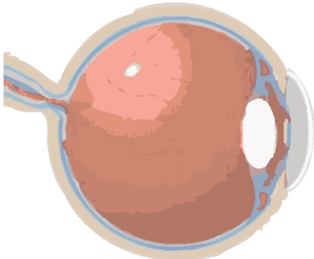

Why Measure Mechanical Properties?

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Mechanical Properties Influence Function

Virtually all biological tissues, including those that form the eye, carry mechanical loads. Disease and aging can change the loads applied and affect the ability of specific tissues to carry load; function can be impaired as a result. Drugs designed to ameliorate or reverse these changes provide one strategy to maintain or restore function, while implants designed to replicate the mechanical properties of virgin tissue, provide another.

In the case of elevated intraocular pressure (IOP), for example, the mechanical loading on the eye is raised and increased deformations occur in the optic nerve head region of the eye. This increased deformation has been hypothesized as the mechanism by which attendant optic neuropathy (glaucoma) arises. To test this hypothesis, researchers at the Toronto Western Hospital, University of Toronto Institute for Biomaterials and Biomedical Engineering and the University of Waterloo Faculty of Engineering formed a team to measure the mechanical properties of key tissues in the eye. The data they collected led to a better understanding of how the optic nerve head (ONH) responds to elevated IOP and it led to improved computational models of the ONH. Further testing and analysis may make it possible to identify patients at risk for glaucoma.

Mechanical testing can be important for:

- Revealing the basic biomechanics of a particular organ, such as the eye, or a part thereof
- Understanding how ultrastructural changes in tissues might affect their ability to generate or carry load
- Assessing the health of a tissue
- Tracking the progress of disease
- Evaluating the effectiveness of pharmaceuticals
- Developing replacement biomaterials

The Basics of Mechanical Testing

In vivo, tissues typically carry tensile mechanical loads and, in the process of doing so, deform. The objective of mechanical testing is to quantify the load and deformation between. This relationship is important because it describes the ability of the structural tissue to maintain its intended geometry when it carries load. If the sclera and associated eye tissues in the ONH region were not sufficiently stiff, excessive deformations could occur in the ONH under elevated IOP, and deformation-induced neuroligical damage could result.

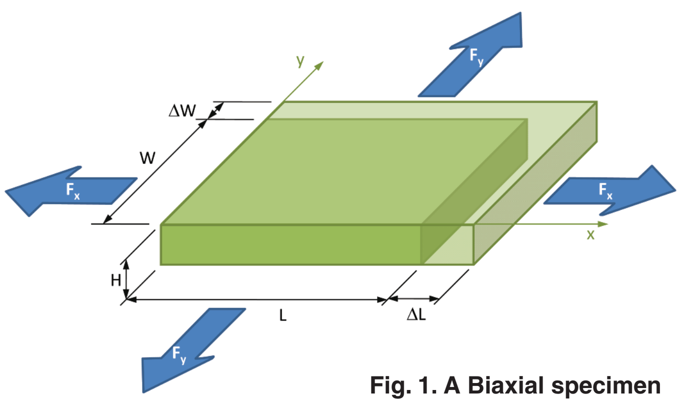


Fig. 1. A Biaxial specimen

Many load-carrying tissues, including those in the eye, are thin and carry loads in both of their in-plane directions.

To replicate this physiological loading requires an apparatus that can simultaneously apply load in two orthogonal directions, i.e., a biaxial tester.

What is Stress?

Stress can be thought of as a normalized load intensity. The load acting in a particular direction is typically divided by the cross-sectional area over which it acts. Often, the cross-section used is that of the undeformed specimen. If the specimen shown in Fig. 1 is loaded in the x-direction, the associated cross-sectional area has a thickness H and a width W. The stress in the x-direction would be

$$\sigma_x = \frac{F_x}{WH}$$

where Fx is the tensile load in the x-direction. Stress is represented by the lower case Greek letter "sigma", and the subscript denotes direction (x in this case). Stress in the y-direction can be calculated in a similar fashion, based on load Fy and the H by L cross-section over which Fy acts, i.e.,

$$\sigma_y = \frac{F_y}{LH}$$

Stress has units of force per unit area: N/m^2 or Pa ($1Pa = 1\ N/m^2$). If tension is applied simultaneously in the x- and y-directions the test is said to be a biaxial tensile test. (If load were applied in only one direction, it would be called a uniaxial test.)

