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Biaxial Studies of Skin's Mechanical Properties

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BIAXIAL STUDIES OF SKIN'S MECHANICAL PROPERTIES

by

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ABSTRACT

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Understanding skin's mechanical properties plays an important role in treating conditions like edema, wound healing etc, in designing of skin grafts and also development of artificial skin. Many methods have been used to study skin's mechanical properties based on the loads applied i.e uniaxial, biaxial and multiaxial tests. The objective of this thesis is to perform biaxial experiments to study the skin's mechanical properties.

In this research, biaxial tests are performed on 12 pieces of abdomen skin specimen from rats. The results show that skin possesses anisotropy, non-linearity and hysteresis. To explain the stress-strain relationship in terms of the strain-energy function the material constants are determined from the experiments and comparison between the theoretical and experimental result shows a high correlation. These results may provide an insight into better understanding skin's mechanical properties.

PREFACE

The master thesis that is lying before you is the result of two years of research at the University of Memphis, Department of Biomedical Engineering.

The writing of this report has gone through several ups and downs. Especially in the beginning I experienced some difficulties in getting all the pieces of the setup together. This period took me approximately two months. Then I was faced with the challenge of making improvements to the setup design to make it more current and efficient.

Groups this thesis will benefit:

With the above accomplished this thesis is written to help people familiarize themselves with the biaxial testing device and its uses. For students both undergraduate and graduate this device could serve as a potential learning tool to better understand the device setup and put it to practical use.

Scope of the thesis:

This thesis covers the basics concepts of continuum mechanics. It familiarizes the reader with the various methods of testing of tissues by providing examples. It also touches on the positive and negative aspects related to using the biaxial testing device.

Supporting Paper:

Shang, Xituan, Yen, M.R., & Gaber, M.W. (2004). Studies of the biaxial mechanical properties and finite element modeling of skin. *Molecular Cell Biomechanics*, **7**, 93-104.

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LIST OF SYMBOLS

ρ_0	Density of the material
W	The strain energy per unit mass of the material
$\rho_0 W$	The strain energy per unit volume of the tissue in the zero stress state
σ_{ij}	Cauchy stress components
T_{ij}	Lagrangian stress components
S_{ij}	Kirchhoff stress components
E_{ij}	Green's strain components
λ	Stretch ratio
k	Boltzman's constant, $k = 1.3807 \times 10^{-23}$ J K^{-1}
θ	Absolute temperature, θ=298K
N	The number of rigid links of length 'l' in the fiber
n	The network molecular chain density

CHAPTER 1 INTRODUCTION

1.1 Introduction to Skin's Anatomy

Skin is the largest organ in human beings. It acts as a barrier between the external and internal environment. As a barrier it offers protection from pathogens and excessive water loss. Its other functions are heat regulation, evaporation control, and regulation of blood pressure within the body.

Human skin

The structure of the human skin is shown in Figure 1.1. The human skin is composed of two primary layers: the epidermis and the dense layer of fibrous and elastic tissue called the dermis. The thickness of the two layers varies depending on the location of the skin. The dermis is 20 times thicker than the epidermis (Shergold, 2004) and dominates the overall behavior. The epidermis is the outer layer of the skin. It acts as a protective barrier for the dermis, against infections and inhospitable environments.

The most abundant dermal constituent is collagen. Collagen is a protein and constitutes 60-80% of dry weight of dermis (Dombiand Haut, 1985; Reihsner et al., 1995). Type I collagen accounts for 80% of total collagen in skin and type II makes up 15% of total collagen. Collagen is responsible for skin's tensile strength of 1.5 to 3.5 x 10^2 MPa and a Young's modulus of up to 1GPa (Lanir Y, 1987). The functions of the dermis are to regulate temperature and supply the epidermis with nutrient-rich blood.

Rheology of Living Tissues

All living tissues are composites exhibiting complex mechanical behavior. In skin the collagen fibers are twisted, and each fiber takes load depending on the condition of the network, thus exhibiting nonlinear stress-strain relationship (Fung, 1972). The nonlinearity of stress-strain relationship is evident if the slope of the load-stretch curve is plotted against the load (Fung, 1972).

1.2 Mechanical Properties of Skin

The mechanical properties of skin are an important indicator of its pathological conditions like edema, wound healing, Ehlers Danlos syndrome etc. (Lanir & Fung, 1974).

Ehlers Danlos syndrome is a disorder marked by extremely loose joints; hyperelastic skin that bruises easily, and easily damaged blood vessels.

Knowledge about the mechanical properties of skin will help plastic surgeons in designing the size, shape and orientation skin grafts to optimize wound closure. When mechanical tension is applied, skin appears to be able to stretch beyond its natural yield and this unique property provides opportunities for closure of large wounds (Gibson et al., 1965; Stark 1977; Hirshowitz et al., 1993). Also studies of skin's mechanical properties are of importance in cosmetic applications to validate the effectiveness of cosmetic products (Hendriks, Brokken, Van Eemeren, Oomens, Baaijens & Horsten, 2003).

Many methods have been used to study the mechanical properties of skin depending on the type of load applied to skin, i.e uniaxial, biaxial and multiaixial extension, indentation, suction and torsional tests. These tests measure in-plane forces and displacements, which can then be converted to a stress-strain relationship to obtain a constitutive equation.

The uniaxial test is probably the most commonly used technique in the study of the mechanical properties of skin. It is simple to use and well suited for measurements of directional mechanical effects (Snyder & Lee, 1975). The results of uniaxial studies have been used to investigate the directional variation of skin extensibility of significance in plastic surgery (Kenedi & Gibson, 1962; Stark, 1977) monitoring the changes to skin due to drugs or radiotherapy (Burlin, Hutton & Ranu, 1977). Study of skin's mechanical properties provides an understanding of the direction of maximum stretching of skin, helping surgeons make smaller incisions for wound closures and in cosmetic surgery.

Testing of skin has been performed both in vivo and in vitro. In vivo testing avoids the issue of tissue environment and release of in vivo skin tension, while in vitro study is relatively simpler and easier. It also helps study the failure state of skin. In vivo and in vitro tests yield some information on the mechanical properties of skin, but not a complete three-dimensional stress-strain relationship (Lanir & Fung, 1973) to better understand the mechanics of skin in surgery and physiology. Also effects of surrounding tissue are unknown in in vivo tests (Lanir & Fung, 1973).

Although uniaxial testing has been intensively studied, they are capable of doing measurements in only one direction at a time. Constituent stress-strain properties for a two-dimensional model cannot be obtained using this method. Since skin can be considered as an incompressible material, any changes in the third dimension can be calculated from changes in the other two dimensions. So a comprehensive three-dimensional constitutive model can be obtained from two-dimensional experiments.

The aim of this thesis is to study mechanical properties of skin using biaxial tests in vitro. In this research dynamic biaxial tests are performed on abdomen skin specimens and results analyzed. The material constants are obtained from the experimental results using Tong and Fung's strain-energy function (Fung, 1975). Through this analysis, the advancements in the field of tissue engineering can be analyzed.

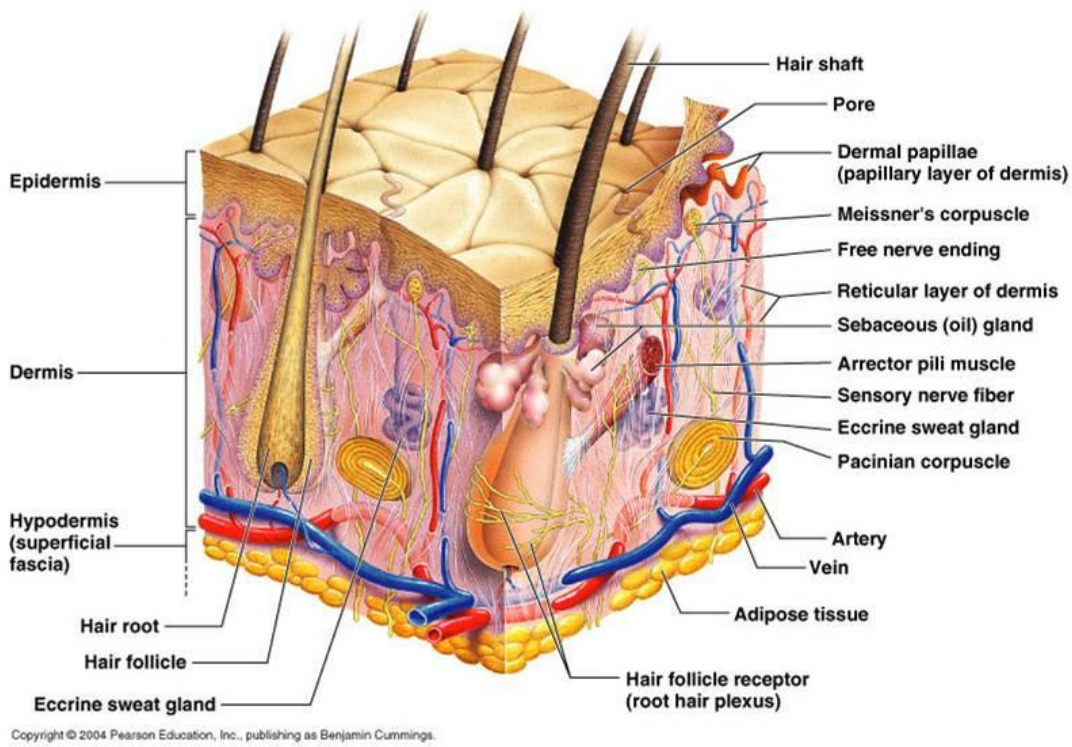


Fig 1.1 Diagram of the skin (Pearson Education Inc).

CHAPTER 2 STUDY OF THE BIAXIAL PROPERTIES OF SKIN

2.1 Soft Tissue Mechanics

Soft tissues are inhomogeneous, anisotropic materials exhibiting nonlinear and viscoelastic properties. These properties make continuum mechanics an ideal approach to study the mechanical properties of soft tissues. Therefore, knowledge of nonlinear solid mechanics by a continuum approach seems essential.

Identification of a suitable strain energy function is the ideal means to study the nonlinear elastic properties of soft tissues. Once the strain energy function is known, the constitutive stress-strain relationships can be directly obtained from the strain energy function.

2.2 Basic Concepts

Stretch Ratio (λ)

Stretch ratio λ is defined as the ratio of the deformed length l divided by the initial length L:

$$\lambda = \frac{l}{L} \tag{1}$$

Nominal Strain (e)

Nominal strain, also known as engineering strain is the ratio of the change in length ΔL per unit of the original length L of the line elements. The nominal strain is positive if the material fibers are stretched and negative if they are compressed (Fung, 1974).

$$e = \frac{\Delta L}{L} = \frac{l - L}{L}$$

Strain Rate ($\dot{\varepsilon}$)

It is the rate of change of strain with respect to time.

$$\dot{\varepsilon} = \frac{d\varepsilon}{dt} = \frac{d}{dt}\left(\frac{l - l_0}{l_0}\right) = \frac{1}{l_0}\frac{dl}{dt} = \frac{v}{l_0} \qquad (2)$$

where l is the length under applied stress, l_0 is the original length and v is the speed of deformation.

Strain Energy Function (W)

Strain energy function is used to study the mechanical behavior of soft tissues. Strain energy function is a function of the strain components e_{11}, e_{22}, e_{33}, e_{12}, e_{23}, e_{31} (Fung, 1975). It is denoted by the symbol W. A strain energy function is used to define a hyperelastic material by postulating that the stress in the material can be obtained by taking the derivative of W with respect to the strain.

2.3 Hyperelastic Behavior

Hyperelasticity is the ability of the material to experience large elastic strain without losing its original properties. A material is hyperelastic if there is a scalar function, denoted by W=W (F), where W is the strain energy function and W (F) is the strain energy density function (Allan Bower, 2009).

Deformation Gradient (F)

Writing the current position of a material point as x and the reference position of the same point as X, the deformation gradient is (Maurel et al., 1998):

$$F = \frac{\partial x}{\partial X}$$

Cauchy Stress (σ)

$$\sigma = \frac{1}{J} F \left(\frac{\partial W(F)}{\partial F} \right)^T$$

where J is the determinant of the deformation gradient.

For isotropic hyperelastic material the stress relationship describing the behavior of hyperelastic, homogeneous materials at finite strains is:

$$S = 2\left[\left(\frac{\partial W}{\partial I_1} + I_1 \frac{\partial W}{\partial I_2} \right) I - \frac{\partial W}{\partial I_2} C + I_3 \frac{\partial W}{\partial I_3} C^{-1} \right] \qquad (3)$$

where I is the identity tensor, S is the second Piola-Kirchhoff tensors, and I_1, I_2 and I_3 are the invariants of C.

Green's Strain (E)

Green's strain tensor to characterize the deformation near a point is:

$$E = \frac{1}{2}\left(F^T \bullet F - I \right)$$

where F is the deformation gradient.

