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**SOFT TISSUE MECHANICS**

**MEE 514**

**Introduction**

The term **tissue** is used to describe a group of cells found together in the body. The cells within a tissue share a common embryonic origin. Microscopic observation reveals that the cells in a tissue share morphological features and are arranged in an orderly pattern that achieves the tissue’s functions. From the evolutionary perspective, tissues appear in more complex organisms. For example, multicellular protists, ancient eukaryotes, do not have cells organized into tissues. Soft tissue is the connective tissue just under your skin that connects the muscles, bones, nerves and blood vessels of the body.  It is also referred to as “fascia”. Any solid component of a living organism, from bones to cells, may be considered as a living tissue. Soft tissues are distinguished from other tissues like bones, for their flexibility and their soft mechanical properties. Soft tissues include connective tissues, muscles, organs and the brain. The functions of soft tissues in an organism also greatly differ from that of hard tissues. Bones build the rigid skeletal structure of the body, cartilage to lubricating the articulations, skeletal muscles to producing strength and to moving the skeleton through the tendons, and organs and brain play physiological functions to maintain and control the organism.

**Soft Tissue in The Human Body**

Soft tissue is a broad term often used for mesenchymal tissue that support and surround more well-defined organs and specific tissues. The cells and structures of soft tissue are present throughout the human body. The major cell types of soft tissues are non-epithelial and of mesodermal origin, denoted as "mesenchymal cells". In the tissue dictionary, soft tissue is exemplified by ***cartilage*** (from the bronchus), ***fibroblasts*** (from the gallbladder), ***adipocytes*** (from gallbladder and rectum), ***ganglion*** (from the rectum) and ***peripheral*** ***nerve*** (from the breast). Other main types of soft tissue include muscle tissue (smooth and striated muscle), which is described separately [1].

***Chondrocytes*** are the main cell type in cartilage. Cartilage, similar to bone, is a specialized form of connective tissue. Cartilage is composed of a non-vascularized extracellular matrix of collagen fibers embedded in a gel-like proteoglycan matrix. Mature chondrocytes synthesize and secrete extracellular matrix, which separates the cells from each other and result in the appearance of isolated chondrocytes surrounded by a ***lacuna***.

***Fibroblasts*** are the main and prototypic mesenchymal cell type that produces collagen fibers. Collagen, which exists in various subtypes, is the main component of connective tissue. Fibroblasts are slender, elongated cells with indistinct cytoplasmic borders and bipolar oval nuclei. Fibroblasts are sparsely distributed in between collagen fibers that support various epithelial tissues and other organs. With the exception of brain tissue, fibroblastic cell types can be found throughout the human body.

***Adipocytes*** are the main cell type in adipose tissue (fat). Adipose tissue is typically homogeneous and finely divided by faint septa. Adipose tissue is spread throughout the body and surrounds most organs and tissues in the human body. In the skin, underlying adipose tissue forms the subcutis as an integral component of the skin. Microscopically adipose tissue is mainly composed of ill-defined lobules of adipocytes surrounded by thin bands of collagen and small blood vessels. The cytoplasm of the adipocyte is compressed at the perimeter of the cell as it is displaced by a single lipid vacuole and only a thin rim of cell membrane is evident in the microscopic image. Adipocytes contain a small, thin and oval nucleus located peripheral to the dominating lipid vacuole, whereas nuclei of capillary endothelial cells are present at intersections between multiple adipocytes.

***Ganglion*** cells are peripheral neurons (nerve cells) that form groups of cells denoted as a ganglion. A ganglion provides a relay and connection point for nerves in the peripheral nervous system. Ganglia are present at various locations throughout the human body. In the gastrointestinal tract they are involved in transmitting signals coordinating bowel movement. Ganglia can often be visualized in between the two layers of smooth muscle surrounding the intestinal mucosa.

