Extract from:

The stiffness of living tissues and its implications for tissue engineering

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The concept of stiffness

The mechanical properties of a material — most notably its stiffness — relate to loads and deformations; that is, the forces exerted on the material and the resulting changes in its shape. To fully understand the stiffness of living tissues, one has first to explore these underlying concepts. The stiffness of a structure derives from the following two premises: first, that when a structure is exposed to a certain load, it will deform; and second, that the ratio between this load and the consequent deformation yields the stiffness of the structure, meaning how much load is necessary to achieve a certain deformation[6]. Although there is no absolute definition of what constitutes a 'stiff' or 'soft' material, rubber and foodstuffs are generally considered soft materials, wood and plastics are of intermediate stiffness, and steel is among the stiffest commonly encountered materials. Clearly, the load necessary to deform a stiff material, such as steel, will be far higher than that required to similarly deform a softer material, such as wood or rubber.

The stiffness of materials commonly used in manufacturing is far higher than that of human tissues or organs, including materials used specifically because of their deformability, such as the plastics used in ketchup bottles. The vast majority of industrial plastics (such as polypropylene, nylon or polyethylene) have elastic moduli in the 0.2–5.0 GPa range, higher than for all biological tissues except bone. By contrast, the stiffness of some foodstuffs is comparable to that of tissues: the elastic moduli of panna cotta range from 100 Pa to 1 kPa (REFS [7,8]), the elastic moduli of gummy bears and bananas are between 50 and 100 kPa, an apple has an elastic modulus of about 1 MPa and a carrot has an elastic modulus of around 7 MPa (REFS [9,10]). Similarly, from a biological perspective, fat is clearly far softer than muscle tissue, which itself is far softer than bone. Naturally, the stiffness of a tissue can be quantified and precisely analysed but, despite the term being widely mentioned in the biomedical field, analyses of tissue stiffness are sometimes insufficiently detailed.

Stiffness is a general structural property that depends not only on the material itself but also on its amount and distribution (shape). For example, the hollow nature of bones confers an increased stiffness-to-weight ratio. During compression or extension (stretching) of a material, the whole cross-sectional area of the material equally sustains the stress, whereas during bending or torsion, the material furthest from the midpoint or centre line sustains most of the stress. For this reason, structural construction elements often have T-shaped or L-shaped cross-sectional shapes, which maximize their stiffness while minimizing the weight and amount of material used. Moduli and stiffness are intimately related concepts and therefore these terms are frequently used as synonyms. However, stiffness is a property of a structure, whereas moduli describe the properties of the material composing that structure. As such, various moduli relating to the intrinsic elastic properties of materials[5] are reflected in the stiffness of the final structure. These moduli are derived from mathematical conversions of load versus deformation relationships obtained from standardized tests on samples of a standardized size and shape (BOX 1).

For example, Young's modulus (E) can be calculated by subjecting a material to uniaxial stress resulting from compression or extension and measuring elastic (that is, reversible) deformation (strain) in the linear region of the stress–strain curve:

$$E \equiv \frac{\sigma(\varepsilon)}{\varepsilon} = \frac{F/A}{\Delta L/L_0} = \frac{FL_0}{A\Delta L}, \quad (1)$$

where σ is uniaxial stress (force per unit surface), ε is strain, F is the force exerted on an object (uniaxial stress), A is the cross-sectional area perpendicular to the applied force, ΔL is the amount by which the length of the object changes (ΔL has a positive value for a stretched material and a negative value for a compressed material) and L_0 is the original length of the object. Thus, the axial stiffness (k) of a longitudinal structure such as a beam can be calculated as

$$k = \frac{EA}{L_0}. \quad (2)$$

Equations 1 and 2 assume a linear relationship between strain and stress (that is, Hooke's law). In real-life scenarios this assumption might not hold for all levels of

Box 1 | Standards in mechanical testing

Standardized protocols must be followed in mechanical analyses if the data so obtained are to be interpreted with a good level of confidence and are to be comparable between different investigational studies. Accordingly, in the past decade, there has been a great effort to introduce standardized methods for the analysis of tissue-engineered products, such as the ASTM standards developed by ASTM Committee F04 and related subcommittees, which aim to improve the safety, quality and consistency of these products. Although these standards were not specifically developed for the purpose of performing mechanical tests on human tissues, they can provide guidance for appropriate testing. For example, ASTM F561-19 covers recommendations for the handling and analysis of post-mortem tissue and living tissue samples surgically removed from humans and animals, whereas ASTM F2150-13 provides guidance on the selection of appropriate test methods for analysing the bulk physical, chemical, mechanical and surface properties of such samples. ASTM D638-14, a standard for testing polymeric materials under uniaxial extension, states that it might not be meaningful to compare the results of tests performed with widely different loads or over widely different timescales. Although biological systems differ substantially from the plastics referred to in ASTM D638-14, when taking a cautious approach, we might reasonably extend these concepts to human tissues. Overall, however, the tissue engineering field seems to be reluctant to adopt similar standards, or even to attempt to follow existing standards. In most published reports, mechanical tests were either not performed according to any consensus standard, or if they were, the standard followed is rarely referenced.