Lagrangian Stress (T)

Once the strain energy function is obtained, the principal components of the Lagrangan stress tensor (T) can be derived as (Allaire et. al., 1977):

$$T_{ii} = \frac{2}{\lambda_i}\left[\lambda_i^2 \frac{\partial W}{\partial I_i} + I_2 \frac{\partial W}{\partial I_2} - \frac{I_3}{\lambda_i^2} + I_3 \frac{\partial W}{\partial I_3}\right] i = 1,2,3 \qquad (4)$$

where λ is the stretch ratio, W is the strain-energy function and I_i are the invariants of C.

Biaxial Compression

In the case of biaxial deformation the deformation gradient is

$$F = \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix}$$

$$\lambda_1 = \lambda_2 = \lambda_3 = 1$$

$$J = \det F = \lambda_1 \lambda_2 \lambda_3 = 1 \qquad (5)$$

2.4 Constitutive Equation of Skin Elasticity

Human skin is non-linear, anisotropic and exhibits viscoelastic behavior. In order to accurately study the mechanical properties of skin, a constitutive equation has to be developed. A constitutive equation is an equation describing the stress-strain relationship in a three-dimensional stress field. Many constitutive laws are available to model the mechanical behavior of skin (Lanir, 1974; Tong & Fung, 1975; Nash & Hunter).

Planar soft tissue mechanics is characterized using strain-energy functions. Strain energy function is a constitutive equation to study the mechanical behavior of soft planar tissues. Strain-energy functions by Demiray & Vito, 1976; Fung, 1993; Tong & Fung, 1976; Arruda & Boyce, 1993; Lanir, 1994 is used to study the nonlinear mechanical properties of skin.

Lanier's Model

Lanier's model suggested that the mechanical properties of skin depend on the collective behavior of all its constituents. Accordingly, Lanier derived two sets of equations (Lanir, 1974). The first model assumes elastin-induced undulation of the collagen fibers with a high density of crosslinks between the two major constituents.

$$Tns = \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} W(\lambda,\theta).\cos(\mu-\theta).[\cos\psi(\lambda\ \ \cos\theta+\gamma\lambda_2\sin\theta)+\sin\psi\lambda_2\sin\theta]d\theta+p(\lambda_1,\lambda_2,\gamma)\cos(\overline{n}_1,\overline{s})$$

$$W_1(\lambda,\theta)=[F(\lambda)/\lambda].N.R(\theta)$$

where Ψ is the strain-energy density, p is the hydrostatic pressure, θ is the angle of maximum principal stress from the x-axis, μ is the Ogden material coefficient, λ_i is the principal stretch and T is the transformation matrix describing the principal stress directions.

The second model assumes natural undulation of the collagen fibers and lower density of crosslinks between collagen and elastin fibers. The stress-strain relationship is:

$$W_2(\lambda,\theta) = K_1.\mathrm{Re}(\theta)[\lambda(\theta)-1]/\lambda(\theta) + K_2 R_c(\theta)[1/\lambda(\theta)] \times \int_{1(\theta)}^{2(\theta)} P(x)[\lambda(\theta)-x]/xdx$$

where Re is the Reynolds number, K_1 and K_2 are stress-like parameters.

Bischoff's Model

Bischoff, Arruda, and Grosh (2002) developed a model to describe the stress-strain behavior of skin assuming skin as an orthotropic hyper elastic material. This model is based on the microstructure of skin. The stress-strain relation is:

$$W = W_0 + \frac{nk\Theta}{4}\left(N\sum_{i=1}^{4}\left[\frac{\rho^{(I)}}{N}\beta_p^{(i)} + \ln\frac{\beta_p^{(i)}}{\sinh\beta_p^{(i)}} \right] - \frac{\beta_p}{N}\ln\left[\lambda_a^{a^2}\lambda_b^{a^2}\lambda_c^{a^2}\right]\right) + B[\cosh(J-1)-1]$$

Where W_0 is the constant, k is the Boltzmann's constant, *Θ* is the absolute temperature, *ρ* the deformed length of the molecule chains, *β* the Langevin function, and B (cosh(J-1)-1) the compressible component of the strain energy.

Danielson's Model

Danielson's model assumes skin to be an elastic membrane. He modeled the stress-strain behavior of skin under two conditions. First for small deformations, skin was assumed as elastic and the constitutive equation is:

$$N^{\alpha\beta} = \frac{\partial W}{\partial \gamma_{\alpha\beta}}$$

$$W = A^{\alpha\beta\gamma\mu}\gamma_{\alpha\beta}\gamma_{\lambda\mu}\exp\left(B^{K\delta}\gamma_{K\delta} + C\gamma^{\rho\phi}\gamma_{\rho\phi}\right)$$

where A and B are material constants, C is a scalar and γ a strain component.

For large deformations, Danielson assumed skin to be viscoelastic. So there would be no conservation of energy. The constitutive equation is:

$$N^{\alpha\beta} = A^{\alpha\beta\lambda\mu}\gamma_{\lambda\mu}\exp\left(B^{K\delta}\gamma_{K\delta} + C\gamma^{\rho\phi}\gamma_{\rho\phi}\right)$$

Tong and Fung's Model

The stress-strain model proposed by Tong and Fung is the most widely used to study soft planar tissues. The model was developed using Lanir and Fung's experimental data from rabbit skin. The study by Lanir and Fung shows that in cyclic loading at constant strain rate the stress-strain relationship is independent of the strain rate.

Lanir and Fung's experimental results suggest that skin is not purely elastic, so Tong and Fung defined a pseudo-strain-energy function to model skin's behavior. This strain-energy function during deformation is applicable only to preconditioned specimens.

Following is the strain-energy function by Tong and Fung used in this study.

$$\begin{aligned}\rho_0 W^{(2)} &= \frac{1}{2}(\alpha_1 E_1^2 + \alpha_2 E_2^2 + \alpha_3 E_{12}^2 + \alpha_3 E_{21}^2 + 2\alpha_4 E_1 E_2) \\ &+ \frac{1}{2} c \exp(a_1 E_1^2 + a_2 E_2^2 + a_3 E_{12}^2 + a_3 E_{21}^2 + 2a_4 E_1 E_2 \\ &+ \gamma_1 E_1^3 + \gamma_2 E_2^3 + \gamma_4 E_1^2 E_2 + \gamma_5 E_1 E_2^2)\end{aligned} \tag{1}$$

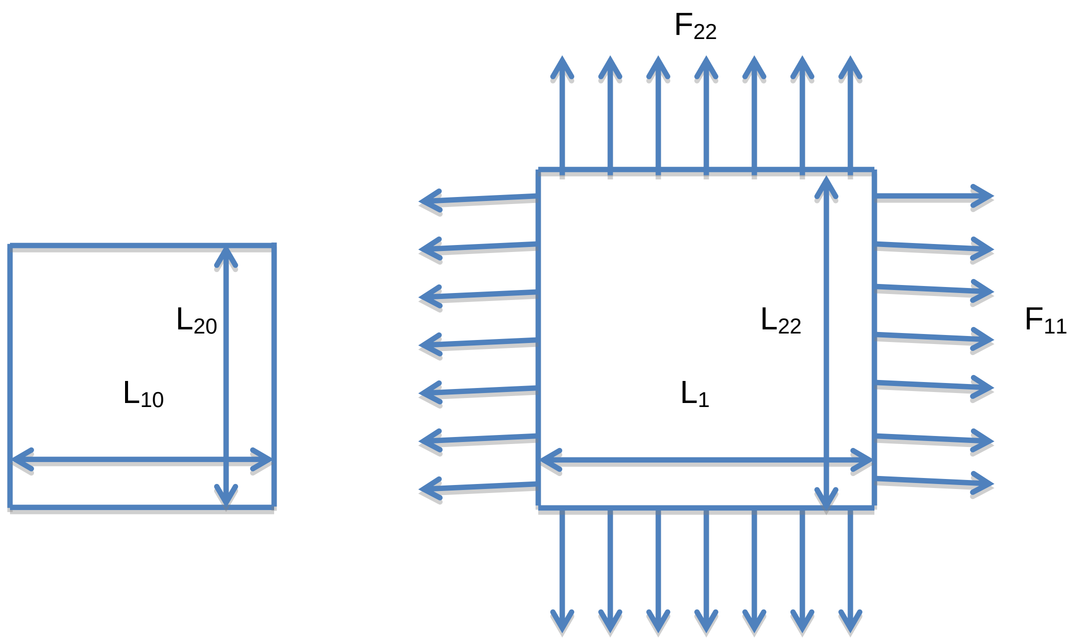
For practical purposes the strain-energy function can be simplified as:

$$\rho_0 W^{(2)} = \frac{1}{2}(\alpha_1 E_1^2 + \alpha_2 E_2^2 + \alpha_3 E_{12}^2 + \alpha_3 E_{21}^2 + 2\alpha_4 E_1 E_2)$$
$$+\frac{1}{2} c \exp(a_1 E_1^2 + a_2 E_2^2 + a_3 E_{12}^2 + a_3 E_{21}^2 + 2a_4 E_1 E_2 \qquad (2)$$

ρ_0 is the density of the material in the un-deformed state. α's, a's, γ's and c are constants. E_1 and E_2 are Green's strains in the x and y direction respectively, and E_{12} is the shear strain. W is the strain energy per unit mass of the material.

In order to derive the constants for above equation based on experimental results, a relation has to be established between stress, strain, stretch ratio and strain energy.

Figure 2.1 shows an illustration of a rectangular skin sample with initial dimensions L_{10}, L_{20}. E_{12}= E_{21}=0 for our experiments (a rectangular sample deformed into another rectangular sample). On application of forces F_{11} and F_{22} on the edges the dimensions become L_1, L_2. The initial and final thickness of the specimen is h_0 and h respectively.



(a) before deformation (b) after deformation

Fig 2.1 Deformation of a rectangular membrane (Fung, 1993, p. 299).

The deformation ratios are:

$$\lambda_1 = \frac{L_1}{L_{10}} \qquad \lambda_2 = \frac{L_2}{L_{20}}$$

Greens's strains are:

$$E_1 = \frac{1}{2}\left(\lambda_1^2 - 1\right) \qquad E_2 = \frac{1}{2}\left(\lambda_2^2 - 1\right)$$

Cauchy's stresses are:

$$\sigma_{11} = \frac{F_{11}}{L_2 h} \qquad \sigma_{22} = \frac{F_{22}}{L_1 h}$$

Lagrangian stresses are:

$$T_{11} = \frac{F_{11}}{L_{20}h_0} \qquad T_{22} = \frac{F_{22}}{L_{10}h_0}$$

The Kirchhoff's stresses are:

$$S_{11} = \frac{\partial\left(\rho_0 W^{(2)}\right)}{\partial E_1} = \alpha_1 E_1 + \alpha_4 E_2 + cA_1 X$$

$$S_{22} = \frac{\partial\left(\rho_0 W^{(2)}\right)}{\partial E_2} = \alpha_4 E_1 + \alpha_2 E_2 + cA_2 X \tag{3}$$

$$S_{12} = \frac{\partial\left(\rho_0 W^{(2)}\right)}{\partial E_{12}} = \alpha_3 E_{12} + ca_3 E_{12} X$$

Where

$$A_1 = a_1 E_1 + a_4 E_2 + \frac{3}{2}\gamma_1 E_1^2 + \gamma_4 E_1 E_2 + \frac{1}{2}\gamma_5 E_2^2$$

which can be simplified as:

$$A_1 = a_1 E_1 + a_4 E_2$$

$$A_2 = a_4 E_1 + a_2 E_2 + \frac{3}{2}\gamma_2 E_2^2 + \gamma_5 E_1 E_2 + \frac{1}{2}\gamma_4 E_1^2$$

which can be simplified as:

$$A_2 = a_4 E_1 + a_2 E_2$$

and

$$X = \exp(a_1 E_1^2 + a_2 E_2^2 + a_1 E_{12}^2 + 2a_4 E_1 E_2 + \gamma_1 E_1^3 + \gamma_2 E_2^3 + \gamma_4 E_1^2 E_2 + \gamma_5 E_1 E_2^2)$$

which can be simplified as

$$X = \exp\left(a_1 E_1^2 + a_2 E_2^2 + 2a_4 E_1 E_2\right)$$

The Kirchhoff's stresses determined experimentally are:

$$S_{11} = \frac{T_{11}}{\lambda_{11}} = \frac{1}{\lambda_1^2}\sigma_{11} \qquad S_{22} = \frac{T_{22}}{\lambda_{22}} = \frac{\rho_0}{\rho}\frac{1}{\lambda_2^2}\sigma_{22} \tag{4}$$

2.5 Skin Thickness

Skin's thickness depends on age, area of the body in consideration, exposure to sunlight and person's health. 90% of the skin's thickness comes from the dermal layer and variations in collagen and elastin levels causes changes in skin thickness.