***Peripheral*** ***nerves*** are branching extensions of the peripheral nervous system. Large nerves originate from the spinal cord and through smaller and smaller branches end up at distal locations in various tissues throughout the body. Peripheral nerves appear as white fascicles bound together by connective tissue. Microscopically, transverse sections of a peripheral nerve show how endoneurial compartments containing axons and ***Schwann*** ***cells*** are surrounded by perineurium to form individual fascicles, which are embedded in epineural fibrous tissue. Most peripheral nerves contain a mixture of myelin and unmyelinated nerve fibers. Myelin is produced by Schwann cells, which account for 90% of nuclei found in peripheral nerves.

**Basic Structural Elements of STM And Collagen as A Fibrous Protein**

Collagen is a protein which is a major constituent of the extracellular matrix of connective tissue. It is the main load carrying element in a wide variety of soft tissues and is very important to human physiology (for example, the collagen content of (human) achilles tendon is about 20 times that of elastin). Collagen is a macromolecule with length of about 280 nm. **Collagen** is a fibrous protein consisting of three polypeptide chains wound around each other. Each of the three chains is a coil itself. Hydrogen bonds form between these coils, which are around 1000 amino acids in length, which gives the structure strength [2]. This is important given collagen’s role, as structural protein. This strength is increased by the fact that collagen molecules form further chains with other collagen molecules and form **Covalent Cross Links** with each other, which are staggered along the molecules to further increase stability. Collagen molecules are linked to each other by covalent bonds building collagen fibrils. Collagen has many functions:

* Form the structure of bones
* Makes up cartilage and connective tissue
* Prevents blood that is being pumped at high pressure from bursting the walls of arteries
* Is the main component of tendons, which connect skeletal muscles to bones

Depending on the primary function and the requirement of strength of the tissue the diameter of collagen fibrils varies (the order of magnitude is 1.5 nm [17]). In the structure of tendons and ligaments, for example, collagen appears as parallel oriented fibers, while many other tissues have an intricate disordered network of collagen fibers embedded in a gelatinous matrix of proteoglycans.

The defining feature of collagen is an elegant structural motif in which three parallel polypeptide strands in a left-handed, polyproline II-type (PPII) helical conformation coil about each other with a one-residue stagger to form a right-handed triple helix .The tight packing of PPII helices within the triple helix mandates that every third residue be Gly, resulting in a repeating XaaYaaGly sequence, where Xaa and Yaa can be any amino acid. This repeat occurs in all types of collagen, although it is disrupted at certain locations within the triple-helical domain of nonfibrillar collagens. The amino acids in the Xaa and Yaa positions of collagen are often (2S)-proline (Pro, 28%) and (2S,4R)-4-hydroxyproline (Hyp, 38%), respectively. ProHypGly is the most common triplet (10.5%) in collagen. In animals, individual collagen triple helices, known as tropocollagen (TC), assemble in a complex, hierarchical manner that ultimately leads to the macroscopic fibers and networks observed in tissue, bone, and basement membranes.

**Stress-Strain Relationship in Collagen Biomaterials**

Collagen is the main structural and load-bearing element of various connective tissues, where it forms the extracellular matrix that supports cells. It has long been known that collagenous tissues exhibit a highly nonlinear stress–strain relationship, although the origins of this nonlinearity remain unknown. Stress and strain measurements are employed in biomaterials, using mechanical methods, in order to determine other mechanical properties of the materials. The extension (strain) exhibited by a given material is measured upon the application of a force (stress), resulting in the collection of a stress–strain curve from which physical properties can be determined [3].

A typical stress–strain curve is J-shaped, where the initial “softer” response is due to entropic elastic deformation and the latter stiffer response correlates with molecular deformation (changes in internal energy), which is followed by plastic deformation and rupture of the material [4]. The typical curves for collagen exhibit a classic J-shape with a small entropic response (typically below strains of about 3%) and a substantial molecular stretching component, which adopts linearity at higher levels of strain. The mechanical function of biological fiber networks is essentially two-fold: (i) at the subcellular (actin, spectrin) and supracellular (collagen, fibrin) scales, the material offers little resistance and high sensitivity to small deformations, allowing it to be easily remodeled locally; (ii) at larger strains it stiffens strongly to ensure cell and tissue integrity [5]. The non-linear stiffening, while observed in many biological systems  is not fully understood yet, with theories focusing on one of two broad mechanisms: (i) microstructural nonlinearities of individual filaments , and (ii) collective non-affine deformations of multiple filaments . To unravel the relative importance of these mechanisms, a range of experimental tools have been developed to quantify the network's mechanical non-linearity in systematic ways and relate the material micro-structure (network density and morphology, fiber behavior) to the mesoscopic stress-strain laws. These tools fall into two broad categories: simple shear in cone-plate or parallel plate geometries, and uniaxial/biaxial stretch.

