On Mechanical Behavior and Characterization of Soft Tissues

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Radhika Chavan[1], Nitin Kamble[2], Chetan Kuthe[3]
and Sandeep Sarnobat[2]

[1]Department of Mechanical Engineering, D Y Patil College of Engineering, Akurdi, Pune, India.
[2]Department of Robotics and Automation, D Y Patil College of Engineering, Akurdi, Pune, India.
[3]Department of Mechanical Engineering, Maharashtra Institute of Technology, Aurangabad, India.

ABSTRACT: The growth and advancements done in solid mechanics and metallurgy have come up with various characterization techniques that help in prediction of elastic properties of different types of materials—isotropic, anisotropic, transverse isotropic, etc. Soft tissues which refer to fibrous tissues, fat, blood vessels, muscles and other tissues that support the body were found to have some control over its mechanical properties. This mechanical behavior of soft tissues has recently shifted the attention of many researchers to develop methods to characterize and describe the mechanical response of soft tissues. The paper discusses the biomechanical nature of soft tissues and the work done to characterize their elastic properties. The paper gives a review of the behavior and characteristics of soft tissues extracted from various experimental tests employed in their characterization. Soft tissues exhibit complex behavior and various complexities are involved in their experimental testing due to their small size and fragile nature. The paper focuses on the conventionally used tensile and compression tests and the difficulties encountered in soft tissue characterization. It also describes the utility of ultrasound technique which is a non-destructive method to characterize soft tissues. Tensile and compression test used to characterize materials are destructive in nature. Ultrasound technique can provide a better way to characterize material in a non-destructive manner

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CORRESPONDING AUTHOR: Radhika Chavan, D Y Patil College of Engineering, Sector No. 29, Nigdi Pradhikaran, Pimpri Chinchwad, Near Akurdi Railway Station, Akurdi, Pune 411044, India. Email: radhichavan18@gmail.com

Introduction

The advancements in material science have shown a considerable growth in the fields of solid mechanics and metallurgy over the past years. Materials are an important application of solid mechanics and their characteristic properties are the necessary preconditions for putting them into application. Existing procedures used for characterization of materials work on some available standards and are restricted to materials having effective orthotropy.[1] Modern materials that have come into development have broken the boundary of conventional category of materials—homogenous, isotropic, and linearly elastic.[1,2] In understanding the structure and characteristics of materials, complex materials like polymers, composites and other biological materials pose a challenge to the long-established characterization techniques.[3]

Research on characterization of biological materials has the potential to create new knowledge and develop new materials that can be a combination of known biological material and other material. Many materials like wood, rigid and flexible foam and soft tissues have been found to depict transverse isotropic and also anisotropic material characteristics. With few constitutive models available to accurately describe their properties, research has directed the attention to the development of models to describe the mechanical behavior of these transverse isotropic and anisotropic materials. A constitutional model was presented to describe the transversely isotropic nature of material with anisotropic hardening. It described the mechanical response of a transversely isotropic material that exhibits hardening.[4] A method was developed to compute the crack-opening displacement tensor for a transverse isotropic material and calculate its overall elastic properties. No explicit analytical expressions could be found to calculate the overall elastic characteristics of transverse isotropic materials.[5] The characteristics of transverse isotropic materials were calculated by first calculating the components of the compliance tensor of transverse isotropic platelets. The approach was done on hydroxy apatite platelets embedded in the transverse isotropic collagen matrix. Maxwell homogenization technique and non-interaction approximation were used to compute the elastic properties and results were then compared to the experimental data from literature. The model was based on crack-opening displacement tensor that related the average displacement discontinuity vector at the site of crack to the vector of uniform traction.[6]

Soft Tissues

Various types of Soft Tissues are found in human body. They are made up of collagen and elastic proteins and are composite in nature.[7] Collagen is found to play a major role in muscle elasticity and thus determines the mechanical properties of muscles.[8] They are heterogeneous in nature and are made from cells dispersed in a filament like and hydrated extracellular matrix (ECM). The configuration and structural organization of ECM varies with each tissue and complements their distinct functionality. With variation in the composition of soft tissues, a micromechanical variation can be seen in the properties. A variation in stiffness with the fiber direction due to the collagen and elastin present in the ECM is observed. So they are also categorized as transverse isotropic materials.[9]

To describe the complex nature of soft tissues, a number of mechanical mathematical models have also been proposed. Brain tissue is studied extensively whereas studies on other soft tissues are comparatively scanty and more recent. Attempts have been made to theoretically portray the material response of soft tissues by considering them as single phase continuum. To describe the linear viscoelastic response, few models that are based on fractional calculus are also suggested. A mathematical model describing nonlinear viscoelastic behavior of kidney and liver tissues includes few parameters with closed-form formulation. It is capable of characterizing linear as well as non-linear viscoelastic behavior of soft tissues as tested in the parallel plate configuration. A strain hardening bi power law, that can be used to predict time and strain dependent response of tissues is proposed.[10]

Rheological experiments conducted on polyvinyl alcohol cryogel (PVA) samples that are found to best mimick soft tissue properties showed frequency dependence of the material and non linear properties. The rheology tests conducted on Bose Electroforce 5500 test instrument investigated material response to dynamic micro oscillations and macro oscillations that depicted non linear behavior around varying static loads. The large non linear deformation response in time was also investigated and both the tests were carried out with a frequency sweep to investigate frequency dependence of the polyvinyl alcohol cryogel material. The load relaxation tests conducted showed a small visco elastic response of the PVA samples due to the material being highly elastic. This response is however expected to disappear for in vivo data obtained from soft tissues as they have stronger viscous component.[11]

A model proposed for mechanical behavior of soft connective type of tissue, depicts that the variation of glycosaminoglycan (GAG) components within the tissue has a control over its mechanical properties. Strain energy stored in the fibrils of the loaded tissue is shifted to the potential field created by charged GAG components. The potential field is created due to their electrostatic interaction with the fibrils. Non-linear variation of tissues and invariance of the stress relaxation behavior with deformation are also recognized in the model.[12] In a study done on the determination of effect of microstructure on mechanical behavior of tissues, it was found that the models at fiber level exhibited behavior that is consistent with the assumption of transverse isotropic nature whereas fascicle level models exhibited the behavior of transverse anisotropic nature.[13]