Calculation of stress requires knowledge of the cross-sectional area of the specimen. In a rectangular specimen, it requires determination of the width and thickness of the specimen. The simplest method to measure the skin thickness is using a ruler on skin fold. The ultrasonic biometric ruler is an accurate, non-invasive method of measuring the thickness of skin (Harold Alexander & Miller, 1979). Radiological method has also been used to measure skin's thickness. Comparison of the ultrasonic and radiology methods by Alexander and Miller shows substantial correlation (r=0.99) in the measurement of skin thickness.

2.6 Experimental Methods

Several methods have been used to study the mechanical properties of skin both in vivo and in vitro: indentation test, torsional test, and suction test, uniaxial, biaxial and multiaxial tests. The simplest and easiest to perform is the indentation test (Highley, 1997). It has been used as a diagnostic tool to study changes in skin's mechanical properties in skin diseases. Because of the importance of the mechanical properties of skin to medicine, most information is available for mammalian skin from common laboratory animals such as rats, rabbits and cats.

Uniaxial Testing of Skin

The uniaxial test is the most commonly used in research to study the mechanical properties of skin. It is simple to use and study of directional mechanical properties. The uniaxial extensometer used to study the mechanical properties of skin works by applying displacements to the skin using extensible pads attached to the skin using tape. The force needed for deformation is measured by the load cell as the skin deforms (Evans & Siesennop,1967; Baker et al., 1988; Gunner et al., 1979).

Ridge and Wright (1966) used an extensometer to study the mechanical properties of skin. The skin samples were 1cm in length and 4cm in width. The stress-strain curves obtained from the model were characterized using the equation $e = C + kL^b$, where e is the extension, L the load, and C, e and b constants.

Gunner et al. (1979) studied the mechanical properties of skin in vivo using an extensometer. The tabs on the instrument are 20mm long and 10mm wide, spaced 10mm apart. They reported force-time and extension-time data that was converted to nominal stress versus stretch assuming an initial thickness of 1mm and an initial width equal to distance between the extensometer tabs. Ohura et al. (1980) used a tension meter to study the skin wound healing problems in vivo. In vivo study of the skin's mechanical properties is affected by distortions imposed by body movements during testing (Wilkes, Brown & Wildnauer, 1973).

Dunn et al. (1985) excised normal skin samples from human cadavers, and performed uniaxial tensile tests on samples 20mm long and 10mm wide. Studies were made to account for the directional variation in mechanical behavior. Data was reported as stress-strain: the stress corresponding to nominal stress.

Belkoff and Haut (1991) performed uniaxial tensile tests on skin samples taken from the back of the rats. They used dumbbell shaped specimens 22.4mm in gauge length and 4.8mm in gauge width in their study. Tests were done by placing the samples in the grips and applying a force of 0.1N. The test was carried out until the specimen failed, and then the load-deformation curves were recorded. The data obtained provided an opportunity to study the mechanical properties of the skin specimens.

Biaxial Testing of Skin

Lanir and Fung (1976) were the first to perform biaxial testing of skin. They developed a biaxial X-Y table to test square shaped tissue in vitro. The sample was placed in a saline bath and connected by hooks to silk threads. The silk threads were then connected to the X-Y table that has the force transducer, displacement motor and a pulley system. The stress-strain relationship was measured by changing the displacement in one direction, while keeping it constant in the other direction or by changing the displacement in both directions simultaneously. The results of Lanir and Fung's experiments were of importance to biaxial testing of skin.

The results of Lanir and Fung's experiments show that: 1) the stress-strain relation of skin is extremely non-linear. 2) the stress-strain relation is independent of the strain rate. 3) hysteresis is observed at all strain levels. 4) the stress in a uniaxial tension test is considerably lower than the stress in the biaxial stretch test for a given stretch ratio. 4) the temperature effect on the mechanical properties of skin depends both on the rate of temperature change and the stress and strains in the tissue.

According to Sacks and Sun (2003) biaxial mechanical data is essential to determine the three dimensional constitutive equation of tissues. Biaxial testing is considered superior to indentation and uniaxial testing (Lanir & Fung, 1974).

Neilsen, Hunter, and Smaill (1991) developed a biaxial testing system with some variations to the Lanir's model. The model used only four attachments on each edge, sample mounting was simplified, the point forces were monitored individually, the loading was controlled using software and the stress field could be controlled individually. This equipment was used to test deformable membrane samples of size ranging between 12 to 25mm.

Wan Abas (1994) made biaxial stretch test studies of human skin in vivo. The results of his studies illustrate that skin exhibits, non-linear, anisotropic and viscoelastic behavior. The results also suggest that the response to a biaxial stretch in vivo was similar to in vitro test results.

Hung, Chong, Steinhart, Trexler, and Billiar (2005) designed a planar biaxial testing device to study the mechanical properties of soft connective tissues. The testing device had a four-axis control system; temperature controlled testing chamber, low force capability (<0.5N), automated sample attachment and real time computer control.

The BioTester designed by cellscale is used to characterize soft tissues and biomaterials. The BioTester displays live time, can measure sample sizes ranging between 3 to 15mm, fast and accurate mounting of sample and real time graphing of data.

Multiaxial Testing of Skin

A multiaixal rig (Kvistedal & Nielsen, 2003) in Fig 3.7 designed to study the mechanical properties of skin has 16 displacement actuators, each equipped with a force transducer to measure the force. The deformations resulting were recorded using a camera. The results were reported in terms of stress and strain. The data was then used to study the mechanical properties of skin. Other multiaxial methods to study the properties of skin are suction and indentation tests.

Suction test (Hendriks, Brokken, Oomens, Baaijens & Morales-Serrano, 2003) is used to study the mechanical properties of skin in vivo. The experimental setup consists of a pressure chamber and an ultrasound device connected to it. The skin sample is attached to the chamber filled with water.

Suction is applied to the chamber with a syringe; pressure applied measured using a pressure gauge. The displacements are measured from the images obtained from the ultrasound. This method is used to study non-linear behavior of skin.

Indentation testing by (Pailler-Matte & Zahouani, 2008) is used to study the adhesive behavior of human skin in vivo. The test uses a smooth spherical steel indenter. The device is displacement controlled and records force as a function of indentation depth. A linear variable differential transformer is used to measure the displacement.

Multiaxial testing has the advantage that samples can be tested in multiple directions without having to remount the sample for each test. What all the papers suggest is that skin shows a nonlinear stress-strain relationship, incompressible, anisotropic and inhomogeneous, and is subject to a prestress. Table 2.1 summarizes the studies of the mechanical properties of skin. Table 2.2 gives an account of the measured young’s modulus of the entire skin.

Table 2.1 Summary of the studies of the mechanical properties of skin

Author	Species	Load Type	Properties
Ridge & Wright (1966)	Abdominal skin from cat	Uniaxial tension	Stress strain curve for skin extended at a constant rate of 0.2mm inches/min. The extension process could be divided into three phases: 1) Straightening out and orientation of collagen fibers. 2) Extension of oriented collagen fibers. 3) Yielding.
Ohura (1980)	Human	Uniaxial tension	Study wound healing problems in vivo. Comparison of stress-strain curves of skin and hypertrophic scar tissues suggests: These differences in the stress-strain curve are due to the ordered alignment of collagen fibers in hypertrophic scar tissue.
Lanir & Fung (1976)	Rat abdomen	Biaxial tension	The stress-strain relation of skin stretched at a rate of 0.02, 0.2 and 2 mm/sec is non-linear, anisotropic and hysteresis is observed in the stress-strain curves. The temperature effect of the properties of skin depend on rate of temperature change and stress- strain in the tissue

Table 2.1 Summary of the studies of the mechanical properties of skin

Author	Species	Load Type	Properties
Wan Abas	Human skin	Biaxial tension	The study suggests skin is non-linear, anisotropic and viscoelastic.
Kvistedal & Nielsen	Human skin	Multiaxial	The material exhibits viscoelastic behavior showing stress relaxation and creep. Variation in material parameters indicates differences in mechanical properties between similar individuals. Mechanical properties of skin are independent of the contribution of the fat layer.

Table 2.2 Summary of in vivo measured Young's moduli of the entire skin

Method	Young's Modulus (MPa)	Test Region	Reference
	E‖ =20	Leg	Manschot (1985)
Tensile	E⊥=4.6	Leg	Manschot (1985)
	E=0.42	Dorsal forearm (<30 yr)	Agache (1985)
Torsion	E=0.85	Dorsal forearm (>30 yr)	Agache (1985)
Torsion	E=0.02-0.11	Dorsal forearm	Sanders (1973)
	E=1.99. 10^{-3}	Male thigh	Bader & Bowker (1983)
Indentation	E=1.51. 10^{-3}	Male forearm	Bader & Bowker (1983)
	E=1.09. 10^{-3}	Female forearm	Bader & Bowker (1983)
Suction	E=18-57	Forearm	Grahame & Holt (1969)
	E=0.15	Forearm	Barel (1998)
Suction	E=0.25	Forearm	Barel (1998)

CHAPTER 3 BIAXIAL EQUIPMENT SYSTEM

3.1 Introduction to the Biaxial Equipment System

The BIAX-II was custom designed at the University of California, San Diego in 1999. Figure 3.3 is the picture of the equipment. The components of the BIAX system are 1) X-Y table, 2) control amplifier, 3) video dimensional analyzer, 4) computer I, 5) video camera, and 6) computer II. Figure 3.1 shows the diagram of the system setup.

In the original design of the system the computer II was equipped with a image acquisition card and image analysis software OPTIMAS used to grab the video signal recorded by the video cassette recorder (VCR). The drawback of this design is that the VCR needs to be stopped manually at certain times to grab the video frames. It is very difficult to accurately stop the VCR at a certain time, causing errors. We made improvements to this design by using another computer with a image acquisition card (ATI TV wonder, ATI Technologies Inc.) and video capture software (Video Capturix, Capturix Technologies Inc., Portugal), so the video signal could be automatically grabbed and extracted from the VCR.

Force Measurement

The coupling of the specimen to the force transducer is seen in figure 3.2. The BIAX-II system is displacement controlled and records force. The BIAX-II picks up three signals, force x, force y and the video signal. It measures the forces in the x and y directions by force transducers attached to one of the platforms.

The force transducer reads between 0-1000g weights. The goodness of fit for the force channels is 0.9997 ± 0.0002 and 0.9993 ± 0.0003 respectively.

Stretching Mechanism

The specimen is immersed in saline solution in an open compartment. Staples to 6 to 8 silk threads, which are connected to the loading mechanism, hook each edge of the specimen. A square target is made at the specimen center using steel wires. Each string is adjusted individually so the marker placed at the center of the specimen remained rectangular at rest and in tension. This was done to ensure uniformity in the distribution of strain.

Non-Contact Strain Measurement

The non-contact measurements of strains are fulfilled by the video dimensional analyzer, video camera and computer II equipped with the image acquisition card and image analyzing software (Scion Image, Scion Corp, Frederick, MD).

In the BIAX-II system, the VDA is used to obtain the specimen deformation in the y direction. The VDA works together with the video camera signals to convert the distance between two dark-light or light-dark transitions in the image into voltage, which can be recorded and converted to displacement. Knowing the scan rate and the starting and stopping of the counting circuit, the distance between the markers placed on the specimen can be computed by the VDA.

The computer II is equipped with the image acquisition card and image capture software, wherein the video signal can be automatically grabbed and extracted from the VCR at 30 frames per second. The grabbed images are then analyzed using the Scion image software. The computer II measures the strains in the x direction using the Scion image analyzer.

Biaxial Testing Protocol

The HP-VEE software in the setup in capable of doing both uniaxial and biaxial tests. Two types of tests are performed. 1) Step test: the strain in one direction is kept constant each run and varied every 4-5 successive runs; 2) Ramp test: the strain in both directions is varied simultaneously. Figure 3.4 shows an example of biaxial ramp test code. Figure 3.5 shows an example of biaxial step test code.

These experiments yield information on the constitutive equation of the skin and the material properties (hysteresis, non linearity etc) of skin.

The testing software has an initialization program to help setup the specimen on the loading mechanism: centering and tensioning of the specimen to avoid shear. If the shape of the specimen does not change during the course of the experiments, i.e. a rectangle deformed into a rectangle, shear was absent. The second program is used to test the specimen to study the mechanical properties. The required test data are entered and the output stored in data files, was later analyzed. Figure 3.6 shows an image of the biaxial rig software GUI.