Simple shear deformations are commonly used to study purified protein networks. This technique requires low sample volumes and provides a consistent set of experimental tools and generic protocols to probe the visco-elastic properties of soft gels in both the small-strain (linear) and large-strain (non-linear) regimes, and in addition, normal stresses can be measured. Recent data collected by Janmey et al. [6] show in particular that sheared biopolymers exert negative normal forces, a fact that is in contradiction with the hyperelastic behavior of other well studied elastomers. The broad availability of experimental data in that geometry has encouraged a large number of related theoretical and numerical studies, focused primarily on the linear response of the material. However, since simple shear rheology assumes that the material undergoes purely isochoric deformations in the limit of small strains, it only allows for partial exploration of material behavior. In particular, these experiments do not allow one to study completely the non-linear regime (strain typically larger than 10%) that is most relevant in many biological situations (single cell or tissue deformation). And furthermore, it does not allow for a probe of the dilatational rheology of the networks.

In contrast, at mesoscopic scales, uniaxial and biaxial testing are most common for tissue mechanical characterization [5] and have been used to study reconstituted collagen networks [7], the simplest tissue equivalents [7]. In contrast with simple shear, uniaxial stretch generically leads to non-isochoric deformations, and hence allows one to measure quantities such as the material Poisson ratio which can have values as large as  for strongly deformed collagen gels. These values arise in highly anisotropic materials, as reported for instance for solid foams [8], and it is somewhat surprising to see similar behavior in in vitro collagen gels which display little or no anisotropy in their undeformed state.

**Cartilage and Its Applications in Articulating Joints**

Articular cartilage is a thin layer of specialized connective tissue with unique viscoelastic properties. Its principal function is to provide a smooth, lubricated surface for low friction articulation and to facilitate the transmission of loads to the underlying subchondral bone. Articular cartilage is unique in its ability to withstand high cyclic loads, demonstrating little or no evidence of damage or degenerative change.

The biomechanical behavior of articular cartilage is best understood when the tissue is viewed as a biphasic medium. Articular cartilage consists of 2 phases: a fluid phase and a solid phase. Water is the principal component of the fluid phase, contributing up to 80% of the wet weight of the tissue. Inorganic ions such as sodium, calcium, chloride, and potassium are also found in the fluid phase. The solid phase is characterized by the ECM, which is porous and permeable. The relationship between proteoglycan aggregates and interstitial fluid provides compressive resilience to cartilage through negative electrostatic repulsion forces.

The initial and rapid application of articular contact forces during joint loading causes an immediate increase in interstitial fluid pressure. This local increase in pressure causes the fluid to flow out of the ECM, generating a large frictional drag on the matrix. When the compressive load is removed, interstitial fluid flows back into the tissue. The low permeability of articular cartilage prevents fluid from being quickly squeezed out of the matrix. The 2 opposing bones and surrounding cartilage confine the cartilage under the contact surface. These boundaries are designed to restrict mechanical deformation.

Articular cartilage is viscoelastic and exhibits time-dependent behavior when subjected to a constant load or deformation. Two types of mechanisms are responsible for viscoelasticity in articular cartilage: flow dependent and flow independent. The flow-dependent mechanism depends on interstitial fluid and the frictional drag associated with this flow. The drag resulting from the interstitial fluid is known as biphasic viscoelastic behavior. The flow-independent component of viscoelasticity is caused by macromolecular motion—specifically, the intrinsic viscoelastic behavior of the collagen-proteoglycan matrix. As a result, the fluid pressure provides a significant component of total load support, thereby reducing the stress acting upon the solid matrix.