Typically, such an instrument will stretch a tissue specimen by prescribed amounts in two orthogonal directions and will measure the loads associated with specific degrees of elongation. For a particular degree of specimen elongation in the x-direction, the instrument measures the associated load in that direction. It does the same for elongations and loads in the y-direction.

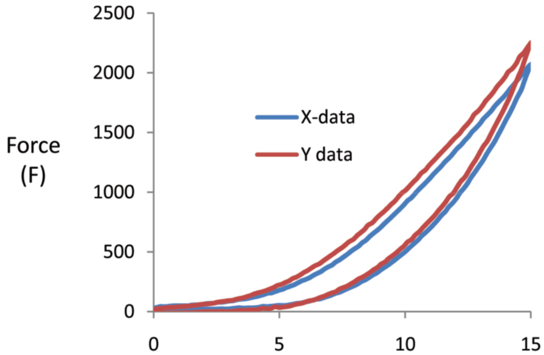


Fig. 2. A Force Displacement Graph

The force-displacement data so obtained are typically normalized by converting the loads into stresses (a measure of load intensity per cross-sectional area) and the elongations into strains (often expressed as elongation percentages).

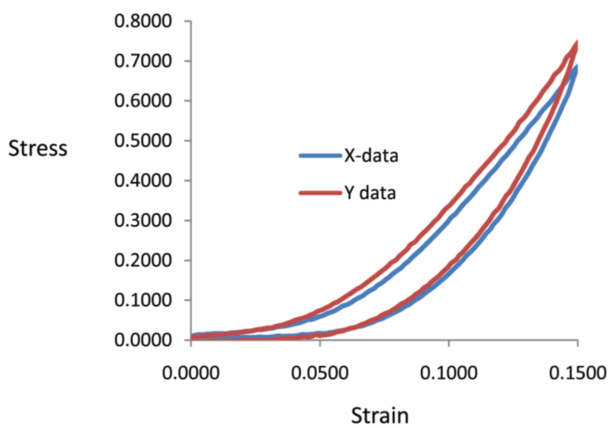


Fig. 3. A stress strain graph

What does a Stress-Strain Graph Mean?

A stress-strain graph can be considered a scaled or normalized representation of the load-deflection data obtained by a test instrument. For example, the load F_x in the x-direction is typically divided by WH to determine the stress σ_x while ε_x, the strain in the x-direction is the specimen elongation ΔL divided by its original length L. **The normalization factors are designed so that if two specimens have the same mechanical properties but are different sizes, their stress-strain graphs will be the same.** If tissue deformations are large, more complex normalization procedures are necessary. Load in one direction can, in general, produce deformations in both the x- and y-directions. As a result stress-strain graphs for one direction will depend on the amount of the deformation applied to the specimen in the other direction. **The stress-strain graph for a particular tissue provides a fundamental measure of how tissue load and deformation are related to each other in that tissue.**

The slope of the stress-strain graph at any point indicates the "stiffness" of the material. Physically, it indicates the change in load required to produce a specified change in elongation. Interestingly, the stiffness of a material may change as it is elongated. This phenomenon is common in biological materials, and it is typically attributed to the recruitment of additional fibres that have straightened as a material is stretched.

When a tissue is stretched simultaneously in two directions, separate stress-strain curves are obtained for each direction. The pair of curves shown in Fig. 3 are for sclera and they demonstrate that the tissue stiffness is higher in the y-direction than it is in the x-direction, a compelling reason to use biaxial tests, rather than uniaxial tests. The data also demonstrate that the stiffness of sclera increases as it is stretched.

The effects of age, disease or treatment protocols on the mechanics of a tissue can be identified by comparing the stress-strain curves associated with each type of tissue, and computational models based on the appropriate curves can be used to predict the mechanical consequences of disease or of proposed interventions.

Tests on human sclera show that its properties vary from one location to another. In order to map these variations, which are important to mechanical function of the eye, it is necessary to use small (6mm square) specimens, something that the CellScale BioTester has been specifically designed to do.

When computational models – such as finite element

What is Strain?