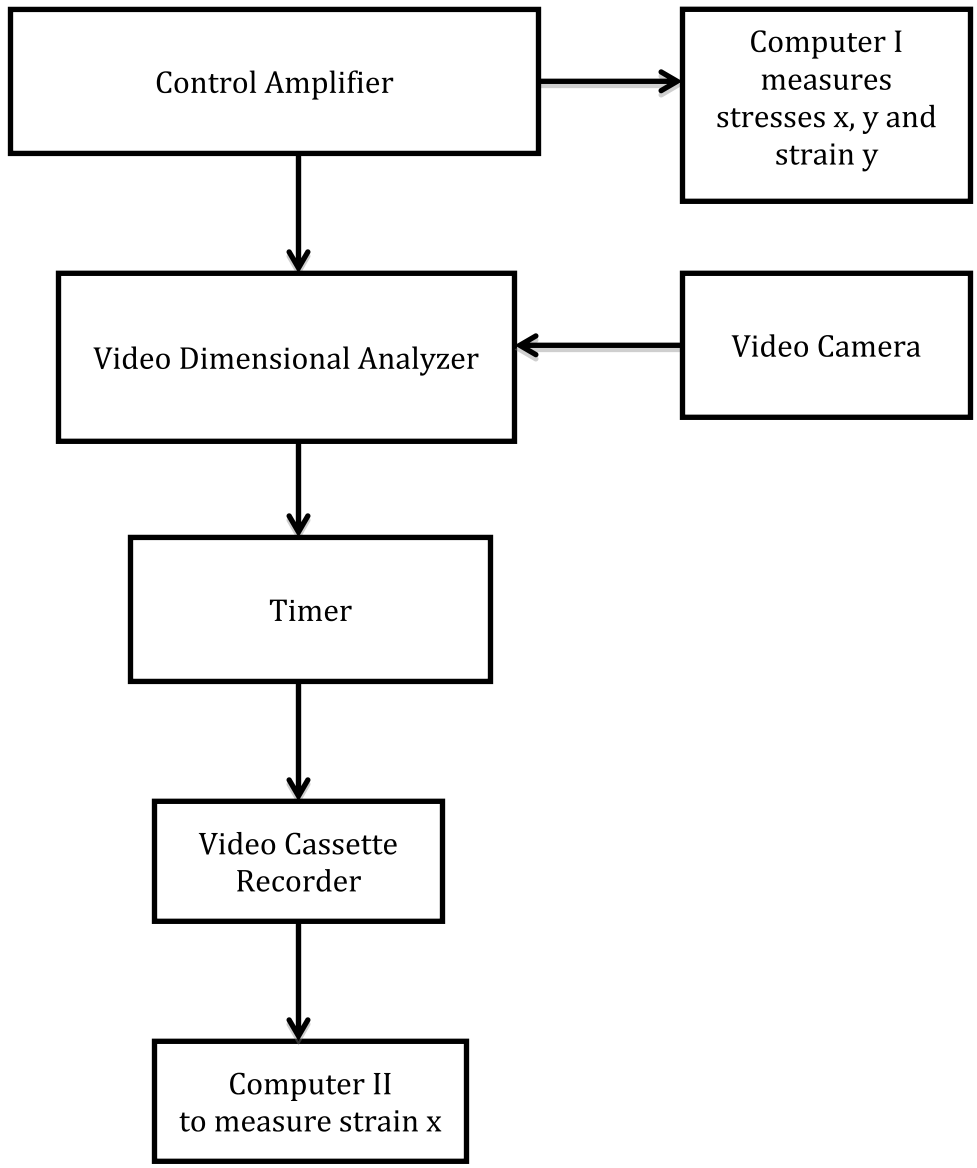


Fig 3.1 Block Diagram of the Biaxial Testing system setup.

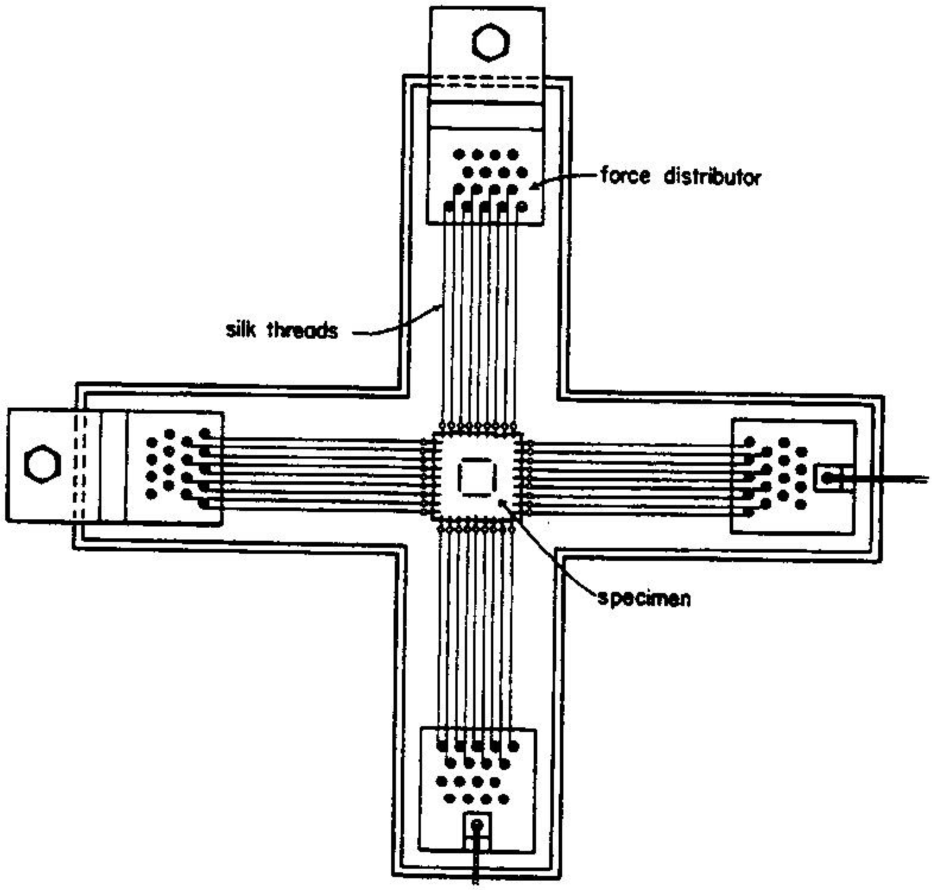


Fig 3.2 Setup of specimen hooking and force distribution (Lanir & Fung, 1974).

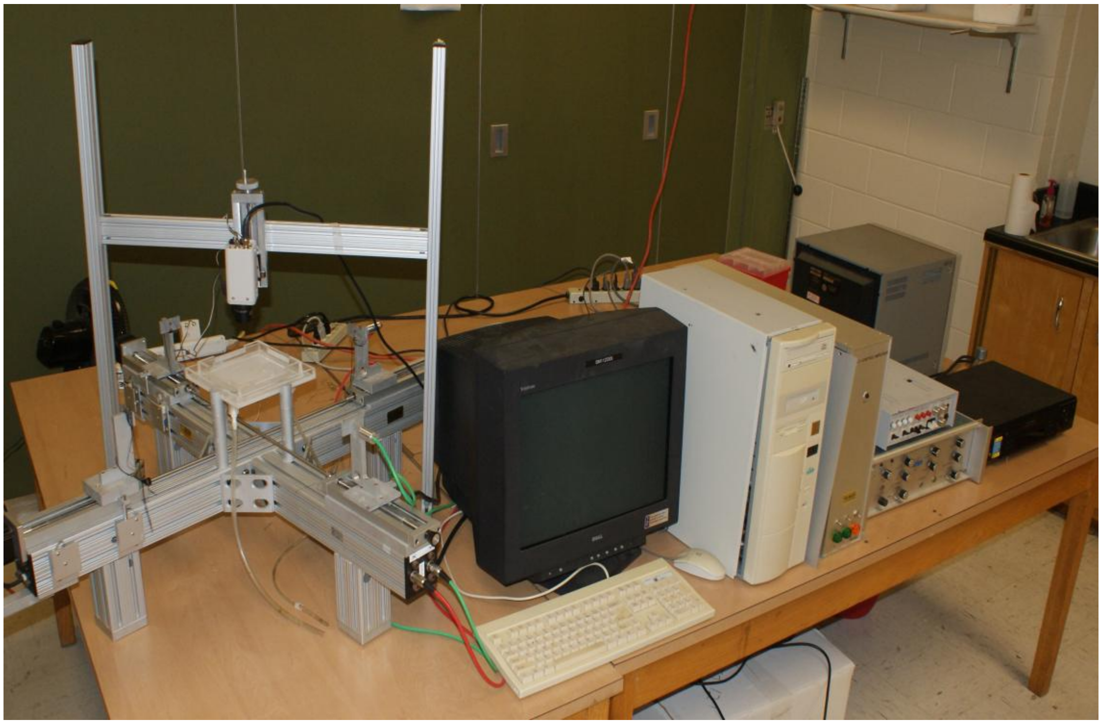


Fig 3.3 Experimental equipment overview.

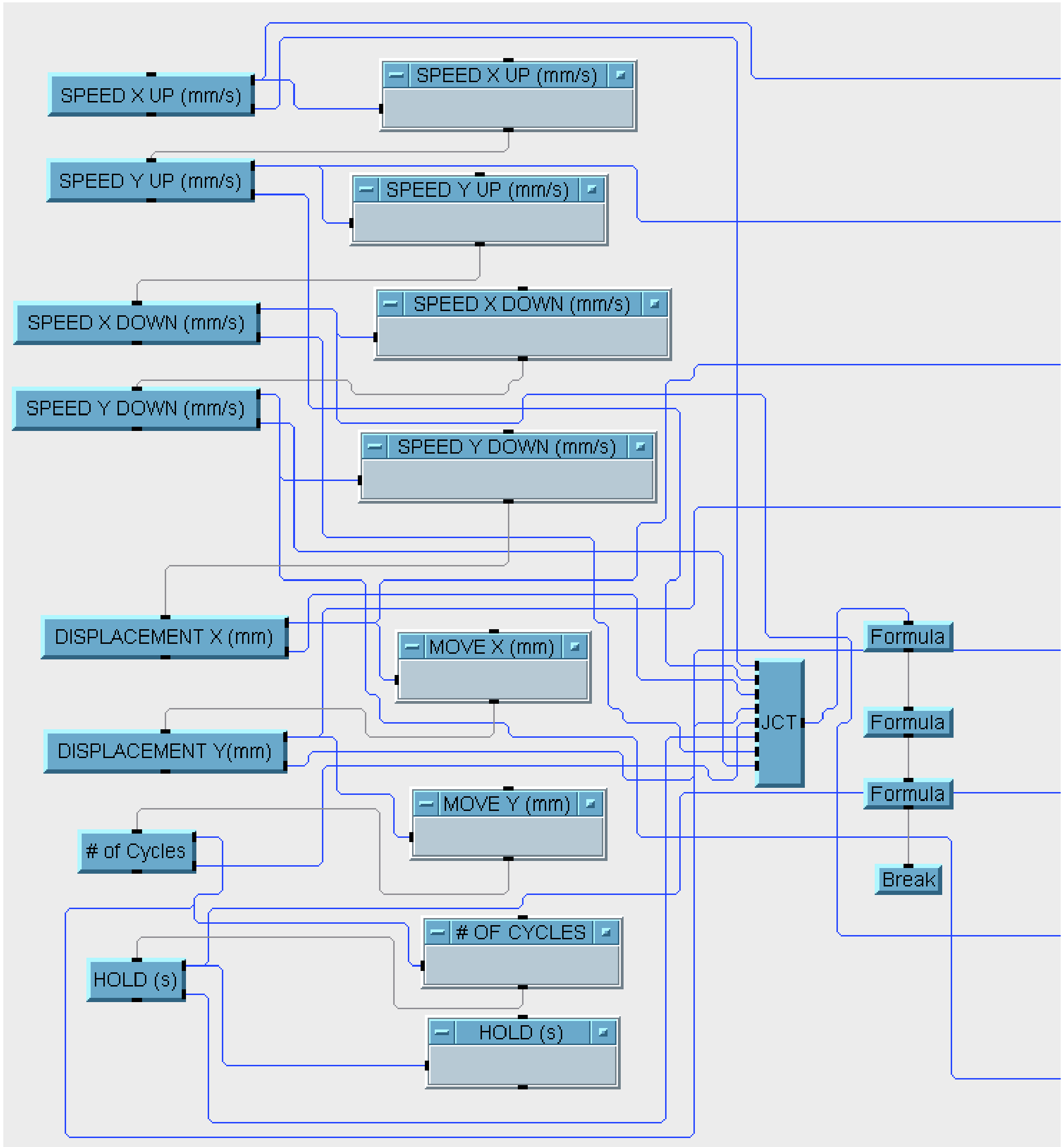


Fig 3.4 Example of biaxial ramp test code.

