EXPERIMENTAL MECHANICS OF BIOLOGICAL SYSTEMS

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Summary

Development of many areas of Biology is related to the ability to perform various tests on the biological objects and many of these tests are based on the principles of mechanics. The history of the testing to biological objects utilizing the principles of mechanics goes back to the 16th century when Galileo Galilei used pendulum to study the pulse rate. The symbiotic relationship between the experimental mechanics and biology lead to modification of many traditional test methods with the aim to adapt them to the study of the behavior of diverse biological systems. This chapter will review various engineering principles governing testing and evaluation of biological materials and structures and will show how these principles are applied to the study of bone, cartilage, ligaments, tendons, muscles, cells, etc.

Several experimental techniques for study of bone, soft tissues and cells are discussed here. Mechanical and ultrasound methods for testing of bones mechanical behavior are presented. The complexities of soft tissues testing are discussed for both static and dynamic cases. Recent advances in the digital imaging technology and their application to study mechanics of soft tissues are presented here and advantages are discussed.

Cell comprising the human body are sensitive to the application of the mechanical loads. As it was found, these loads are very important for cell proliferation and communication. Recent advances in the load application and study of the effects of the mechanical loads on the cell in-vitro are presented in the last part of this work.

1. Introduction

Biology and Medicine coexisted together and benefited one from another for many centuries. Very few scientific experimental methods were involved in the earlier times and people mainly relied on the observations. Last centuries brought about fast development of various experimental techniques that were based on the principles of mechanics. The symbiotic relationship of experimental mechanics and biology resulted in development of variety of sound experimental techniques that are capable of providing accurate data about behavior of diverse biological systems.

This chapter will review various engineering principles governing testing and evaluation of biological materials and structures and will show how these principles are applied to the study of bone, cartilage, ligaments, tendons, muscles, cells, etc. Developments in these areas were vital in creation of the new interdisciplinary field called biomechanics. It is based on the contributions from a number of fields, such as natural sciences, engineering, mathematics, etc. The following chart (Figure 1) shows this interrelationship.

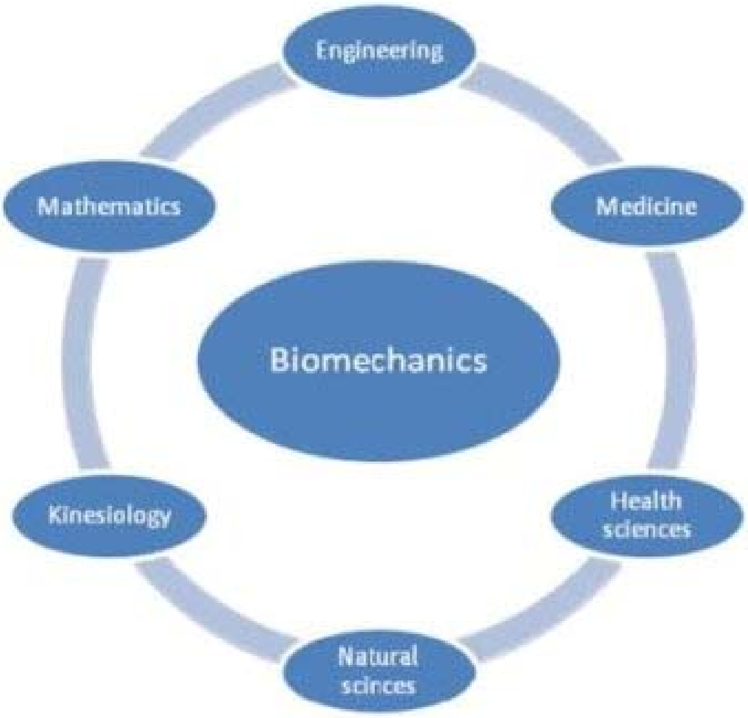

Figure 1. Biomechanics and its fields.