A most common yet undesirable feature observed in anisotropic and hyperelastic constitutive models for soft tissues is the auxetic behavior and the unphysical transverse expansion that happens during uniaxial tension. Auxetic behavior is the orthogonal expansion that occurs during uniaxial tension. It is a common and undesired feature of hyperelastic models and is a subject of recent studies. Auxetic behavior that causes out of plane expansion is found to be affected by the stiffness ratio of fiber to matrix. It is accentuated by strain stiffening fibers in a constant stiffness matrix. Studies have also reported the secondary effect of fiber dispersion and compressibility. It is also referred to as negative poison's ratio and is considered as unphysical in soft tissue model content. Introduction of volumetric compressibility was found to increase the auxetic behavior whereas fiber dispersion was found to reduce it but not eliminate it completely. A bilinear strain stiffening fiber matrix was proposed that controlled fiber to matrix stiffness ratio thus eliminating the auxetic behavior.[14]

Soft tissues depict some amount of shrinkage when dissected from their location. The shrinkage is seen as a result of the release of residual stresses when they are removed from their pre stressed physiological configuration. Application of a pre force or pre stress to the sample while testing can help to get rid of the residual stresses. A finite element simulation done to replicate the uniaxial tensile test on soft tissues depicted the influence of various pre stretches on the mechanical properties. It is also found that most tissues like pelvic tissues and abdominal tissues are stressed not uniaxially but multiaxially. So multiaxial testing would provide richer information in this regard.[15]

The following chart shown in Figure 1 depicts the different types of soft tissues found in human body.

Mechanical Behavior of Soft Tissues

A live tissue is found to have control over its mechanical behavior. This mechanism to control mechanical behavior may change or even collapse in case of any disease. Soft tissues depict stress relaxation behavior which is the rise of stress in the tissue when stretched to a certain deformation and then fall as the deformation gets constant.[12] The biomechanical behavior shown by soft tissues plays an important part in the mechanical integrity analysis of a living body. Thus, it is very important to assess the biomechanical characteristics of tissues.[16] The skeletal system which includes the skeletal muscles, contributes significantly in the well-being of mammals. It plays a significant role in recovery from injuries and also provides them with the tolerance to various risk factors.[17,18] For this purpose, it is important to study the characteristics of different biological materials.

The material properties of biological materials are highly variable and also poorly defined.[19] Various biological factors like tissue composition, sex, age, conditions of disease, body mass index and body mass are found to influence the stiffness of soft tissues.[20] A study done on hip tissues depicts that the

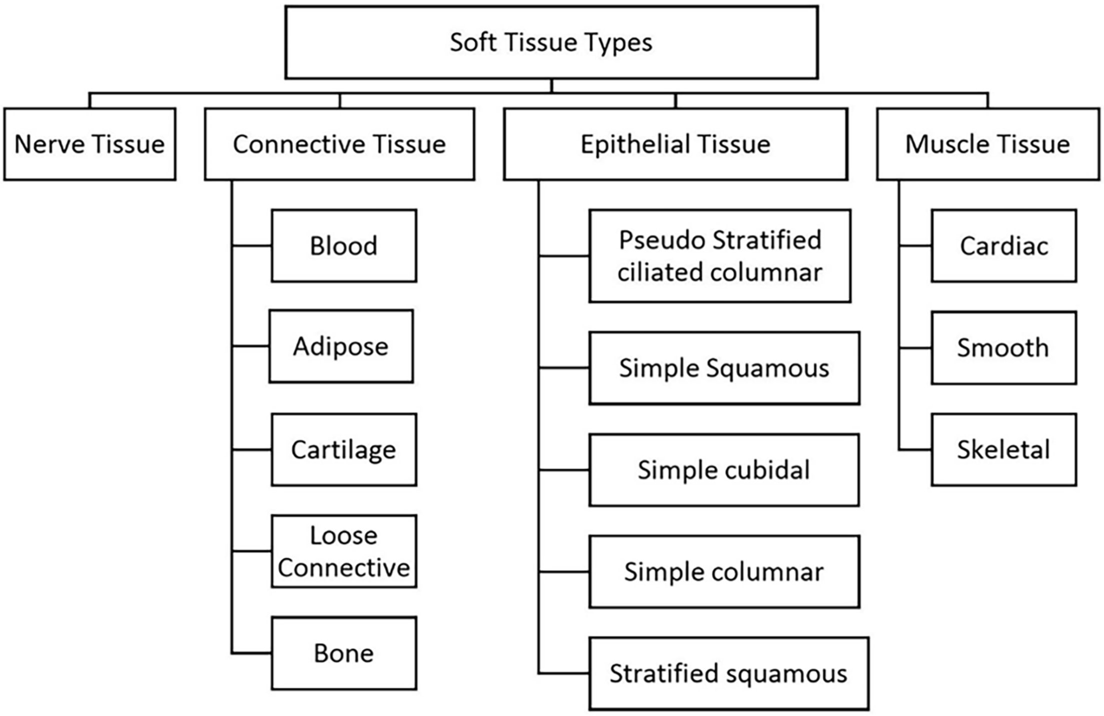

Figure 1. Different types of soft tissues found in human body.

shock absorption capacity of soft tissues decreases with age or one can say that stiffness increases with age. Some findings show that stiffness decreases with age but that can be due to the difference in measurement techniques. Aging effect occurs as the structure and collagen content present within soft tissues changes with age which causes stiffness to increase.[21] The dependence of factors like age and sex, the post-mortem interval or body side on the passive load-deformation properties was studied on human temporal muscle. The samples were obtained from the human cadavers in age range: 4 months to 94 years and were investigated on a quasi-static tensile test. The elastic modulus, ultimate tensile strength and strain at maximum force was found to be independent on the factors predicted and thus the biomechanical characteristics in simulation models may not always require adjustments for age, sex, post mortem interval or body side.[22]

Microscopic details of the structure of tissues are very important as they are the essential factors affecting elastic properties.[23] In vitro testing of soft tissues deals with lot of difficulties as they are very sensitive and the difficulties occur due to their own highly non-linear viscoelastic and anisotropic nature. Soft Tissues show strong time dependent mechanical behavior that arises due to their fluid flow induced poroelasticity and viscoelasticity. The time dependent properties affect the physiological functions of soft tissues and are even linked to different pathological processes.[24] Time dependent behavior of tissues can contribute significantly to tissue engineering and tissue pathology that offers promising alternatives to the present replacement strategies used in biomechanical areas that is, synthetic or bioprosthetics.[25] Skeletal muscle tissues are continuously under tension either as antagonist or agonist (working in opposite ways to bind cell receptors giving rise to different responses) during the eccentric contractions. Passive tensile strain is generated at the connection of muscles to tendons which contributes to the maintenance of highly organized muscle fibers. The muscles even show the capacity to regenerate, remodel and repair after an injury.[26]