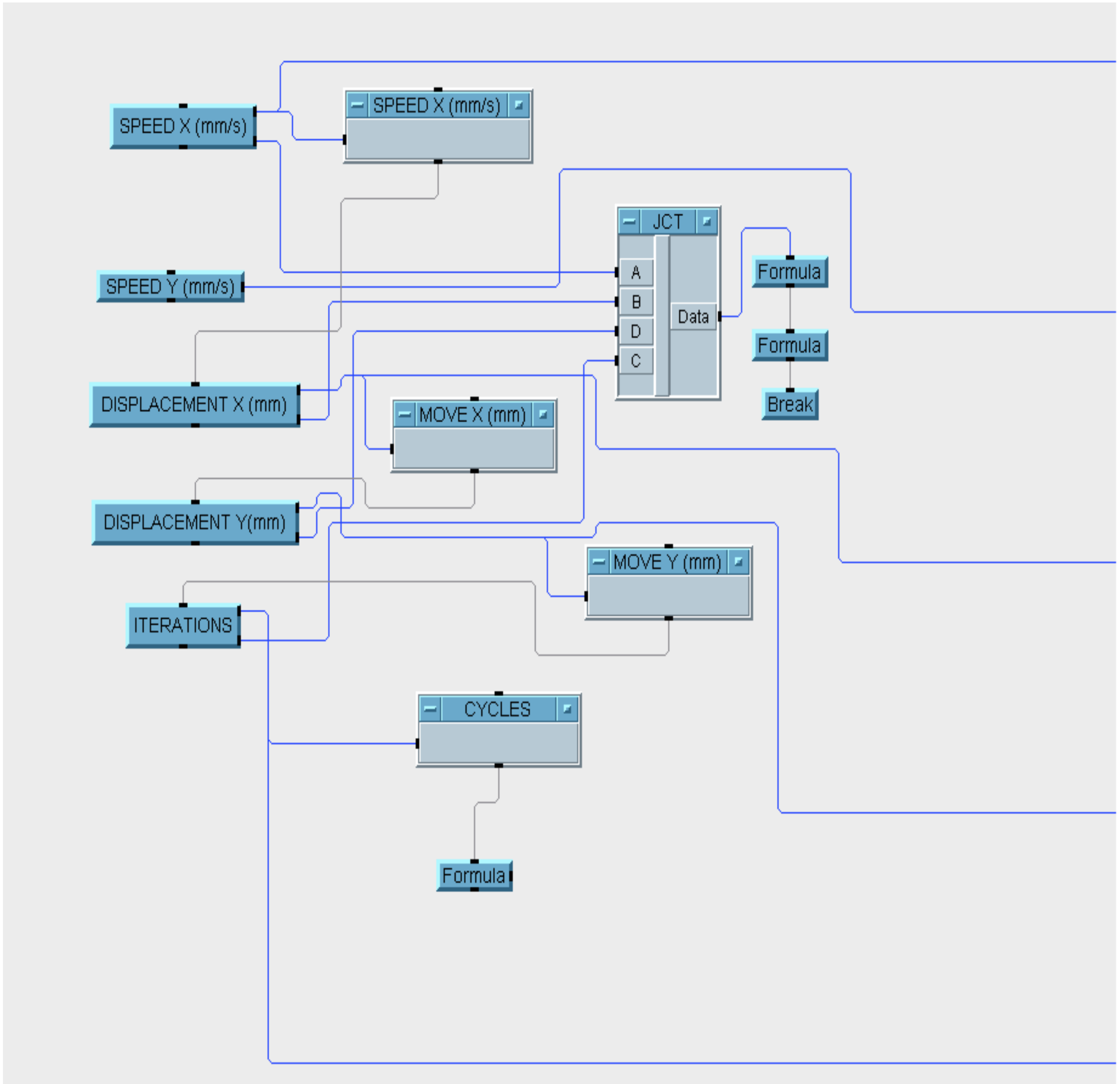


Fig 3.5 Example of biaxial step test code.

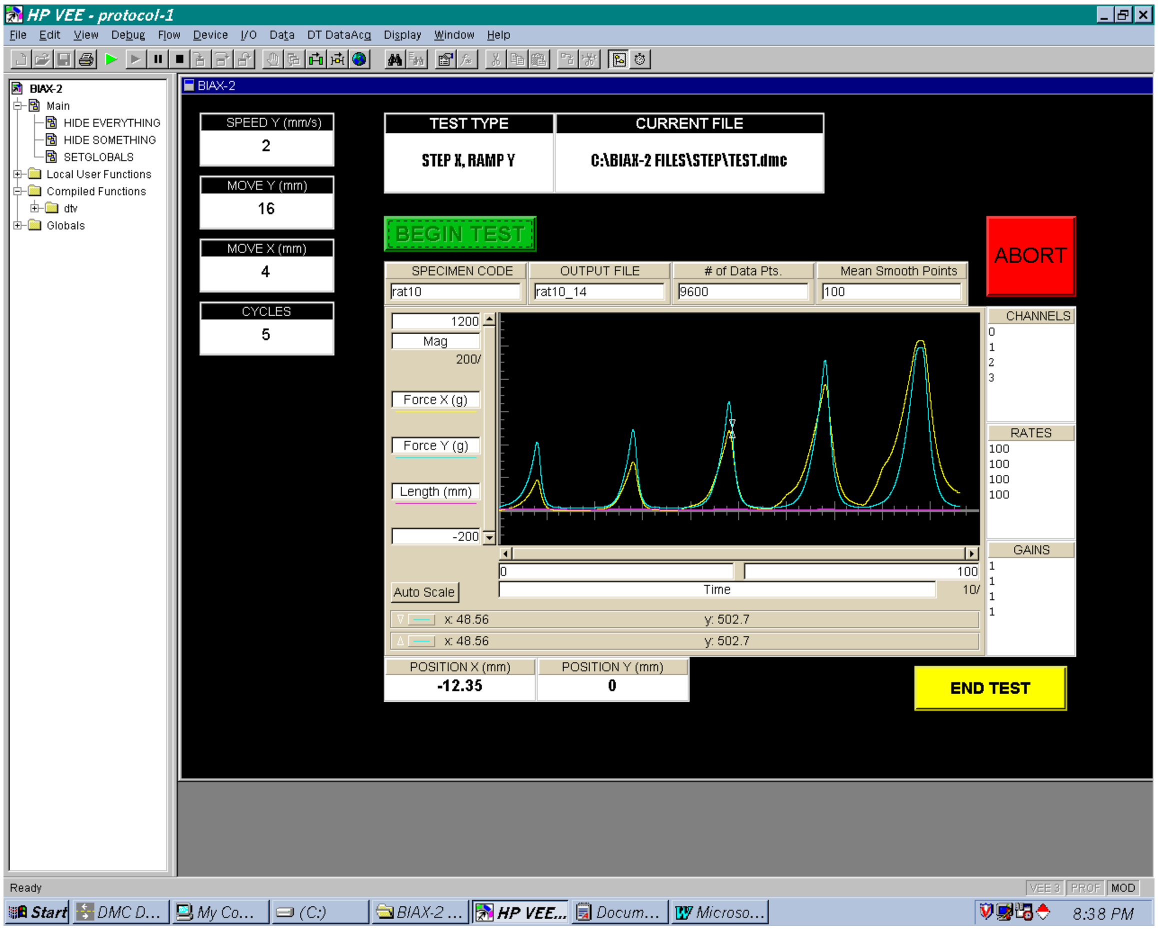


Fig 3.6 Biaxial rig software GUI.

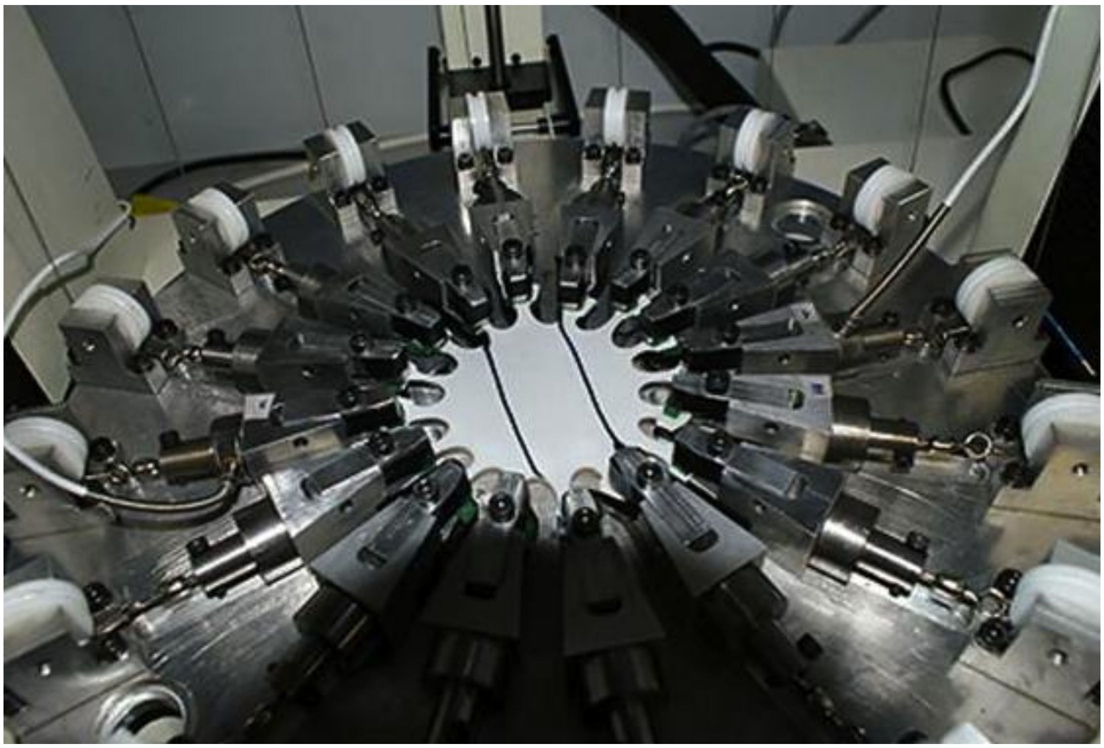


Fig 3.7 Multiaxial Testing Rig Setup (Y. A. Kvistedal, P. M. F. Nielsen).

CHAPTER 4 BIAXIAL IN-VITRO EXPERIMENTS

4.1 Specimen Preparation

The Sprague-Dawley adult male rats weighing 250 to 300 grams were used in the study. The rat specimens were obtained from the Integrated Microscopy Center, University of Memphis. A square specimen of size 50mm × 50mm was cut from the abdomen, and allowed to rest in saline solution for 1 hour. The relaxed dimensions of the specimen are measured, including the thickness. 8 skin samples were used in this study.

The thickness was measured using a force sensor connected to an oscilloscope. The specimen was placed on a flat surface and the sensor head adjusted to contact the specimen (as illustrated in Figure 4.3). When the specimen was in contact with the force sensor an electrical signal was output, which was read on the oscilloscope as voltage. When measuring the thickness at a different point on the specimen, if voltage read on the oscilloscope was the same, then both points has been applied the same pressure. The test was repeated at 10 different points on the specimen, and the thickness read on the scale averaged (Table 4.1).

The specimen was hooked by staples to 6-8 silk threads, which are attached to the loading mechanism in the X-Y table (as illustrated in Figure 4.4). According to Lanir and Fung (1974) swelling is observed during the first 4 hours of placing the specimen in saline solution, affecting the mechanical properties considerably. So the specimen was allowed to rest in saline solution for 4 hours, before conducting force measurements.

A square target 10mm × 10mm was marked at the center of the specimen using 4 200μm wires. The steel wires were bent at the center into a right angle. One leg of the wire was pressed into the specimen, while the other was placed on top of it. According to St. Venant's principle strain measurements are made at the center of the tissue specimen, as it is free of distortions (Downs J., et al., 1990).

4.2 Dynamic Test Procedure

Soft tissues do not exhibit any unique configuration to serve as a reference for measuring the stress and strain (Fung, 1974). So the loading and unloading process is repeated several times to obtain homeostasis. This process is preconditioning.

Preconditioning of the sample is done to obtain repeatability of results (Lanir & Fung, 1974). Preconditioning is repeating a test procedure several times, until the results converge. Soft biological tissue when subject to repeated mechanical loading exhibits unequal mechanical properties. So preconditioning is essential to overcome the problem of soft tissue handling and ensuring repeatability of results. Each time a testing procedure is changed or a different specimen is used, a different preconditioning protocol should be followed (Fung, 1974).

After preconditioning two types of tests are performed on the specimen.

1. Step Test: the specimen was cycled from preload to maximum load and back to preload in one direction, while the strain in the other direction was kept constant each run and varied in 4 to 5 runs.

2. Ramp test: the specimen was cycled from preload to maximum load and back to preload in both the directions simultaneously.

As the target area occupied only a small portion of the specimen center, the edge effects caused by the staples was neglected (Lanir & Fung, 1974). Also if the specimen shape did not change during the course of the experiment, the stresses and strains were assumed as uniformly distributed.

4.3 Experimental Results and Discussion

Experimental data from biaxial studies on rat specimens is presented in figures 4.4 to 4.10. The specimens are tested according to test protocols. The relaxed dimensions of the specimen are considered in all experimental results. The head to tail direction of the rat is the x direction and the transverse direction is the y direction.

The test results are shown in Figures 4.5 to 4.10. Figure 4.5 shows the biaxial stress-strain response for loading and unloading. Figure 4.6 presents the comparison between uniaxial and biaxial force-stretch ratio curves. Figure 4.7 shows biaxial loading curves for three fixed strains. Figure 4.8 represents biaxial force-stretch ratio response for three fixed strain rates. Equibaxial (equal level of tension applied to each test axis) stress-strain response is shown in figure 4.9.