Mechanical properties of living tissues form a central subject in biomechanics. The mechanical properties of the musculo-skeletal system, skin, lungs, blood and blood vessels have attracted much attention. Even though the systematic study and even creation of the term "biomechanics" did not occur until the mid of the 20th century, significant contributions to the area of biomechanics were done by number of famous scientists. Galileo Galilei (1564-1642) applied the consistency of the pendulum period to the measurements of the pulse rate. About 1593, Galileo constructed a thermometer, using the expansion and contraction of air in a bulb to move water in an attached tube. As early as 1680, Giovanni Alfonso Borelli (1608-1679) in the "On Motion of the Animals" (1680) (De motu animalium (Rome, 1680-1681)) successfully described the muscular movement and body dynamics; he described how balance is maintained by forward movement of feet providing support. Robert Hooke (1635-1703) is well known for his contributions to the field of mechanics and being perhaps the greatest experimentalist of the 17th century, introduced the term "cell" in biology (Hooke,R. (1665)). Leonhard Euler (1707-1783), Swiss mathematician who made enormous contributions to a wide range of mathematics and physics including analytic geometry, trigonometry, geometry, calculus and number theory, was first to describe the pulse waves in arteries (Vischer, D. Daniel Bernoulli and Leonard Euler). Herrmann von Helmholtz (1821-1894) described the focusing mechanism of the eye, the tri-chromatic theory of color vision, the mechanism of hearing and more. His work showed that muscle force was derived from chemical and physical principles (Cahan, D. (ed.) (1994)). As early as 1836, the Weber (Weber, W. and Weber, E. (1836)) brothers (Germany) described gait cycle and provided accurate measurements of the gait timing. However, the first description of gait could be found in the Aristotle's (384-322 BCE) statement "If a man were to walk on the ground alongside a wall with a reed dipped in ink attached to his head, the line traced by the reed would not be straight but zigzag, because it goes lower when he bends and higher when he stands upright". An important contribution to the study of the ways the muscles in the body work was development of the electromyography (du Bois-Raymond E.H. (1849)) in 1849 by du Bois-Raymond (1818-1896). Better understanding of the ways the muscle generate force was developed by Archibald Vivian Hill (1886-1977). He introduced modeling of muscle contraction and muscle mechanics. His work was recognized by a Nobel Prize in physiology/medicine in 1922. In 1878 Eadweard Muybridge (1830-1904) developed a methodology that allowed recording human and animals in motion. He recorded horse trotting using 24 cameras. Nikolai Alexandrowitsch Bernstein (1896-1966) suggested that performance of any movement results from an infinite variety of possible combinations of neuromuscular and skeletal elements. The system should, therefore, be considered as self-organizing, with body elements coordinated in response to specific tasks (Bernstein, N.A. (1967)). Bernstein coined the term "biomechanics", describing the application of mechanical principles and methods to biological systems.

The above short historical excursion does not even pretend to mention all the important people and their contributions to the development of the experimental techniques and methods used in biological systems. Next chapters will introduce the specific techniques developed and used in contemporary scientific studies of various biological systems.

2. Bone

Bone is historically the most studied tissue since it has simpler behavior compared to soft tissues. Also, more is known about bone mechanics in relation to its structure. Bone is also a good starting point because it illustrates the principle of hierarchical structure function that is common to all biological tissues.

The structure of bone is very similar to reinforced concrete that is used to make a building. When the building is first assembled, an initial frame of long steel rods is put in place. Cement is then poured around these steel rods. The rods and the cement form a tight union, producing a structure that is strong and resilient enough to withstand some motion while maintaining strength. Without the steel rods, the cement would be brittle and fracture with only minor movement. Without the cement, the steel rods would have inadequate support and would bend.

The same organization is true of bone. The steel rods that support the building are collagen rods in bone (25-30% dry weight of bone). The cement that surrounds and supports the rods is formed by minerals (including calcium and phosphorous) from the blood that crystallize and surround the rods. These minerals give the bones strength while the collagen rods provide resiliency (60-70% dry weight of bone). Such structure may be describe in mechanics as a composite material and, therefore, the experimental methods developed for the testing of composite materials may be easily adapted for testing of the bone's mechanical properties. The situation becomes more complex since human skeletal system consists of 206 bones that can be geometrically divided into different types: short, flat, irregular and long bones. Bone is identified as either cancellous (also referred to as trabecular or spongy) or cortical (also referred to as compact). The basic material comprising cancellous and compact bone is identical, thus the distinction between the two is the degree of porosity and the organization. The material properties of bone are generally determined using mechanical testing procedures and sometimes, ultrasonic techniques have also been employed. Force-deformation (structural properties) or stress-strain (material properties) curves can be determined by means of such tests. Bone shows a linear range in which the stress increases in proportion to the strain. The slope of this region is defined as Young's modulus or the Elastic modulus. However, the properties of bone and most biological tissues depend on the freshness of the tissue. These properties can change within a matter of minutes if allowed to dry out. Cortical bone, for example, has an ultimate strain of around 1.2% when wet and about 0.4% if the water content is not maintained.

The bone testing procedures were developed by engineers and, therefore, were mainly based on the methods and techniques developed for man-made materials. These methods were somewhat modified and changed to accommodate the peculiarities of the samples one can extract from the bone.