Soft tissue characterization is very crucial in the areas of regenerative medicine, robot assisted surgery, surgical simulations and tissue engineering. The properties can be used in cell regeneration and differentiation with engineering of tissue scaffolds and also to develop new biomaterials for replacement of damaged tissues. In surgical simulations, the mechanical characteristics of soft tissues can help to estimate their interaction with surgical tools.[27] Accurate prediction of soft tissue properties can find crucial applications in tissue engineering where engineered tissues can be made with properties to match the native replaced tissues. The stress strain response obtained from soft tissue characterization is found to be dependent upon the arrangement of fibers in alignment with the loading direction.[28]

Proper characterization can help fully understand the mechanical characteristics of soft tissues which also play a crucial role in cell behavior. Accurate mechanical properties can aid surgeons in their training and practice by creating a virtual reality based environment. It also helps in diagnosis of diseases as the changes in mechanical properties can help in early detection and aids treatment.[29] The mechanical properties are an important aspect of cellular biology as they control the cell shape, growth, migration, differentiation and even apoptosis.

The structural differences of each cell play a major role in their physiological functions A specially designed tensile tester was used that performed tensile test on vascular smooth muscle cells and cervical cancer HeLa cells to determine tensile stiffness and strength of the cells. It was observed the structural arrangement generates differences in the mechanical characteristics and adhesion forces of the cells. The cell adhesion forces are very important to regulate the cell physiology and tissue development.[30] The mechanical properties of vascular cell walls were found to change along with their dimensions in response to blood pressure. Being highly heterogeneous in nature, the cell properties depend on the arrangement of stress fibers and microtubules in the skeletal network. To understand the mechanism of mechanical adaptation, it is thus necessary for future studies to establish a connection between cell and tissue mechanics.[31]

Characterization of Soft Tissues

Significant development is seen in synthesis and characterization of materials to the size of nano particles and their composites. Commonly used imaging techniques that describe the morphological behavior include scanning electron microscopy (SEM), transmission electron microscopy (TEM), scanning tunnel microscopy (STM), atomic force microscopy (AFM) and field emission scanning electron microscopy (FESEM). These nano sized particles are used with composites to improve their properties and it is possible only with the advancement of characterization techniques.[32] Atomic Force Microscopy senses small interaction between the surface and instrument that can aid in characterization of nano mechanical properties of the surface. FM techniques have expanded the capabilities of nano mechanical mapping of materials. A variety of materials can be characterized with the use of FM-AFM technique.[33]

The Elastic Modulus of a material is a very important parameter to characterize the material for various applications. Various direct and indirect methods are used to experimentally characterize a material. Speed of sound can also be used to compute the elastic properties of a material but the measurements are uncertain. Uncertainties are related to the calibration procedures, an estimation of contact depth. Reproducibility of the measurement is the only significant contributor to the accuracy of measurement in these cases. Characterization of biological tissues is done by ultrasound testing, tensile testing, whole organ compression or induction testing. Tensile test being the simplest and most commonly used method is destructive in nature whereas ultrasound is non-invasive and can be successfully used with tissues.[34] From studies it is also found that soft tissues are fiber oriented and it is convenient to treat them as hyper elastic materials.[35] Combination of experimental and numerical investigation of the mechanical nature of soft tissues are done to provide the location, time and history dependent behavior.[36]

This section gives a description of the most commonly employed characterization techniques from the literature review.

Tensile and compression testing

Various uniaxial and biaxial tests have been done to study the transverse isotropic and anisotropic properties of soft tissues. Tensile test is the most conventionally used test to characterize materials and has largely been employed in characterization of soft tissues. In tensile testing, the ends of soft tissue samples are gripped at the ends in a fixture and pulled apart until it attains a predetermined strain value or reaches failure. The test is simple in procedure but destructive in nature. It can incorporate multiple axes in tension during experimentation leading to high repeatability and versatility. The stress strain curves can help in obtaining mechanical properties directly without any involvement of mathematical models. The only disadvantage is inaccuracy in gripping of samples, slippage and misalignment. Also when the tissue is extended in uniaxial direction, the fiber in lateral direction shows contraction but that can be overcome with biaxial testing. Compression test is another conventionally used test to characterize soft tissues. It works on the same principle of tensile test with only difference that the sample is kept between two flat platens unlike of that gripping by sample holder. Difficulties associated with compression testing are with sample preparation and effect of friction. The sample has to be free of irregularities on surface and need to be cut to strict specifications.[29] Typical uniaxial tensile test setup is as shown in Figure 2 and Schematic of unconfined and confined compression test setup is as shown in Figure 3.

Little attention is given to compressive behavior of tissues, but it is important for complete characterization of soft tissues. Quasi static and dynamic unconfined compression tests were conducted on pocrine muscle sample. The results showed that the weakest direction is 45° to the fiber direction. It explained the connection between adjacent fiber fascicles which caused shearing during compression. A sub microscale tensile testing apparatus was developed that used a flexure mechanism along with a 3D printed sample holder to grip the samples of tissues. Precise force measurements were possible due to the flexure mechanism that overcame the drawback of measuring minuscale forces during tensile testing of soft tissues. The 3D printed clamps had extruded patterns that gripped onto the samples and allowed for reduced slippage and gave quick and repeatable results during testing. Different lung samples of mice were tested and the elastic modulus was found to increase with the age of mice (3.56 kPa in 3 months to 4.01 kPa in 30 months). It was also found that the mice that suffered from emphysema showed highest elastic modulus in comparison to other healthy mice.[27]

In a study done to assess elastic deformation properties in chicken pectoralis muscle, deformation data was obtained on