Figure 4.5 shows the loading and unloading force-stretch ratio curves for a specimen with a varied stretch ratio in one direction and a fixed stretch ratio of 1 in the transverse direction. At stretch ratios above 1.2, nonlinearity is evident. Also hysteresis is observed between the loading and unloading curves, wherein loading and unloading occur at different force-stretch ratio paths. Considerable hysteresis is noted at all strain rates.

Figure 4.6 is a comparison of uniaxial and biaxial loading-unloading force-stretch ratio curves. The results show that the force in the uniaxial tension test is significantly lower than in the biaxial stretch condition.

Figure 4.7 represents the loading stress-stretch ratio plot for a skin specimen with three fixed stretch ratios (1.0000 to 1.1000 divided equal) in the x direction and varied stretch ratios in the y direction. The figure 4.7a is a plot of the Lagrangian stress T_{xx} vs L_y. The figure 5.5b is a plot of Lagrangian stress T_{yy} vs L_y. The plots indicate that, as the stretch ratio increases in the x direction, the curves move upward and left.

Figure 4.8 presents the force-stretch ratio relation for a skin specimen subject to three fixed strain rates. The figure is a plot of the force vs the stretch ratio. From the figure it is evident that there is only a minimal dependence of the force-stretch ratio relations on the strain rate.

Figure 4.9 presents the equi-biaxial loading stress-strain plot for a skin specimen. The figure is a plot of the Lagrangian stress vs the nominal strain. From the figure, non-linearity is evident as the strain increases.

Evaluation of constants of the strain-energy function

Two sets of experimental curves are needed, i.e stress-strain relations with E_1 fixed and one with E_2 fixed. Seven constants a_1, a_2, a_4, α_1, α_2, α_4 and c are to be determined. Using experimental data obtained from biaxial testing of skin samples, and the iterative method, the coefficients of the strain energy function are determined.

Since in the experiments it is assumed to a fair approximation that the material shape does not change during the course of experiments, shear strain E_{12}=0 and also with only tensile test data available the constant α_3 cannot be determined. Also all the γ's are set to zero and it is assumed that $\alpha_1 = \alpha_2$. The data obtained experimentally are fit by equations at selected points on the curves shown in Fig 4.10. The points A and C are placed at regions on the curve where the stress changes rapidly. Similarly the points B and D are located at regions of low stress. The experimental data at points B and D are used to determine constants α_1, α_2, α_4, while the data at points A and C help in calculating the constants a_1, a_2, a_4, and c.

Using the six pieces of experimental information obtained from the equations 2 and 4 mentioned in chapter 2, the iterative method can be used to determine the material constants a_1, a_2, a_4, α_1, α_2, α_4 and c (Fung & Tong, 1976).

$$X_1 = \left[\left(\frac{\partial S_1}{\partial e_1} - \alpha_1\right) / \left(S_1 - \alpha_1 e_1 - \alpha_4 e_2\right)\right]_A atA \qquad (1)$$

$$X_2 = \left[\left(\frac{\partial S_2}{\partial e_2} - \alpha_2\right) / \left(S_2 - \alpha_4 e_1 - \alpha_2 e_2\right)\right]_C atC \qquad (2)$$

$$X_3 = \left[\left(S_2 - \alpha_1 e_1 - \alpha_2 e_2\right) / \left(S_1 - \alpha_1 e_1 - \alpha_4 e_2\right)\right]_A atC \qquad (3)$$

$$2a_1 = \left[X_1 - \left(e_1 + a_4 e_2 / a_1\right)^{-1}\right]\left[e_1 + a_4 e_2 / a_1\right]_C^{-1} atA \qquad (4)$$

$$2a_2 = \left[X_2 - \left(e_2 + a_4 e_1 / a_2\right)^{-1}\right]\left[e_2 + a_4 e_1 / a_2\right]_C^{-1} atC \quad (5)$$

$$\frac{a_4}{a_1} = \left[\left(X_3 e_1 - \frac{a_2}{a_1} e_2\right) / \left(e_1 - X_3 e_2\right)\right]_A \quad (6)$$

An initial guess of the material parameters α's and a's are made to solve a fixed boundary coordinates problem. Then small changes are made to the value of the material parameters α's, and for each change a boundary force problem is solved using a set of deformed states from the experimental data to obtain a's. When the parameter values are obtained, the procedure is repeated until the values converged. Similarly the material parameters c and α's are evaluated.

The values of the constant are independent of the positions of the points on the curves (Fung, 1974). Material constants are obtained from 8 test results (Table 4.2).

The constants obtained in an experiment depend on a specific test protocol and preconditioning. So using a different test protocol and a new preconditioning process will change the values of the constants in the strain-energy function. Thus the constants determined are phenomenological as they are sample specific and can only be used to predict the outcome of other observations. The constants from a sequence of experiments used to predict the outcome of another sequence of experiments on the same specimen, does not produce uniform results. The reason for this non-uniformity is that the skin is not elastic (Tong & Fung, 1976). A material is defined as elastic if the stress-strain relationship is independent of the strain rate or strain history.

Figure 4.11 presents the comparison between the theoretical and experimental loading force-strain curves. The figure is a plot of the force vs the stretch ratio. The data are computed from the experimental information (1-6).

Table 4.1 Thickness measurements using the force extensometer

Sample number	Thickness (mm)
1	0.0025
2	0.0028
3	0.0024
4	0.0025
5	0.0026
6	0.0025
7	0.0021
8	0.0026

Table 4.2 Material constants from strain-energy function

Experiment number	Constants					
	a_1	a_2	a_4	$\alpha_1 = \alpha_2$	α_4	c
1	3.14	11.1	1.25	9.12	1.80	0.0045
2	4.32	15.20	3.20	15.20	5.63	0.0022
3	6.21	11.34	0.802	9.30	2.10	0.0051
4	4.60	25.41	0.505	10.8	3.50	0.0001
5	9.12	12.2	1.12	7.50	3.21	0.0031
6	6.30	15.21	0.654	10.42	5.24	0.0009
7	5.70	24.3	3.08	12.2	4.10	0.0062
8	4.45	12.4	0.910	10.24	3.41	0.0015

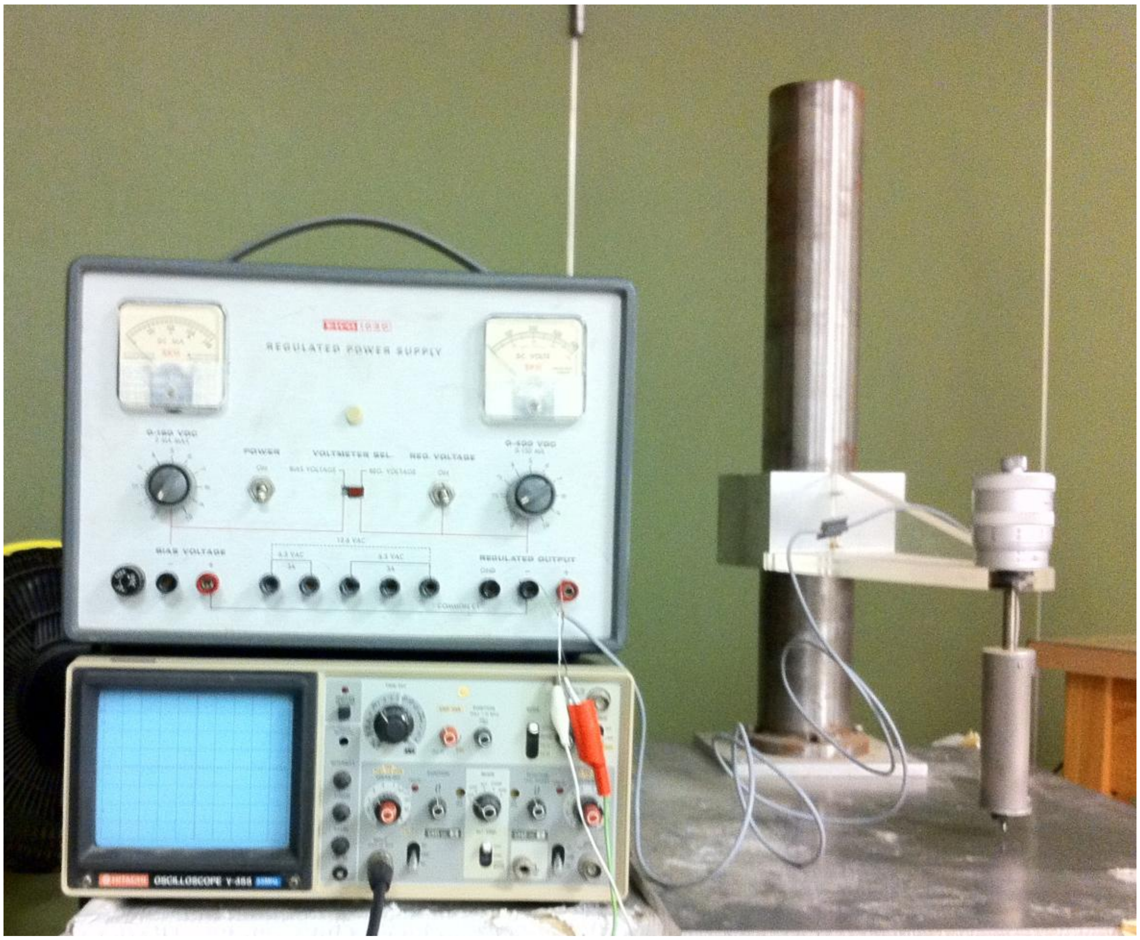


Fig 4.3 Force sensor setup to measure sample thickness

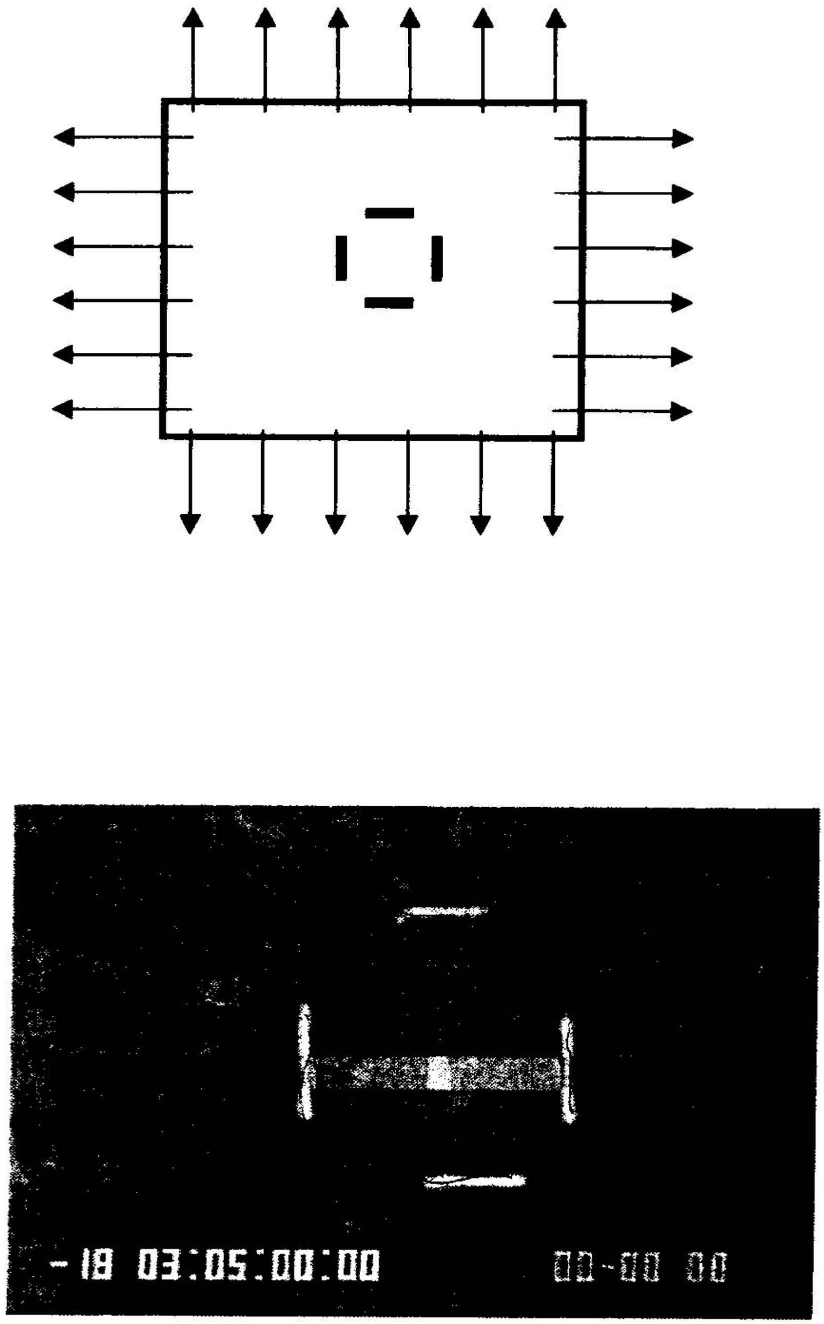


Fig 4.4 Upper: Sketch of specimen with markers. Lower: Overview of specimen on image monitor (Xituan Shang, 2004).