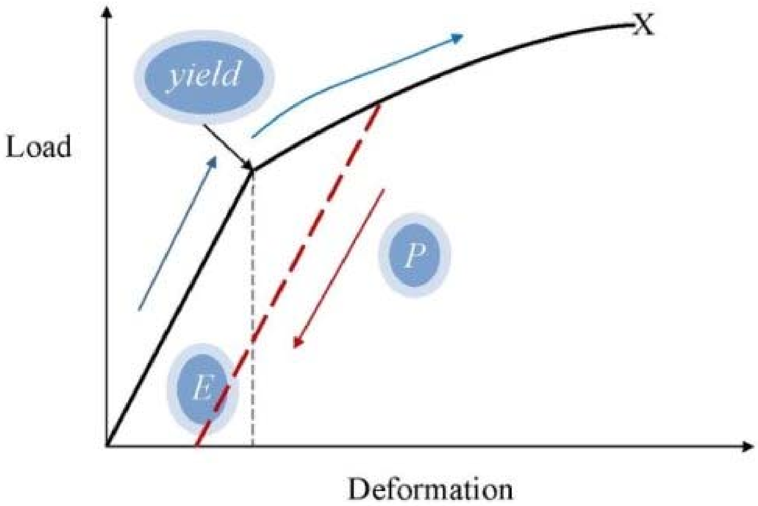

Figure 2. Typical stress-strain or load–deformation curve for a wet bone. Arrows indicate the direction of loading and unloading, the X marks failure of the bone sample.

The relationship between the applied load and resulted deformation of any structure is called a load-deformation curve. The typical load-deformation curve for the wet bone has two distinct regions: elastic strain (E) and plastic strain (P) regions (Figure 2).

The test consists of applying the tensile load to the specimen. One end of a bone specimen is clamped in a loading frame and the other subjected to a controlled displacement δ. A force transducer connected to the loading frame in series with the specimen provides a reading of the load P corresponding to the displacement δ. Two main approaches are utilized depending on the testing machine using load or displacement as the controlled variable.

The measures of stress σ and strain ε, are determined from the measured the load and deflection using the original specimen's cross-sectional area A_0 and length L_0 at the beginning of the test

$$\sigma = \frac{P}{A_0}$$

and

$$\varepsilon = \frac{\delta}{L_0}$$

When the stress σ is plotted against the strain ε, an engineering stress-strain curve is obtained. This curve looks pretty similar to the one shown in Figure 2; however, the horizontal axis represents the strain and the vertical - the stress.

In the early (elastic region) portion of the curve, bone usually obey Hooke's law, i.e. the stress is proportional to strain with the constant of proportionality being the modulus of elasticity or Young's modulus, denoted as E:

$$\sigma = E\varepsilon$$

As strain is increased, bone eventually deviates from the linear elastic proportionality, this point being termed the yield. This nonlinearity is usually associated with stress-induced "plastic" flow in the specimen. If the load is reduced after this point (red arrow in Figure 2), the unloading path will follow the red dotted line that is parallel to the elastic loading path (blue arrow). After the yield point the bone is undergoing an irreversible change in its internal molecular and microscopic structure. Increase in load will eventually lead to fracture, as indicated by an X at the end of the curve (Figure 2).

Analysis of the stress-strain curve provides number of important bone characteristics:

- modulus of elasticity E – defined by the slope of the curve in the elastic region,
- yield strength σ_{y} – stress corresponding to the yield point,
- ultimate strength σ_{u} – stress corresponding to the failure of the specimen.

The obtained results are dependent on the direction of the loading, i.e. the direction the bone sample was cutout from the original bone. It was found that the bone's mechanical properties are direction dependent, i.e. the bone is not isotropic material, but rather anisotropic one. This fact makes testing and characterization of the bone samples even more challenging.

Another difficulty in measuring mechanical properties of a bone is variety of bones structures in the musculoskeletal system. Two main variations are: compact and cancellous bones. Compact bone forms the outer shell of bones; it consists of a hard virtually solid mass, while the cancellous bone, found beneath compact bone, consist of a network of bony bars (trabeculae) with many interconnecting spaces. Thus, the modulus of elasticity of the cancellous bone is much lower than that of the compact bone and it depends on the actual structure of the cancellous bone. These structural properties depend on the particular anatomical region.

2.1. Specimen Preparation

Mechanical properties of the bone are function of the type of bone, its location, age, and testing environment (temperature and humidity). Therefore, it is nearly impossible to measure ‘absolute’ bone property; one should realize that the obtained data is always relative in a sense that the comparison may be done only for samples that were harvested, kept and tested under the same conditions. Only tests performed immediately after harvesting the bone sample may be considered as providing the ‘absolute’ bone properties. Next best is to preserve the test specimen at -20^0C in asaline-soaked gauze. Extensive research had shown that such procedure cause no or limited change to the measured mechanical properties (Sedlin, E. D., and C. Hirsh. 1966) (Pelker RR, Friedlaender GE, Markham TC, Panjabi MM, Moen CJ.).