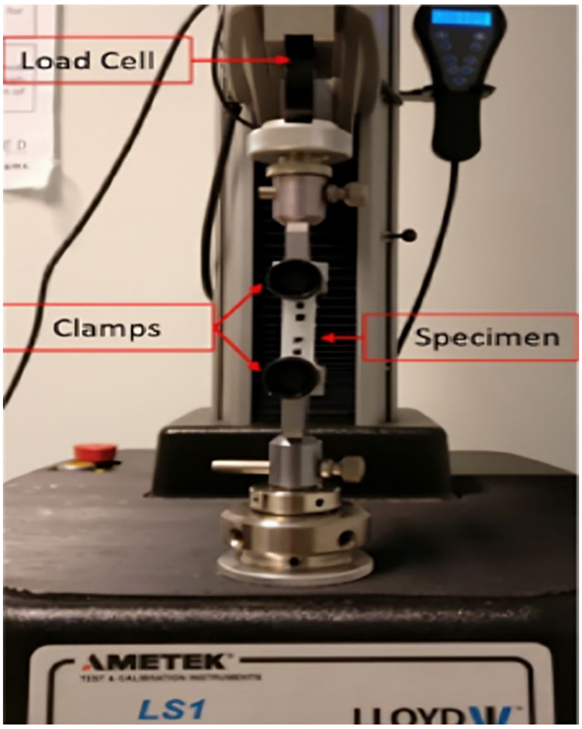

Figure 2. Typical uniaxial tensile test setup.[29]

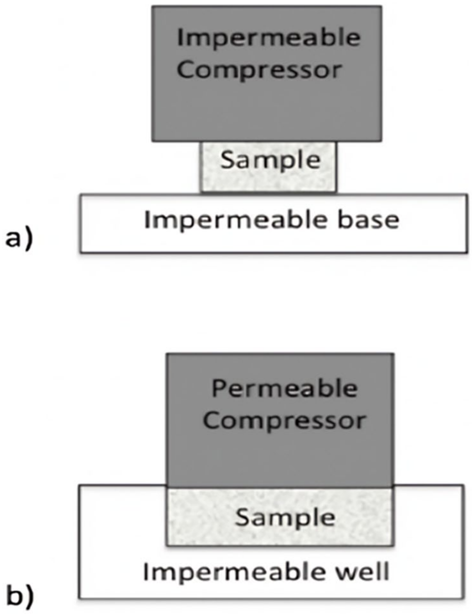

Figure 3. Schematic of: (a) unconfined and (b) confined compression test setup.[29]

skeletal muscle which was very dissimilar from porcine tissue. Uniaxial tension and compression tests were done along the fiber direction, cross fiber direction and even at 45° to the fiber direction. The tests showed significant asymmetry in tension and compression as response obtained from tension test was two orders higher in magnitude than that obtained from compression test. The tissue exhibited highest stiffness in cross fiber direction in compression test and 45° to the fiber direction in tensile test. Broadly transverse isotropy was observed for muscles in fiber direction.[37] Tension and compression asymmetry leads to the lack of knowledge of how externally applied tension or compression transforms to the deformation of muscle fibers and ECM. It was observed that muscle fibers flatten in compression thereby aligning them in perpendicular direction to the deformation whereas muscle fibers are stretched under tensile deformation thereby aligning them in parallel direction to the deformation.[38] Tension compression asymmetry can be detected at specific length scales using tension and compression tests. A study that reported tension compression asymmetry at different length scales, significance difference was observed in the characteristics at tissue and fiber level. The asymmetry increased non linearity with deformation at tissue level, whereas it was found to decrease initially and then rise again at the fiber level. It was observed that the ECM plays a significant role in affecting the tension compression asymmetry. Biological tissues are frequently considered incompressible because of their high intracellular and extracellular water content. However, literature shows an ongoing discussion on this topic.[39]

To study the direction dependent mechanical response of ECM samples and the result of lateral compression, tensile experiments were performed. Variations were observed in the results which were attributed to the variation of ECM of the tissue. The endo and perimysial ECM of the skeletal muscle tissue was isolated by decellularisation and then tested for mechanical characterization. ECM samples tested at 45° to the fiber direction showed stiffest response whereas the stiffest response was observed at the cross fiber direction for intact muscles.[40] It was observed from studies that the nature of anisotropy of soft tissues remains unclear and strong viscoelastic effects are also observed. In a tensile test done on longissimus dorsi muscle harvested from pigs, a non-contact optical method was used to measure strain and the test was performed along and cross fiber direction and also at 30°, 45°, and 60° to the fiber direction. The transverse or cross fiber direction was found to be stiffest with failure occurring at low stretches. The longitudinal fiber direction was comparatively less stiff with failure occurring at high stretches. A sinusoidal stress variation response was obtained at intermediate angles.[28]

While most studies depict experiments done at the tissue level and fiber level, very few studies discuss the microscale and nanoscale structure of muscles that control their characteristics. It was found that the intercellular structural difference of a cell had an effect on the mechanical properties. Experiments to study the effect of fiber bundles, proteins and myofibrils are less common. The biceps femoris muscles extracted from the legs of female pigs were subject to cyclic tension and compression experiments. It was found that muscle fibers depict 5% volume decrease at the point of maximum deformation under tension and 37% volume decrease at the point of maximum deformation under compression.[41] Figure 4 shows the experimental setup used for uniaxial tensile and compression test.

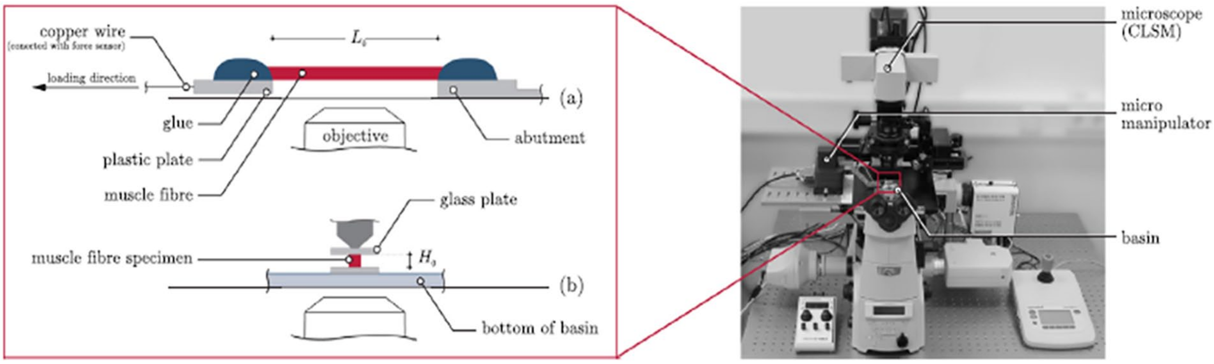

Figure 4. Experimental set up for: (a) uniaxial tension and (b) compression experiments.[41]

In an investigation done on single viscoelastic myoblast obtained from mouse skeleton, a cell specific and confocal based finite element model was used to analyze the effect of cyclic compression. It was observed that the proportion of average tensile strain in all the cell membrane components and the percentage of damaged elements decreased under the cyclic compression tests than that compared to the static compression tests. Thus, the mechano-sensitivity of cells and its interaction with the surrounding environment determines cell morphology and fate.[42] The nonlinear characteristics of skeletal muscle and material properties were found to be strain dependent. The time dependent and independent properties of Tibialis anterior muscle obtained from white rabbits was studied. On computing the relaxation ratios, it was observed that the mid range relaxation and instantaneous modulus increased with strain whereas relaxation at prolonged time periods decreased with strain. The overall relaxation ratio and time constant did not change with strain.[43] The results obtained from the various experimental tests done on soft tissues are listed in Table 1.