Strain is a measure of deformation. In a tensile test, it is the ratio of the change in specimen length in the loading direction to its original length. For the specimen shown in Fig. 1, which had an original length of L in the x-direction and was elongated in that direction by an amount ΔL, the strain in the x-direction would be

$$\varepsilon_x = \frac{\Delta L}{L}$$

The strain in the y-direction would be

$$\varepsilon_y = \frac{\Delta W}{W}$$

Strain is represented by a lower case Greek "epsilon" and since it is the ratio of two lengths, it is dimensionless. Edges that elongate are considered to generate positive changes in edge length (eg., ΔL) and to be associated with positive strains.

models – are constructed, they use these stress-strain curves or mathematical representations of them to calculate how tissues in each region of the model will deform as a result of specified applied loads to the eye.

Some Important Details about Mechanical Testing

The Future is in Small Specimens

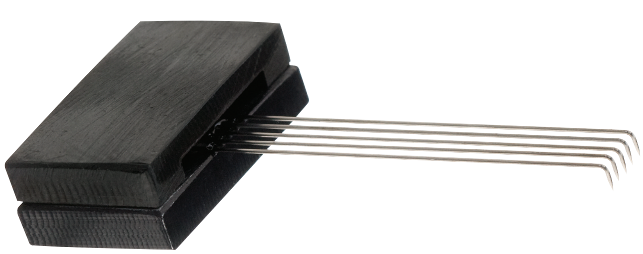
Regional variations in the properties of natural tissues such those of the eye, heart valve and intervertebral disk affect the mechanics of the structures they form. Computational modeling has revealed that subtle property variations can significantly affect the mechanical function of these structures, and so the quest is on to construct maps of regional mechanical properties. Since these variations occur over distances smaller than 10 millimeters, tissue specimens smaller than this size must be used. The CellScale Biotester 5000 can test areas as small as 3mm in size.

Specimen Attachment is Crucial

For a tensile test to provide quality data, stress and strain need to be as uniform as possible throughout the specimen. Good approximations can be made to this ideal if a suitable attachment system is used.

Experiments and finite element models have shown that biaxial samples require at least five attachment points along each side of a biaxial tensile specimen and that these points must be spaced with high accuracy if one is to avoid stress and strain nonuniformities that would degrade data quality. These studies also suggested that when specimens are smaller than approximately 20mm in size, standard suture attachments cannot provide the required attachment accuracy.

The CellScale Biotester 5000 uses the patent pending “BioRake” attachment system so that five accurately spaced attachment points can be made along each edge of the specimen.



Verification of Data Quality

The best ways to ensure data quality are to use precision attachment methods, like the BioRake system, and to monitoring regional deformations in the specimen using an imaging system. Image data from the BioTester 5000 can be analyzed using point tracking and motion analysis software available from CellScale. This software makes it possible to identify regional strain and property variations within specimens and to thereby verify the quality of collected stress-strain data.

True versus Nominal (Engineering) Measures of Stress and Strain

As a test specimen deforms, its length and cross-sectional area change. If stress and strain are calculated using the original specimen geometry, different values will be obtained than if these calculations are based on the deformed (current) geometry. Calculations based on initial area produce are called “nominal” or “engineering” values, while calculations based on current geometry give rise to “true” stresses and strains. When nominal strains are greater than approximately 3%, care must be used is specifying which stress and strain measures are used.

Load Cycle Design

In testing biological tissues, it is common to apply a series of stretch cycles to the material with the objective of restoring the structure of the fibrils and long chain molecules in the tissue to their physiological states and orientations. This crucial process is called pre-conditioning.

Preconditioning is typically followed immediately by one or more sets of stretch cycles of physiological magnitude during which the stress-strain characteristics of the material are measured and saved. In one such set, the material may be stretched equally in both directions, while in other sets, the stretch ratios may be different from each other.

The Biotester 5000 is designed so that preconditioning and any other sequential patterns of loading can be pre-set and run without user intervention.

How CellScale can help you Achieve your Test Objectives

CellScale has developed a turnkey biaxial test platform designed to maximize the quality of the data you collect and to save you and your technicians time and money.

The patent pending rake attachment method, easy-to-use software, and integrated, affordable design makes the BioTester an ideal choice for researchers in many areas of biomaterials testing. Visit our website for more information or contact us to discuss your materials testing needs.