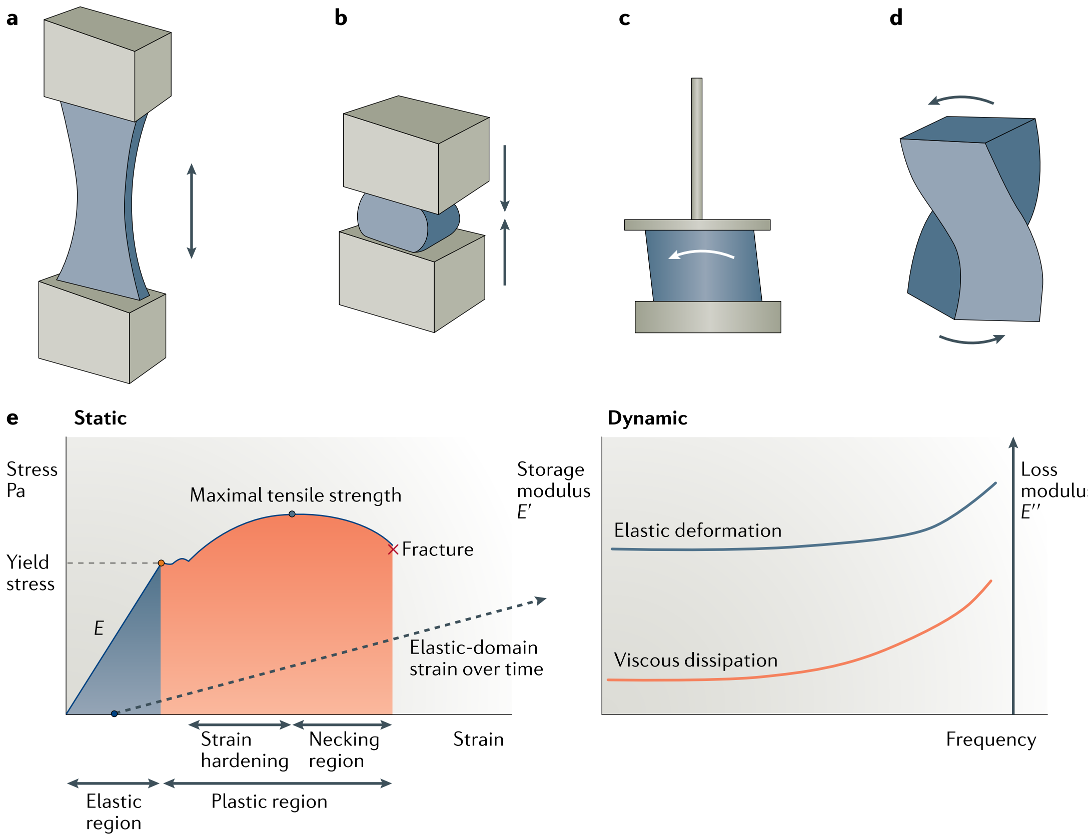

Fig. 1 | Main mechanical deformations and representative curves. The main types of mechanical analysis include tensile (part a), compressive (part b), shear (part c) and torsion (part d) deformations. e | Typically, static deformations (such as tensile or strain deformations) yield a complex curve within which distinct regions can be identified, each of which has important mechanical correlates. Young's modulus (E) is calculated as the slope of the stress-versus-strain curve in the linear (elastic) region. With higher levels of strain, the material enters the plastic domain of the curve, where deformations are no longer reversible and the material deforms permanently until fracture occurs. Alternatively, a dynamic analysis can be performed, in which a strain within the linear (viscoelastic) region is applied repeatedly over time, in cycles often with changes in frequency or temperature, to yield storage (E') and loss (E'') moduli. The storage modulus is related to elastic deformation of the material, whereas the loss modulus represents the energy dissipated by internal structural rearrangements.

strain, particularly for polymers. The proportional limit represents the maximum stress at which stress and strain are proportional, and varies for different materials. Below this limit, the chemical bonds between atoms in the material stretch when under load but will recover completely when the load is released. Above this limit, the bonds will break and slip past each other, leading to non-proportionality. Therefore, the elastic modulus is typically measured at low strain values (0.2%)[4,11].

