Test spin the magnetic bar in the beaker filled with water.
6. Allow thermometer to stabilize on the counter.

Determining proportion of solution ingredients:

Identify relative concentration of distilled water, ethanol and PVA:

a. 5 gr PVA + 47.5 mL water + 47.5 mL ethanol
b. 10 gr PVA + 45 mL water + 45 mL ethanol
c. 15 gr PVA + 42.5 mL water + 42.5 mL ethanol

Preparing solution:

1. Choose suitable sizes of beaker and magnetic bar. (A larger volume of PVA needs more space and a bigger bar in order to prevent sticking of PVA crystals at edges of beaker.)
2. Add distilled water and ethanol to beaker to the required volume.
3. Place the beaker containing solution on hot plate, and place stirrer bar in beaker.
4. Use manual stirrer to mix water and ethanol.
5. Place a chemical plate on the balance and tare.
6. Add PVA crystals from the container to the chemical plate using a spatula, and measure the required amount of PVA.
7. Use spatula to add chemical to beaker. (Do not pour all the chemical at one time as it may stick to and diffuse to beaker edges as the temperature increases)
8. Switch on hot plate; adjust spinning speed to medium and heater to low.
9. Place cork and thermometer at beaker inlet to minimize ethanol loss by evaporation at high temperature, and control the temperature.
10. Increase temperature of solution gradually to 80°C and let PVA crystals dissolve in the solution. (Do not increase the temperature suddenly as doing so may cause chemicals to be diffused at the bottom of beaker)

11. Wait until chemical is fully dissolved and the solution is stable. (The time required for dissolving is from 30 minutes to 2 hours depending on the concentration of PVA)

12. Pour solution into glass storage container, let it cool for a while, and then place the lid.

13. Transfer the container to the fridge to cool from room temperature to -50°C. Leave it there until it solidifies (from 30 minutes to 2 hours)

14. Remove the solution from fridge, let its temperature increase to room temperature, and then return it to the fridge.

15. Repeat steps 13 and 14 (Freeze-Thaw Cycling) until the desired stiffness is reached.

16. Repeat steps 1 to 15 for each type of phantom.

**Clean up:**

1. Wipe down balance and hot plate of chemical residue using damp paper towels, and dispose of towels in chemical waste bin.

2. Wash magnetic stirrers using water and sponge.

3. Return hot plate and balance to the shelf.
Appendix B.

Standard Operation Procedures-Preparation of PVA Phantom and Animal Tissue Samples

Simon Fraser University

Standard Operating Procedure

Preparation of PVA Phantom and Animal Tissue Samples

Date: June 2013

Author: Shima Zaeimdar

Principle Investigator: Dr. Farid Golnaraghi

Summary: The following SOP explains how to prepare phantom and animal samples.

Key Words: Sample dissection

Materials:

Latex gloves, lab coat, protective goggles, bleach, clear disposal bags, red sharp apparatus container, masks, labels, paper towels, cutter, bleach, biopsy punches, permanent marker, forceps, plastic storage container, digital caliper, flat plate, sponge, disposable pipette
Preparing required apparatus:

Place clear disposal bags, red sharp apparatus containing cutters and biopsy punches, paper towels, marker, digital caliper, flat plate and forceps on the counter.

Preparing test samples:

1. Place animal tissue or phantom block on flat plate.

2. Using cutter, make two parallel cuts into the target tissue (for animal tissues, the cuts should be parallel to tissue fibers) or phantom.

3. Separate the parallel faced cut from the rest of the tissue or phantom.

4. Place the sliced tissue or phantom on flat plate.

5. Prepare cylindrical samples by punching the slice from top face using biopsy punches.

6. Remove the final sample from biopsy punch by pushing it out from top end of biopsy punch using a disposable pipette.

7. Prepare rectangular specimens by cutting rectangular cross sections from top face of the original slice to the bottom face.

8. Measure sample sizes using digital caliper, three times each sample, and record the averages.

9. Place the final test samples into small clear bags with labels (showing name and dimensions) using forceps to prevent degradation due to dehydration of phantom and tissue specimens.

Clean up:

1. Package all unused tissue and phantom into clear bags with labels showing name, species and date, and keep in freezer.

2. Wipe all used surfaces with soaped sponge and water, and clean finally by bleach spraying.

3. Place all working tools in water and bleach solution (90:10) for 20 minutes.

4. Rinse tools in hot water tank using a sponge, and dry them with paper towel.

5. Return sharp tools to the red container.
Appendix C.

Standard Operating Procedures-Performing Compression Testing on Tissue-Mimicking Phantoms and Animal Tissue Specimens

Simon Fraser University

Standard Operating Procedure

Performing Compression Testing on Tissue-Mimicking Phantoms and Animal Tissue Specimens

Date: October 2013

Author: Shima Zaeimdar

Principle Investigator: Dr. Farid Golnaraghi

Summary: The following SOP describes how to set up the ELF testing machine for compression testing of PVA phantom and animal samples.