Articular cartilage also exhibits a creep and stress-relaxation response. When a constant compressive stress is applied to the tissue, its deformation increases with time, and it will deform or creep until an equilibrium value is reached. Similarly, when cartilage is deformed and held at a constant strain, the stress will rise to a peak, which will be followed by a slow stress-relaxation process until an equilibrium value is reached. Because articular cartilage tends to stiffen with increased strain, it cannot be described by a single Young’s modulus. Rather, the modulus of the tissue depends on the time at which the force measurement was taken during a stress-relaxation test, which was common practice in the preliminary studies of mechanical testing on articular cartilage. The current method is to apply a known strain, which is immediately followed by a peak in measured force and a slow stress-relaxation process; the force/stress value is recorded when it has reached equilibrium. This process is repeated across a range of strain values, and the equilibrium modulus is calculated as the slope of the stress-strain curve.

The complex composition and organization of cartilage through the middle zones of cartilage contributes significantly to its shear-resistant properties. Stretching of the randomly distributed collagen fibrils provides cartilage with its shear stress response. The tensile force-resisting properties derive from the precise molecular arrangement of collagen fibrils. The stabilization and ultimate tensile strength of the collagen fiber are thought to result from the intra- and intermolecular cross-links

Cartilage is a group of tissues produced by chondrocytes that is characterized by a relative lack of vascularity and consists of cells surrounded by a specialized extracellular matrix composed predominantly of type II collagen and proteoglycan [9] Cartilage is a supporting connective tissue that, together with the bone, forms the framework supporting the body as a whole. A **joint**, also called an **articulation**, is any place where adjacent bones or bone and cartilage come together (articulate with each other) to form a connection. Joints are classified both structurally and functionally. Structural classifications of joints take into account whether the adjacent bones are strongly anchored to each other by fibrous connective tissue or cartilage, or whether the adjacent bones articulate with each other within a fluid-filled space called a **joint cavity**. Functional classifications describe the degree of movement available between the bones, ranging from immobile, to slightly mobile, to freely moveable joints. The amount of movement available at a particular joint of the body is related to the functional requirements for that joint. Thus immobile or slightly moveable joints serve to protect internal organs, give stability to the body, and allow for limited body movement. In contrast, freely moveable joints allow for much more extensive movements of the body and limbs.

**Mechanical Testing Procedures for Soft Tissues**

Mechanical testing regimes can be used to establish the compressive, tensile, bending, or shear properties of a tissue. Skin is a highly anisotropic, viscoelastic, and nearly incompressible material. Commonly excised skin is tested using uniaxial tensile methodologies, where a suitably shaped strip of skin is gripped at both ends and stretched while the load and extension are recorded [5].

Since the major component of all soft tissues is interstitial water, the mechanical response of cartilage is strongly related to the flow of fluid through the tissue. Soft tissues such as cartilage have been traditionally tested using compression testing. The methods for testing in compression are quite varied, with confined, unconfined, and indentation being the most prevalent. Within confined compression, a cartilage sample is placed in an impervious, fluid-filled well and loaded through a porous plate. Since the well is non-porous, flow though the cartilage is in the vertical direction. In unconfined compression, the cartilage is loaded using a non-porous plate onto a non-porous chamber, forcing the fluid flow to be predominantly radia. Indentation is the most frequently used method for evaluating the biomechanical properties of cartilage. It consists of an indenter, smaller than the surface of the specimen being tested, that is brought down onto the specimen. Indentation has many advantages over other methods of compression, including the fact that indentation can be performed in situ, enabling the test to be more physiological [7].

To understand the compressive and tensile properties of a tissue, the Young's elastic modulus is typically calculated by analyzing the linear portion of the stress-strain curve, indicating the elastic resistance to compression or tension, irrespective of specimen size. Both tensile and compressive testing regimes can vary according to the load or deformation applied and the rate of both such parameters. At present, there are many different testing protocols to assess tissue mechanics, which makes it extremely difficult to interpret or compare results from different studies.Furthermore, many mechanical methods currently focus on characterizing the mechanical properties of the tissue by testing the specimen to destruction.

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