Ultrasound testing

Among the various characterization methods available so far, use of ultrasound technique for material characterization has given a correlation between the poisson's ratio and shear wave velocity. In different materials like metals, alloys, glasses and ceramics, the poisson's ratio has been found to decrease with ultrasonic velocities. It is also found that ultrasonic shear wave velocity is a preferable parameter to characterize materials in comparison to ultrasonic longitudinal wave velocity. The ultrasonic velocity works on evaluating the association between the independent elastic constants with the electronic configuration, microstructure, atomic bonding, etc.[44]

The modality of ultrasound technique in material characterization is explained as follows:

a. Sources of ultrasound generation and detection

The sources for generation of ultrasonic waves are mechanical, electrodynamic, electromagnetic, electrostatic, piezoelectric effect, magnetostrictive effect and laser method. Ultrasonic waves in frequency range of 100 kHz to 1 MHz can be generated using mechanical shock or friction whereas electrostatic method can produce ultrasonic waves of frequency range of 10 to 200 MHz. Magnetostriction is mechanical deformation in magnetic field of a ferromagnetic material that can be used to produce ultrasonic wave. Piezoelectric effect is most commonly used in which a laser light is incident on surface of a material. Some portion of the incident light is absorbed to produce tangential stress and bulk strain to produce ultrasound wave through transient heating.

Conventional methods like mechanical and optical methods are used for detection of ultrasonic wave. Devices based on piezoelectric effect are used mostly to detect ultrasound wave.[45]

b. Ultrasonic velocity and measurement

For the case of solid state materials, ultrasonic velocity is found to be associated by the mechanical properties and porosity of material. The elastic constants and stiffness of a material can be obtained from measurement of ultrasound velocities. To understand the low temperature material properties and lattice vibrations, factors like Debye temperature and Debye average velocity are required.

The high frequency and low intensity ultrasonic waves can be used for material characterization in a non-destructive manner. Ultrasonic velocity and attenuation are required for material characterization. Ultrasonic velocities are of four types based on the mode of dispersion—longitudinal, shear, surface, and lamb. Of these, the shear wave and longitudinal velocities are found to be associated to the elastic constants and density of the material. Use of ultrasound technique to obtain deformation data from soft tissues can aid in the characterization of mechanical characteristics of soft tissues.

General electrical methods like pulse technique, interferometer and continuous wave method are used to measure ultrasonic velocities. In continuous wave method, ultrasonic velocity is obtained from the wavelength of the material whereas in pulse technique the transit time and material thickness are used to compute ultrasonic velocity. More recent

Table 1. Results of tensile and compression characterization techniques.

REFERENCE	MEASURABLE PROPERTIES	OBSERVED VALUES	TYPE OF TISSUE	METHOD ADOPTED	FIBER DIRECTION	SAMPLE PREPARATION	FACTORS AFFECTING ACCURACY AND REPEATABILITY
Wheatley et al[18]	Strain rate, Young's Modulus	Elastic Modulus range: 3.47 to 4.96 kPa Mean Yield stress: 8.48 kPa Mean Yield strain: 2.53	Tibialis anterior muscle (rabbit)	Load Relaxation test with relaxation cycles of 3% strain	Long axis coincides with muscle fiber direction	Samples were cut from muscle midbelly (away from surrounding fascia and aponeurosis). Surface of sample needs to be flat for accurate measurement.	Post mortem rigor affects have to be taken care by testing within 2 h of sample preparation
Zwirner et al[22]	Elastic modulus and tensile strength	Elastic modulus = 1.58 ± 0.64 MPa, Ultimate tensile strength = 0.26 ± 0.11 MPa Strain at max force of 26.21 ± 12.48%	Human temporal muscle (TM) and temporal muscle fascia (TMF)	Uniaxial Tensile test	TM—as per direction of muscle fiber TMF—as per direction of collagens	Samples were extracted from human cadavers and cut to dog bone shape for testing	Post mortem rigor effects
Kang et al[27]	Elastic modulus, yield stress, yield strain	Elastic modulus range: 3.64 to 4.40 kPa Yield stress range: 7.45 to 9.94 kPa Yield strain: 1.82 to 2.60	Murine lung	Sub microscale tensile testing	Along the fiber direction	Sample slices were harvested from mice of different ages and were to be cut in shape that can be gripped by the tensile holder.	Sample alignment, sample slippage during gripping
Takaza et al[28]	Cauchy stress, Poisson's ratio	Stress: 77 kPa (cross fiber), 10 kPa (along the fiber) Poisson's ratio: $V_{LT} = 0.47$, $V_{TT} = 0.28$, $V_{TL} = 0.74$	Longissimus dorsi muscle from pigs	Quasi static tensile testing	Samples were cut 10 mm thick and 10 mm wide with length as per ASTM E8 standards	Perpendicular to muscle fiber direction and 30°, 45°, and 60° to the muscle fiber direction	Slippage of sample while testing
Nagayama et al[30]	Tensile stiffness, Adhesion force	Tensile stiffness: VSMC = 48 ± 11 nN/%, Hela = 25 ± 19 nN/% Adhesion force: VSMC = 780 ± 370 nN, HeLa = 380 ± 170 nN.	VSMC (rat embryonic aortic) and HeLa (human cervical carcinoma) cells	Micro tensile testing on a specially designed tensile tester	Not specified	Cells were cultured in a medium and moved with use of a micro needle to the stage of micro tensile tester.	Irregularities in cell shape
Mohammadkhah et al[37]	Poisson's ratio	Poisson's ratio ± std (Tension): 0.5 ± 0.08	Chicken pectoralis muscle	Uniaxial Tensile test	Tensile test- Along the fiber direction.	Freshly harvested samples are cut in cuboid dimensions for tensile test.	Clamping of sample, rigor mortis effect