Key Words: Compression testing, Animal, Phantom

Materials:

Bose Elf3200 Testing machine, Sensotech-50lbf load cell, latex gloves, lab coat, protective goggles, ethanol, bleach, clear disposal bags, red sharp apparatus container, masks, labels, paper towels, cutter, bleach, biopsy punches, permanent marker, forceps, plastic storage container, calculator, sponge, disposable pipette
Preparing test set up:

1. Switch on power supply, amplifier, and signal conditioner for Elf testing machine.
2. Leave Elf machine running for 30 minutes.
3. Switch on computer connected to Elf system, and initiate WinTest 7.1 software.
4. Define load and displacement limits to prevent damage to system and load cell.
5. Install 50-lbf load cell and titanium platens on Elf, and tighten them gently with fingers.
6. Adjust position of lower platen relative to upper platen.

Test protocol:

1. Dissect and organize testing samples following SOP of Preparation of PVA Phantom and Animal Tissue Samples.
2. Place a drop of water on the surface of lower platen using a disposable pipette to prevent friction and dehydration of sample.
3. Pick up sample from clear bag with forceps and place it on the lower platen.
4. Add a drop of water to the sample.
5. Switch on the mover.
6. Using manual mover, make sure that lower platen is at zero position.
7. Based on the desired level of strain, strain rate and sample thickness size, calculate peak displacement and rate of displacement for each sample.
8. Change peak displacement and rate of displacement in the software, based on the calculations.
9. Define data acquisition setting for acquiring 1008 data points, and specify the name of the file in which to save the data.
10. For each sample, keep records of specimen name, dimensions, peak displacement, displacement rate and loading setting.
11. Apply preload of 0.1 N through the software.
12. Establish block waveform for all tests.
13. Start data acquisition and run the test.
14. Once the test is completed, return lower platen to the zero position.
15. Place tested specimen inside waste clear bag with proper label.

16. Gently remove tissue/phantom debris from the platen surfaces using a damp paper towel.

17. Repeat steps 1 to 16 to test each sample.

**Clean up:**

1. Switch off control system power supply.

2. Remove platens and load cell from the system

3. Wipe platens and load cell and surrounding system using paper towels wetted with 10% bleach solution.

4. Dispose of all unused tissue and phantoms.

5. Place all working tools in a 10% bleach solution for 20 minutes.

6. Clean up all the working surfaces using sponge soaked with water.

7. Store collected data in a flash drive.
Appendix D.

Standard Operating Procedures- Performing Compression Testing on Large-Scale Breast Phantom and in-vivo Tissues

Simon Fraser University

Standard Operating Procedure

Performing Compression Testing on Large scale Phantom and in-vivo Tissues

Date: October 2012

Author: Shima Zaeimdar

Principle Investigator: Dr. Farid Golnaraghi

Summary: The following SOP describes how to set up Pressure Profile System Fingertip Sensor and Celesco Cable Extension Transducers to capture compressive responses of large-scale phantom and in-vivo tissues.

Key Words: Compression testing, Large-scale phantom, in--vivo

Materials:

Pressure Profile System Fingertip Sensor, Celesco Cable Extension Transducers
Lab coat, Calculator, Ruler, a piece of wire, Screws and nuts, Stereotactic Needle
Biopsy Training Phantom, Camera, Voltmeter, Power supply.
Preparing test set up and sensors calibration:

1. Fix spring pot at one edge of a wooden box by wood screw driver, screws and nuts.

2. Connect spring pot to a power supply of 5 V and voltmeter.

3. Calibrate spring pot by holding tip of rob and extending it to 10 different vertical positions (10 mm, 20 mm...100 mm); measure traveling distance of rob using a ruler. Draw the graph showing voltages and positions relationship, and obtain the trend line.

4. Wear PPS force sensor on index finger.

5. Switch on the laptop.

6. Switch on PPS sensor switch and connect it to the laptop.

7. Start Chameleon TVR v.1.3.16.3 software.

8. Choose index finger in Chameleon and calibrate force sensor by making force value zero and then exerting a certain force on the calibration load cell.

Test Protocol:

1. Wear index finger force sensor and fix the finger at spring pot rob tip using a wire.

2. For in-vivo experiments: subject should place palm/forearm/bicep over the counter below spring pot in vertical direction.

3. For phantom experiments, testing area of phantom should be vertically positioned below spring pot.

4. Mark the level of strain that should be compressed on the subject/phantom.

5. In Chameleon, clear previous data readings, make a preview of current data acquisition and tare force readings.

6. Switch on the camera and initiate video recording of the experiments.

7. Start the experiment by lowering index finger connected to spring pot rob in a vertical position towards the object.

8. Press the tissue/phantom up to more than 10% strain level with constant strain rate, and unload it at the same strain rate.

9. Save acquired force data in the laptop, and save them in a flash drive.
10. Extract voltages from videos and correlate to displacement using calibration equation.

11. Repeat steps 2 to 9 for different tissues and phantom regions.

Clean up:

1. Remove index finger from spring pot.
2. Switch off PPS switch and remove it from the laptop.
3. Place force sensor in the shelf.
4. Switch off voltmeter and power supply.