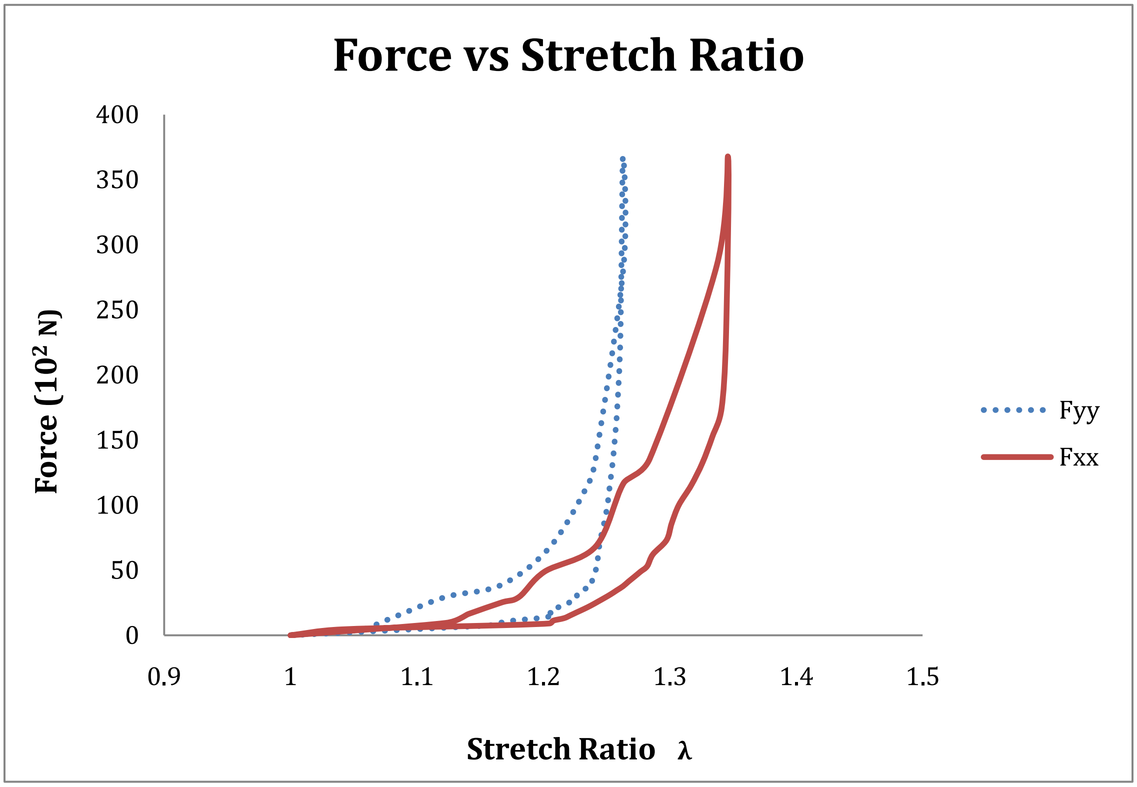


Fig 4.5 Loading and unloading force-strain curves for a skin specimen subject to varied stretch ratios in one direction and a fixed stretch ratio in the transverse direction (transverse stretch ratio is 1.00).

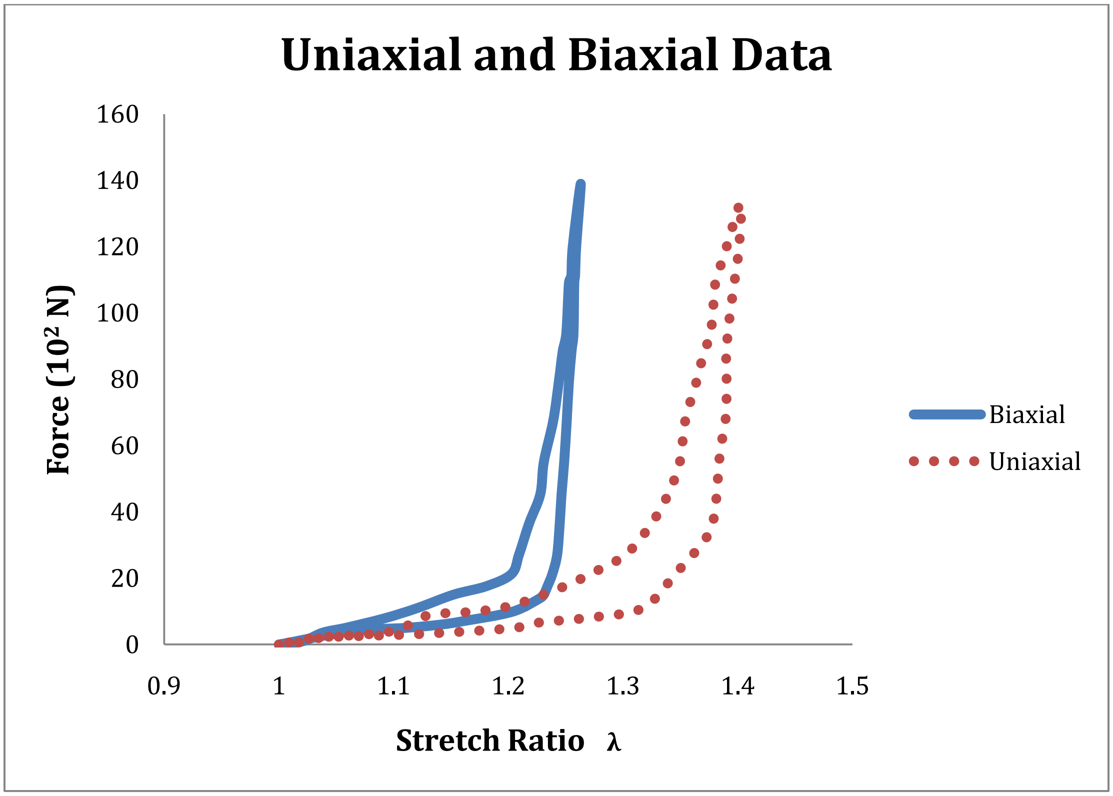
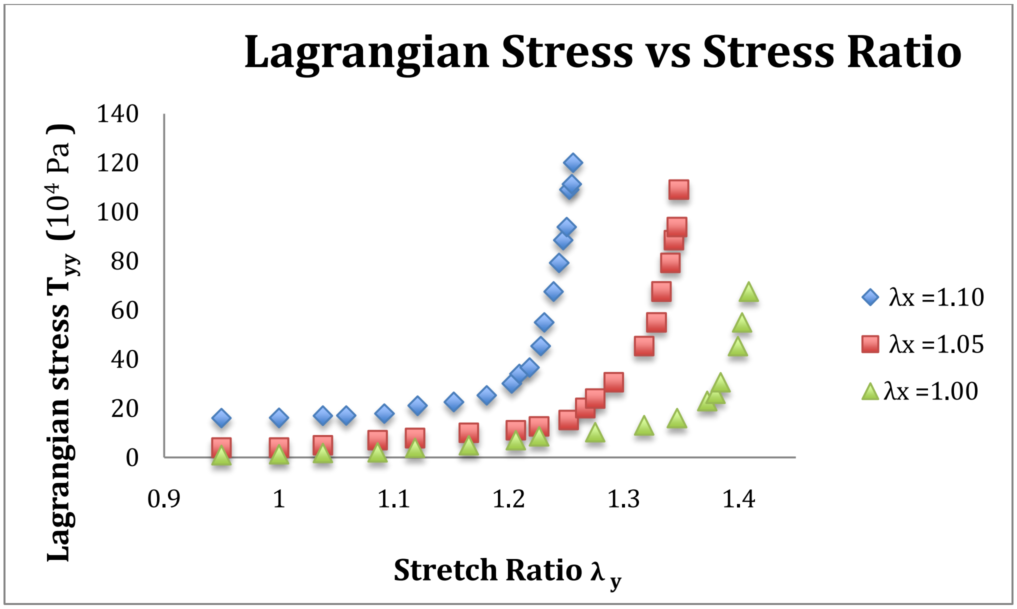


Fig 4.6 Comparison between loading and unloading stress-strain curves for a skin specimen subject to uniaxial and biaxial tests. The stretch ratio in one direction is varied and the transverse stretch ratio is fixed (transverse stretch ratio is 1.00). Solid lines is the response to biaxial tests. Broken lines is the response to uniaxial tests



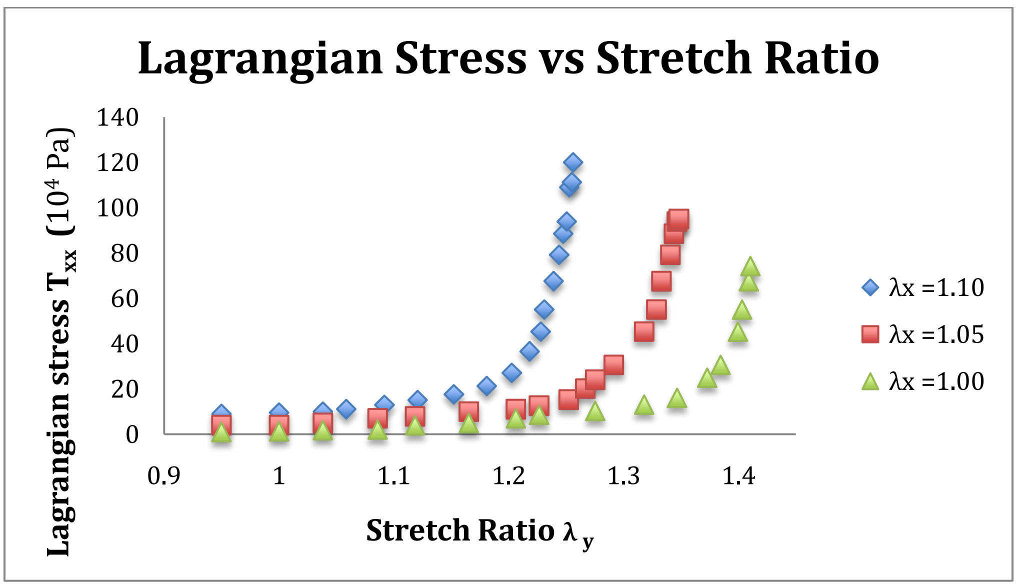


Fig 4.7 Loading stress-strain response for a skin specimen subject to three fixed stretch ratios in the x direction and varied stretch ratios in the y direction. The upper figure is a plot of the Lagrangian stress T_{yy} against the stretch ratio λ_y. The lower figure is a plot of Lagrangian stress T_{xx} vs the stretch ratio λ_y.

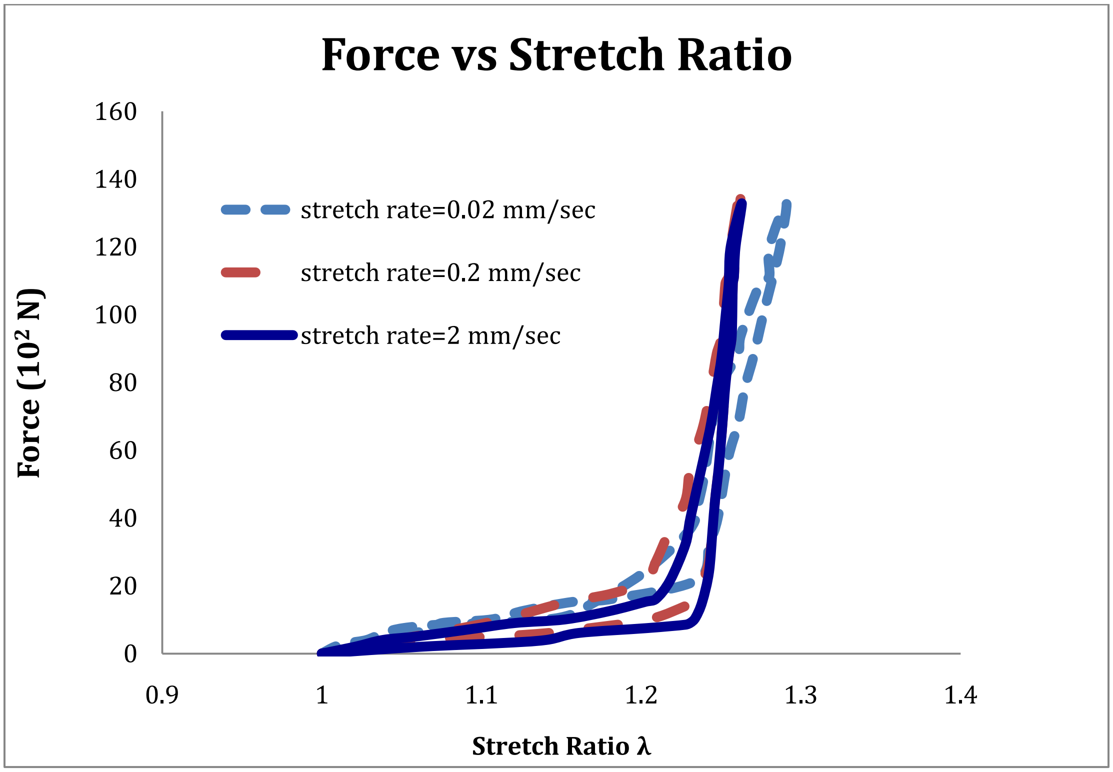


Fig 4.8 Loading and unloading stress-strain curves for a skin specimen subjected to three-fixed strain rates. The stretch ratio in one direction is varied and the transverse stretch ratio is fixed (transverse stretch ratio is 1.00).