(Continued)

Table 1. (Continued)

REFERENCE	MEASURABLE PROPERTIES	OBSERVED VALUES	TYPE OF TISSUE	METHOD ADOPTED	FIBER DIRECTION	SAMPLE PREPARATION	FACTORS AFFECTING ACCURACY AND REPEATABILITY
Mohammadkhah et al[37]	Poisson's ratio	Poisson's ratio ± std (Compression): 0.51 ± 0.08	Chicken pectoralis muscle	Uniaxial compression test	Compression test—cross fiber direction and 45° to cross fiber direction	For compression test, samples are cut in cubic dimensions with height/length aspect ratio 1:2.	Surface irregularities Friction between sample and platens
Kohn et al[40]	Maximum stress (P_{max}), stretch λ_{max}	P_{max} [kPa] = 100.0 ± 24.0 (at 0°), 13.9 ± 3.0 (at 45°), 32.6 ± 18.7 (at 90°) λ_{max}: 1.9 ± 0.1 (at 0°), 13.0 ± 0.0 (at 45°), 1.2 ± 0.0 (at 90°)	Porcine muscle	Axial tension test, Transverse compression test	Along the fiber direction, 45° to fiber direction and 90° to fiber direction	Cuboid samples were cut and treated with NaOH	Sample slippage and friction
Böl et al[41]	Load—deformation, volume change	Volume change at maximum deformation = 5% under axial tension, 37% under axial compression	Bicep femoris muscle of pig	Cyclic and relaxation loading test in axial tension and axial compression	Along the fiber direction	Intact fibers were dissected with forceps and scalpel	Sample slippage
Tamura et al[43]	Young's modulus	Max $E = 338.9$ kPa at stretch of 24%, Min $E = 26.0$ kPa at stretch of 16%	Rabbit hind limb muscle fiber bundle	Stress relaxation test using custom tensile tester	Along the fiber direction	Hamstrings were cut from rabbit hind limbs and then cylindrical muscle fiber bundles were removed using fine forceps and dissection needles.	Misalignment from loading axis

techniques like laser interferometry and resonance spectroscopy are used in materials like textured alloys, thin film etc.[45]

c. Ultrasonic attenuation

When an ultrasonic wave propagates from source through a medium, its intensity decreases due to loss of energy. These energy losses occur due to absorption, scattering and diffraction that occurs in the medium. Other possible causes of attenuation are electron-phonon, phonon-phonon and magnon-phonon interaction, lattice imperfections, grain boundary losses and thermos elastic relaxation. Like velocity measurement, ultrasonic attenuation can be measured using continuous wave method and pulse technique. Ultrasonic attenuation and velcoities provides insight into physical properties and microstructure of materials.[45]

d. Merits and demerits of ultrasonic technique for material characterization

The low intensity and high frequency ultrasound waves used for material characterization provides for a non destructive characterization approach compared to the conventional tensile and compression tests. Ultrasound elastography method used for displaying relative stiffness of soft tissues is helpful for detection of tumors and other possible abnormalities and diseases.[45]

Diagnostic ultrasound is considered to be safe unlike x-ray methods that produce ionizing radiation. Other advantages of ultrasound testing include its portability, consistency, limited access and the ability to detect surface and subsurface defects.

Some of the limitations of ultrasonic testing include the requirement of extensive training, difficulty to be used on thin materials and requirement of smooth surface to couple transducer. But in spite of these, the method proves to be cost effective and time saving in comparison to the conventional destructive characterization tests.

Contemporary image based methods

Methods like magnetic resonance elastography (MRE) and ultrasound shear wave elastography (SWE) have been used to measure passive properties of the skeletal muscle. Mechanical behavior of muscles is an important aspect of their physiology as they help and stabilize the skeleton. In vivo mapping of the passive mechanical properties is provided by elastography measurement techniques. The major application of magnetic resonance elastography is the ability to provide 3D mapping of muscle visco elastic properties (in vivo), also including the variation in properties within the muscle and between muscles. Diffusion tensor imaging can be used for obtaining detailed muscle architecture. MRE can provide 3D mapping of the elastic and viscous characteristics of muscles on combination with diffusion imaging. Shear wave elastography compared to MRE, is better as it is low cost, easy to access, reliable at rest and during passive muscle stretching. Ultrasound SWE provides 2D mapping of muscle characteristics and gathers data faster than MRE. In 2D mapping, it does not provide viscous properties which is a limitation to quantify anisotropy of muscle. However, analysis of shear wave propagation in relation to the direction of muscle fiber can help to measure anisotropy in fusiform muscles.[46]

Shear wave speed is found to be associated directly with shear modulus and thus the stiffness of soft tissue. With most shear wave elastography techniques being 2 dimensional, the propagation of shear wave in tissues occurs in 3 dimensions. The 3D SWE facilitates more accurate speed estimation and determination of stiffness. It provides comprehensive evaluation of 3D field of view of target tissue reducing the risk of sampling error that occurs in 2D SWE. Only limitation of 3D SWE is low ultrasound volume rate (limited to tens of Hertz), otherwise it is best suited for evaluation, monitoring and diagnosis of various diseases specifically for liver fibrosis and cirrhosis. The low volume rate occurs due to the low receiving capacity of hardware beam formers. To overcome this challenge, an external vibration based technique based on conventional imaging sequence is employed with a low volume rate in which Nyquist frequency (half of sampling frequency), kept lower than shear wave frequency caused aliasing of detected signal. The aliasing is corrected by phase shifting the signals received from different vibration cycles to reconstruct a non aliased signal. The proposed technique based on external vibration for conventional scanner provided robust 3D shear wave reconstruction. The challenge of low volume rate is overcome by leveraging the periodic nature of the sinusoidal wave by the use of external vibrator.[47]

Efforts are seen to provide individual specific soft tissue characterization at clinical level to reduce the uncertainties that are related to data based on population. Despite of the progress made in research and characterization of biological materials several other factors like body areas of the specific tissue, body positioning during the experiments and its consequence on the mechanical properties, certain physical disabilities and disease factors need to be explored further.[48]

Discussion and Conclusion

Characterization of biological materials has led open the pathways to knowledge that can be used to develop new materials which are in combination with biological materials. Soft tissues are widely studied and are found to have control over their mechanical properties. Their properties are highly variable and poorly defined. But they play a crucial role in the mechanical integrity analysis of the body. So accurate characterization of biological tissues is crucial. The mechanical nature of soft tissues contributes to the studies of tissue engineering. Biggest challenge faced in tissue engineering is the assessment of mechanical characteristics of engineered tissues prior to their clinical trials. Mechanical characteristics of soft tissues have been found to vary with the constituents by which the tissue is made of. Also, the effect of factors like disability, disease, body position on the mechanical characteristics of soft tissues needs to be explored further. Determination of tissue properties has a wider scope of application in areas like regenerative medicine, robot assisted surgery, surgical simulation and tissue engineering. Their properties can also be used in cell regeneration and differentiation to develop new materials for replacement of damaged tissues.