2.2. Testing Procedures

The simplest testing procedure for accurate measurement of the bone properties is the tensile test, however the bone samples have to be relatively large and they have to be machined to precise dimensions. Bending tests are often used for measuring mechanical properties of relatively small bones due to difficulties to machine them to the appropriate for tensile testing size. Torsion test is used to evaluate the bone’s mechanical properties in shear. Shear property may be also evaluated by using specially developed samples that allow introducing state of pure shear at the desired section. As an alternative to the pure mechanical testing, ultrasonic procedures were developed to measure the bone’s Young modulus on the base of the speed of the wave in the sample.

2.2.1. Mechanical Testing

In a recent study the specimens were prepared from the cadaveric tissue (Nyman J.S., Roy A., Tyler J.H., Acuna R.L., Gayle H.J., Wang X.). Using a circular diamond saw an axial bone strip (2.3 mm thick) was collected from the anterior aspect of the mid-diaphysis. The strip was milled by a CNC machine into a tensile specimen (the grip regions were 10 mm × 5 mm; the gage region was 10 mm × 2 mm; and the tapers were 10 mm long with a fillet radius of 20 mm). A bench-top mechanical testing system was used to conduct tests in tension. The loading and unloading rates were 0.5 mm/min and 480 N/min, respectively, to ensure a consistent strain rate during loading. Strain was recorded with an extensometer to produce engineering stress versus engineering strain curve. Ultrapure water dripped on the specimen at a nominal rate of 4 mL/min throughout the mechanical testing to keep it hydrated. The obtained data (applied load vs. elongation) was used to extract the pertinent mechanical properties.

Three point bending test also may be used to determine the apparent elastic modulus and breaking strength when loading the sample to failure. Cortical bone specimens were taken from the tooth-bearing part of the mandibular body in number of piglets and adult female swine. They were cut into rectangular blocks and placed in the special test rig for testing. From the measurements of the applied load and associated deflection, the modulus and breaking strength were determined (Rose E.C., Hagenmüller M., Jonas I.E., Rahn B.A.).

2.2.2. Ultrasonic's Testing

The high degree of porosity of cancellous bone makes elastic property measurement difficult by traditional mechanical testing methods. An ultrasonic technique is often used to evaluate mechanical properties of anisotropic, rigid, porous materials, such as cancellous bone. The technique utilizes piezoelectric transducers operated in a continuous wave mode at a frequency of approximately 50 kHz. Both longitudinal and shear waves can be propagated and received with the transducers allowing both Young's moduli and shear moduli to be determined with the technique. A comparison between moduli measured with the ultrasonic technique and moduli measured with traditional mechanical testing shows the ultrasonic method to be quite accurate in elastic property determination (Ashman R.B., Corin J.D., Turner C.H. (1987)).

Alternatively, the pulsed ultrasound is also used to measure the speed of sound for evaluation of the elastic modulus of the bone (Rose E.C., Hagenmüller M., Jonas I.E., Rahn B.A., 2005)**Error! Bookmark not defined.**. The emitter and receiver were placed in immediate contact with the specimen and 2 MHz pulsed ultrasound was used. Acoustic coupling was improved by using a coupling gel. Measurement of the time of flight and distance between the emitter and receiver allowed to calculate the speed of sound 'V' and use it to evaluate the Young modulus using the following equation.

$$V = \frac{E}{\rho},$$

where E is Young modulus ρ is density.

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Biographical Sketch

Professor Arkady Voloshin was born in Moldova in 1946. He got his undergraduate degree in mechanics and physics of metals from Faculty of Mechanics and Physics, Leningrad Polytechnic Institute, Leningrad, USSR in 1969 and PhD in solid mechanics at School of Engineering, Tel-Aviv University, Tel-Aviv, Israel in 1978.

In 1969-1970 he was a Senior Research Officer at the Institute of Non-Destructive Testing in Kishinev, USSR. Since 1973 he was working as research assistant at School of Engineering, Tel-Aviv University, Israel. Between 1979 and 1984, he was a Professor at Department of Engineering Science and Mechanics in Iowa State University and since 1984 Arkady is a Professor of Mechanical Engineering and Mechanics at Lehigh University. During his academic life Arkady published over 70 papers in peer-reviewed journals and presented and published 160 papers in the proceedings of various conferences and congresses. He also contributed invited chapters to seven books.

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