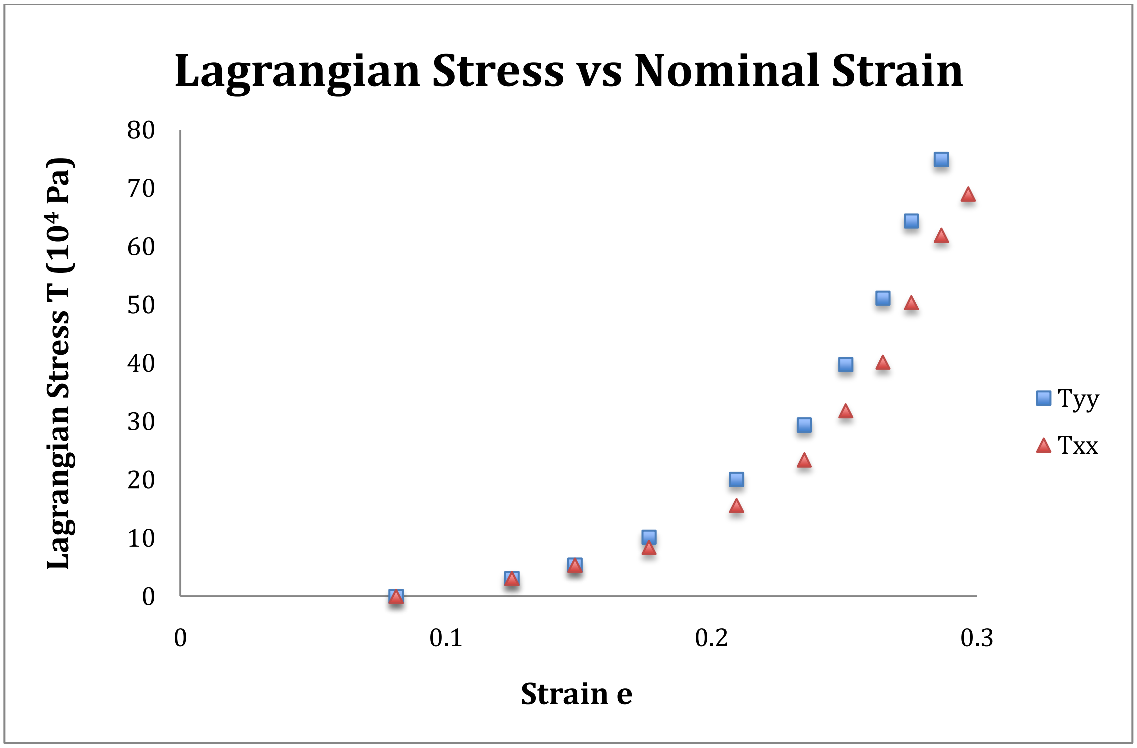


Fig 4.9 Equibiaxial loading stress-strain curves. The Lagrangian stress is plotted against the nominal strain e.

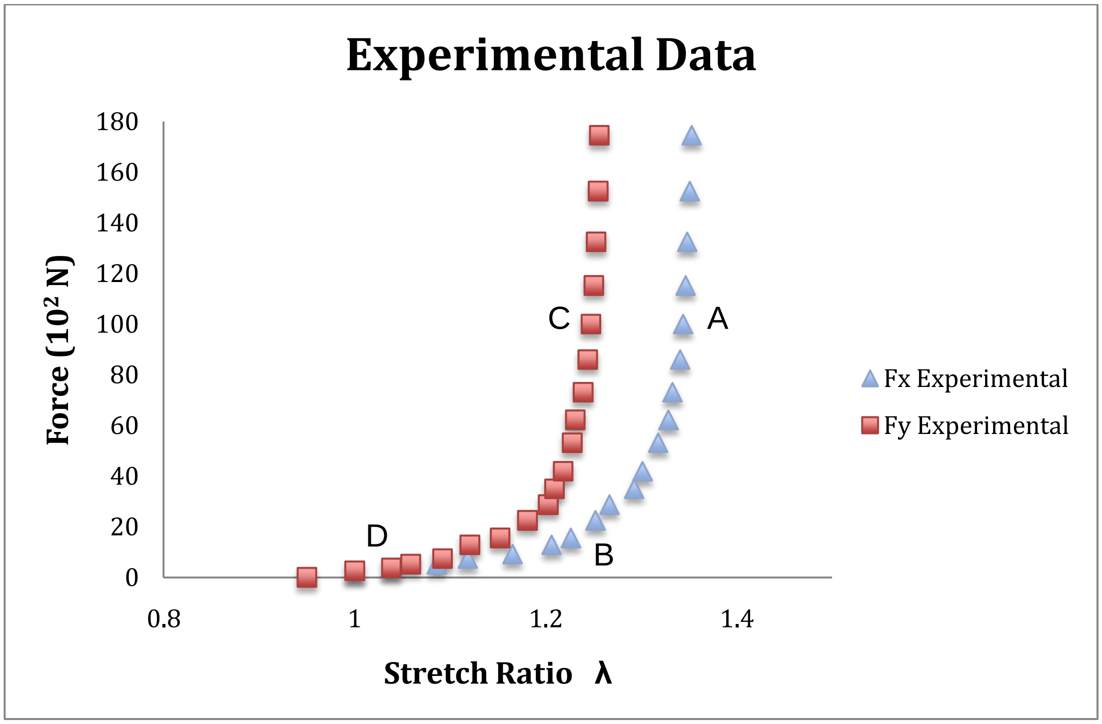


Fig 4.10 Loading force-stretch ratio response for a skin specimen subject to a varied stretch ratio in one direction and a fixed stretch ratio in the transverse direction (transverse stretch ratio is 1). The choice of points A, B, C and D used to determine the constants is illustrated in the figure.

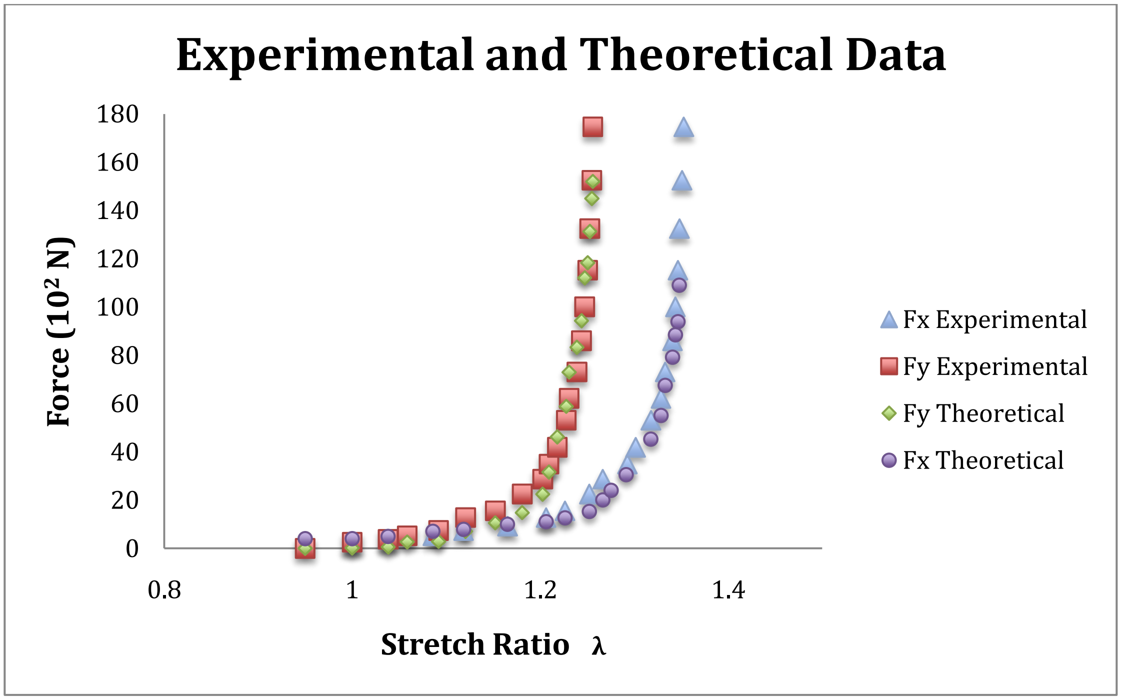


Fig 4.11 Comparison between the experimental and theoretical loading force-stretch ratio curves for a skin specimen.

CHAPTER 6 SUMMARY

6.1 Conclusions

Biaxial studies play an important role in understanding the mechanical properties of skin. The biaxial testing machine and constitutive equations have been successfully used to obtain material parameters. Comparing the theoretical and experimental results validates minimization.

In this thesis, rat skin specimens are used to study the biaxial mechanical properties of skin. The relaxed dimensions of the specimen are used in tests, as equilibrium conditions are impossible to attain. The specimen is allowed to rest in saline solution for 4 hours to eliminate swelling. Preconditioning is significant in obtaining repeatable test results. Different test protocols are used in the biaxial tests and the results are presented.

Biaxial stress-strain relations show nonlinearity for constant strain rate tests. The material displays considerable hysteresis at all strain levels. As the strain levels increase stress increases and the material exhibits non-linearity. Material stiffening is observed as the tensile stresses increase. Stress in uniaxial tests is considerably lower in comparison to biaxial stretch tests.

Tong and Fung's strain-energy function was used to understand the stress-strain relationship of skin, and determine the material constants of each specimen. The constants were obtained from the experimental data using Tong and Fung's strain-energy function and Kirchhoff's stresses. An iterative scheme was setup to determine the material constants using six simple algebraic expressions obtained from two sets of equations, by making initial guesses of the constants until the values converged, when the iteration stops. Since the constants thus obtained from one set of experiments, if used to predict the stress-strain behavior of another set of experiments the results were not uniform. Also the skin's mechanical property is dependent on the preconditioning protocol. So the material constants determined are phenomenological, since they are specific to a particular skin specimen and testing procedure and can only be used to make predictions about the results of other experiments.

The skin was modeled as a two-dimensional incompressible membrane. A major simplification when considering the structure of the human skin was modeling the combined mechanical behavior of all the three layers. A better model should include all the three layers, and the underlying subcutaneous fat, since the three layers exhibit different material composition.

With latest advancements in the field of biaxial testing of tissues and modern equipment's available to test smaller size samples (3-15mm) in a shorter time frame and less machining required (BioTester, cellscale.com), I am a little apprehensive about the further use of the current design for research purposes, In spite of the disadvantages with the current model, it seem a promising learning tool for undergraduate students, helping them understand the process of testing of tissues.

6.2 Future Work

The biaxial testing system designed at the University of California, San Diego to study the mechanical properties of skin has some advantages and disadvantages that need to be addressed to obtain a more robust model in future research.

The setup was appealing and successful, as it was one of the latest biaxial testing devices out in the market in the 90's. Also it was cost effective, easy to understand and use and was versatile (certain actuators can be stationary while an individual axis stretched).

Firstly there is a limitation on the specimen sizes that can be measured using this biaxial testing machine. Specimen size limitations make it difficult to use the rig to test other biological tissues: for example lung tissue with the thickness of the order of a few micrometers is challenging to mount on the testing rig. Small sample numbers are used in this study. Higher sample numbers and use of human specimens in future research work can provide a better understanding of skin's mechanical properties. Also shearing was not considered in this biaxial study. So a new testing method to model the effects of in plane shear stress must be incorporated in future work.

Secondly, the design is relatively large in total size, which would be difficult to meet the constraints of a smaller laboratory setup.

There has always been an issue with the method of attaching the specimen to the grips. Improper sample attachment can cause stress concentration at certain points and also the strains in the sample to be non-uniform. It can alter the sample geometry and cause uneven loads at the attachment sites (Hoffman & Grigg, 1984).

There are several improvements needed to make better use of the testing device for future studies of the biaxial mechanical properties of skin. The technique used to mount the specimen onto the loading mechanism requires improvement, as it is both time consuming and could cause slippage if the threads are not tight enough or if the attachment is too tight, might damage the tissue. Further research in sample attachment could provide a more suitable method of attaching the specimen onto the biaxial testing device. This would make handling and testing other biological tissues effective. Also simulating the in-vivo skin conditions such as, effect of the surrounding tissue and should be incorporated in future research.

A simple modification to the setup is to upgrade the camera to obtain better quality images, for measurement of strains. Also the lighting needs to be improved to enhance picture quality.

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