Various uniaxial and biaxial tests are conducted to study the characteristics of soft tissues. Tensile tests and compression tests are the most conventionally used methods but they are destructive in nature. Use of ultrasound technique is also found in literature for material characterization. A correlation between the ultrasound velocities and elastic properties of material is available which can serve as a basis for characterization of biological tissues using ultrasound technique in a non-destructive way. The method is cost effective and time saving in comparison to the conventional tensile and compression tests used for material characterization.

Only a few constitutive models are available that accurately describe the properties of soft tissues and so research has directed the attention to the development of models that describe the mechanical nature of these transverse isotropic materials. Due to the non- linear visco elastic nature of soft tissues, all models require experimental determination of the material functions in a wide range of deformation. Studies done on auxetic behavior limitedly address the root cause and focus more on secondary factors like fiber dispersion and compressibility. Investigations are required for study of tissue behavior inside a poroelastic framework. A standardization of the methods of characterization of soft tissues is required.

Author Contributions

Study conception and design: Radhika Chavan, Nitin Kamble, Chetan Kuthe, Sandeep Sarnobat; Data Collection: Radhika Chavan, Nitin Kamble, Chetan Kuthe; Data analysis and Interpretation of results: Radhika Chavan, Nitin Kamble, Chetan Kuthe, Sandeep Sarnobat; Draft manuscript preparation: Radhika Chavan, Nitin Kamble; All authors reviewed the results and approved the final version of the mansucript.

ORCID iD

Radhika Chavan https://orcid.org/0000-0001-5597-4900

REFERENCES

1. Shuguang L, ElinaSitnikova. Material categorization and material characterization- chapter 3. In: *Representative Volume Elements and Unit Cells. Concepts, Theory, Applications and Implementation.* Duxford: Woodhead Publishing Series in Composites Science and Engineering; 2020:43-65. https://doi.org/10.1016/B978-0-08-102638-0.00003-7
2. da Costa PR, Reis L, Montalvão D, Freitas M. Ultrasonic fatigue cruciform specimens biaxial fatigue VHCF fatigue in-plane. *Int J Fatigue*. 2021;152:106423.
3. Landais C. *Ultrasonics Method for Characterization of Complex Materials and Material Systems: Polymers, Structured Polymers, Soft Tissue and Bones*. Digital Commons at University of Nebraska; 2011:12-92. Accessed January 27, 2024. https://digitalcommons.unl.edu/engmechdiss/20
4. Li P, Guo YB, Shim VPW. A constitutive model for transversely isotropic material with anisotropic hardening. *Int J Solids Struct*. 2018;138:40-49.
5. Seyedkavoosi S, Vilchevskaya E, Sevostianov I. Randomly oriented cracks in a transversely isotropic material. *Int J Solids Struct*. 2018;150:222-229.
6. Seyedkavoosi S, Sevostianov I. Modeling of the overall elastic behavior of a transversely isotropic material reinforced with arbitrarily oriented transversely isotropic platelets. *Mech Mater*. 2019;132:77-85.
7. Fung YC . *Biomechanics: Mechanical Properties of Living Tissues*. Springer; 1993: 321-538.
8. Kovanen V, Suominen H, Heikkinen E. Mechanical properties of fast and slow skeletal muscle with special reference to collagen and endurance training. *J Biomech*. 1984;17:725-735.
9. Franulovic M, Basan R, Prebil I, Trajkovski A, Maronić T. Materials characterization – from metals to soft tissues. *Mater Discov*. 2017;7:30039-30047.
10. Nicolle S, Vezin P, Palierne J-F. A strain-hardening bi-power law for the nonlinear behaviour of biological soft tissues. *J Biomech*. 2010;43:927-932.
11. Capilnasiu A, Hadjicharalambous M, Fovargue D, et al. Magnetic resonance elastography in nonlinear viscoelastic materials under load. *Biomech Model Mechanobiol*. 2019;18:111-135.
12. Egan JM. A constitutive model for the mechanical behaviour of soft connective tissues. *J Biomech*. 1987;20:681-692.
13. Cleary R, Wallace R, Simpson H, Kontorinis G, Lucas M. A longitudinal-torsional mode ultrasonic needle for deep penetration into bone. *Ultrasonics*. 2022;124:106754.
14. Fereidoonnezhad B, O'Connor C, McGarry JP. A new anisotropic soft tissue model for elimination of unphysical auxetic behaviour. *J Biomech*. 2020;111: 110006.
15. Gong XL, Zhang XZ, Zhang PQ. Fabrication and characterization of isotropic magnetorheological elastomers. *Polym Test*. 2005;24:669-676.
16. Zhu Y, Kang G, Kan Q , Yu C. A finite viscoelastic-plastic model for describing the uniaxial ratchetting of soft biological tissues. *J Biomech*. 2014;47: 996-1003.
17. Berridge BR, Van Vleet JF, Eugene H, eds. Cardiac, vascular, and skeletal muscle systems. In: *Haschek and Rousseaux's Handbook of Toxicologic Pathology*. 3rd ed, Volume 3. Academic Press; 2013;1567-1665. https://doi.org/10.1016/B978-0-12-415759-0.00046-7
18. Wheatley BB, Morrow DA, Odegard GM, Kaufman KR, Haut Donahue TL. Skeletal muscle tensile strain dependence: hyperviscoelastic nonlinearity. *J Mech Behav Biomed Mater*. 2016;53:445-454.
19. Schwenninger D, Schumann S, Guttmann J. In vivo characterization of mechanical tissue properties of internal organs using endoscopic microscopy and inverse finite element analysis. *J Biomech*. 2011;44:487-493.
20. Teoh JC, Lee DY, Lee T. The influence of sex, body mass and body mass index on plantar soft-tissue stiffness in healthy people in their 60s. *J Biomech*. 2016;49:3022-3025.
21. Lim KT, Choi WJ. Soft tissue stiffness over the hip increases with age and its implication in hip fracture risk in older adults. *J Biomech*. 2019;93:28-33.
22. Zwirner J, Ondruschka B, Scholze M, Hammer N. Passive load-deformation properties of human temporal muscle. *J Biomech*. 2020;106:109829.
23. Xie JY, Zhang JJ, Xiang W, et al. Effect of microscopic pore structures on ultrasonic velocity in tight sandstone with different fluid saturation. *Pet Sci*. 2022;19:2683-2694.
24. Islam MR, Virag J, Oyen ML. Micromechanical poroelastic and viscoelastic properties of ex-vivo soft tissues. *J Biomech*. 2020;113:11009.
25. Cox MA, Driessen NJ, Boerboom RA, Bouten CV, Baaijens FP. Mechanical characterization of anisotropic planar biological soft tissues using finite indentation: experimental feasibility. *J Biomech*. 2008;41:422-429.
26. Christ GJ, Passipieri JA, et al. Soft tissue reconstruction: skeletal muscle engineering. In: *Stem Cell Biology and Tissue Engineering in Dental Sciences*. 2015;567-592. https://doi.org/10.1016/B978-0-12-397157-9.00047-3
27. Kang JS, Navindaran K, Phillips J, Kenny K, Moon KS. Characterization of mechanical properties of soft tissues using sub-microscale tensile testing and 3D-printed sample holder. *J Mech Behav Biomed Mater*. 2023;138:105581.
28. Takaza M, Moerman KM, Gindre J, Lyons G, Simms CK. The anisotropic mechanical behaviour of passive skeletal muscle tissue subjected to large tensile strain. *J Mech Behav Biomed Mater*. 2013;17:209-220.
29. Navindaran K, Kang JS, Moon K. Techniques for characterizing mechanical properties of soft tissues. *J Mech Behav Biomed Mater*. 2023;138:105575.
30. Nagayama K, Ohata S, Obata S, Sato A. Macroscopic and microscopic analysis of the mechanical properties and adhesion force of cells using a single cell tensile test and atomic force microscopy: remarkable differences in cell types. *J Mech Behav Biomed Mater*. 2020;110:103935.

31. Matsumoto T, Nagayama K. Tensile properties of vascular smooth muscle cells: bridging vascular and cellular biomechanics. *J Biomech*. 2012;45:745-755.
32. Chandrasekar M, Jusuarockiam N, et al. Role of characterization techniques in evaluating the material properties of nano particle based polymer materials. In: *Polymer Nano-Composite Based Smart Materials*. From Synthesis to Application. Woodhead Publishing Series in Composites Science and Engineering; 2020:21-39. https://doi.org/10.1016/B978-0-08-103013-4.00002-9
33. Snitka V, Ulcinas A, Mizariene V. Characterization of materials' nanomechanical properties by force modulation and phase imaging atomic force microscopy with soft cantilevers. *Mater Charact*. 2002;48:147-152.
34. Carson WC, Gerling GJ, Krupski TL, et al. Material characterization of ex vivo prostate tissue via spherical indentation in the clinic. *Med Eng Phys*. 2011;33:302-309.
35. Teng Z, Trabelsi O, Ochoa I, et al. Anisotropic material behaviours of soft tissues in human trachea: an experimental study. *J Biomech*. 2012;45:1717-1723.
36. Weickenmeier J, Jabareen M, Mazza E. Suction based mechanical characterization of superficial facial soft tissues. *J Biomech*. 2015;48:4279-4286.
37. Mohammadkhah M, Murphy P, Simms CK. The in vitro passive elastic response of chicken pectoralis muscle to applied tensile and compressive deformation. *J Mech Behav Biomed Mater*. 2016;62:468-480.
38. Takaza M, Cooney GM, McManus G, Stafford P, Simms CK. Assessing the microstructural response to applied deformation in porcine passive skeletal muscle. *J Mech Behav Biomed Mater*. 2014;40:115-126.
39. Böl M, Kohn S, Leichsenring K, Morales-Orcajo E, Ehret AE. On multiscale tension-compression asymmetry in skeletal muscle. *Acta Biomater*. 2022;144:210-220.
40. Kohn S, Leichsenring K, Kuravi R, Ehret AE, Böl M. Direct measurement of the direction-dependent mechanical behaviour of skeletal muscle extracellular matrix. *Acta Biomater*. 2021;122:249-262.
41. Böl M, Iyer R, Garcés-Schröder M, Kohn S, Dietzel A. Mechano-geometrical skeletal muscle fibre characterisation under cyclic and relaxation loading. *J Mech Behav Biomed Mater*. 2020;110:104001.
42. Jia J, Chong S, Yu L, Yao Y. Cell membrane tensile strain under cyclic compression: a viscoelastic myoblast finite element model. *Med Novel Technol Devices*. 2022;16:100155.
43. Tamura A, Hongu JI, Matsumoto T. Theoretical elastic tensile behavior of muscle fiber bundles in traumatic loading events. *Clin Biomech*. 2019;69:184-190.
44. Kumar A, Jayakumar T, Raj B, Ray KK. Correlation between ultrasonic shear wave velocity and Poisson's ratio for isotropic solid materials. *Acta Mater*. 2003;51:2417-2426.
45. Pandey DK, Pandey S. Ultrasonic – a technique of material characterization. In: Dissanayake DW ed. *Acoustic waves*. Publisher Sciyo; 2010:397-427.
46. Bilston LE, Bolsterlee B, Nordez A, Sinha S. Contemporary image-based methods for measuring passive mechanical properties of skeletal muscles in vivo. *J Appl Physiol*. 2019;126:1454-1464.
47. Huang C, Song P, Mellema DC, et al. Three-dimensional shear wave elastography on conventional ultrasound scanners with external vibration. *Phys Med Biol*. 2020;65:215009.
48. Arnold N, Scott J, Bush TR. A review of the characterizations of soft tissues used in human body modeling: scope, limitations, and the path forward. *J Tissue Viability*. 2023;32:286-304.