Young's modulus is one of the most common measures of intrinsic material stiffness. Because it is independent of structure, Young's modulus is widely used to characterize the stiffness of both manufactured materials and tissues (FIG. 1a,b). However, in addition to uniaxial stress, biological tissues might also be subjected to deformations resulting from shear forces (FIG. 1c). The shear modulus (G) is calculated similarly to Young's modulus in that stress (force per unit area) is divided by strain. However, whereas for Young's modulus stress and strain are both normal to the cross-sectional area, for the shear modulus they are parallel and associated with an angular change. In isotropic materials, E and G are related to each other through Poisson's ratio (ν), given by the following equation:

$$E = 2G(1 + \nu). \qquad (3)$$

For many common materials, Poisson's ratio is similar to that of incompressible rubber ($\nu = 0.5$); thus, E is frequently approximated to $3G$. Together, the shear and elastic moduli represent the properties of materials under two different types of load, which might suffice to give a general idea of the rigidity of various biological tissues. However, different loads can be simultaneously applied to the same tissue: for example, cartilaginous tissues are routinely subjected to both compressive and shear deformations[12]. Furthermore, such complex loads can cause materials to deform in multiple ways, such as torsion and bending (FIG. 1d). In the case of bending, one side of the sample is subjected to compressive stress and the opposite side is subjected to tensile stress. The mineralized constituent of bone, for example, as for other brittle materials, is highly resistant to compressive stress but not so much to tensile forces; consequently, bending can result in fracture. As such, it is important to consider different types of stress even in the same tissue. The most relevant types of stress in tissue engineering are those that are similar to physiological loads.

Furthermore, it is important to outline that some of these mechanical relationships do not apply directly to most biological tissues. Equation 3, for example, applies only to isotropic materials but is frequently erroneously used for anisotropic materials — those with mechanical characteristics that differ according to the direction of measurement. For example, biological tissues have very complex, anisotropic structures in which many types of matter are present and unevenly distributed; they differ greatly from the typically isotropic, continuum structures used in manufacturing (in which few types of matter are present and are continuously distributed in the containing space). As such, even though it is useful to discuss the relationships that exist between mechanical concepts at this simple level, these will, in practice, be affected by length scale, anisotropy, spatial variations, non-linear behaviour and other characteristics of biological tissues, discussed further in this Review.

Viscoelasticity is another important point to discuss, as the natural response of all solid materials to stress is not purely elastic but also has a viscous component, which is also the case with living tissues. When elastic and viscous components are both prominent in defining the mechanical behaviour of a material, the material is generally referred to as 'viscoelastic'. This type of material can be fully characterized only by time-dependent tests, which apply strain and measure the required force as a function of time (stress relaxation) or apply stress and measure strain as induced changes in shape (creep) as a function of time, or through dynamic mechanical analysis[13]. In dynamic mechanical analysis, loads are applied and released cyclically, often at differing frequencies or temperatures, which facilitates measurement of the viscoelastic response of a material during faster deformations than those derived by creep and stress relaxation tests.

The viscoelastic response of a material is used to derive the dynamic or complex modulus, which is usually represented by storage and loss moduli. For uniaxial forces, the storage modulus (E) represents the elastic, instantaneous and reversible response of the material: deformation or stretching of chemical bonds while under load stores energy that is released by unloading. The loss modulus (E) represents the viscous time-dependent response of the material and is related to irreversible rearrangements and remodelling of their internal structure, such as the slippage of polymer chains past each other. Similarly, for deformations resulting from shear forces, the shear storage modulus (G) and the shear loss modulus (G)[14] are frequently evaluated by rheology and oscillatory experiments (TABLE 1). As biological tissues generally have viscoelastic responses, these tests are extremely relevant in the biomechanical field.

Table 1 | Commonly used techniques for the mechanical characterization of living tissues

Technique	Concept	Modulus	Sample	Dimension	ASTM standard test methods [a]	Refs
Tensile deformation	Classic stress–strain analysis. Uniaxial stress is applied to stretch the material and a relationship is established with the resulting strain	E (elastic)	Mostly ex vivo tissue	Macroscale	D412, D638, D882, D1623, D1708, D3039, D3039M	220
Compressive deformation	Classic stress–strain analysis. Uniaxial stress is applied to compress the material and a relationship is established with the resulting strain; the compressor is as the same size as or larger than the sample	E (elastic)	Ex vivo tissue	Macroscale	D695, D1621	221
Dynamic mechanical analysis	Cycles of tensile and compressive deformation	E , E (viscoelastic)	Ex vivo tissue	Macroscale	D5024, D5026	222
Shear rheometry	Application of small-amplitude oscillatory shear stress and quantification of the resulting strain	G , G (shear, viscoelastic)	Ex vivo tissue	Macroscale	D5279	223,224
Pipette or micropipette aspiration	Establishment of the relationship between the pressure of aspiration and the aspirated volume of the sample	E (elastic)	Ex vivo tissue	Macroscale or microscale	NA	225,226
Indentation	Indentation of the tissue with a probe of defined geometry and calculation of the relationship between indentation depth and probe load (the probe must be smaller than the sample)	E (elastic)	Mostly ex vivo tissue	Nanoscale to macroscale	E2546-15	105,227
Atomic force microscopy	Atomic-level indentation (nanoindentation) or shear rheology (atomic force microscopy-based rheology)	E (indentation), G , G (shear)	Dry or wet ex vivo tissue	Microscale, nanoscale	NA	5,141,228
Magnetic resonance elastography	Magnetic resonance visualization of tissue deformation resulting from the introduction of shear waves into the tissue derived from external vibrations	G , G (shear, viscoelastic)	In vivo tissue	Macroscale (millimetre)	NA	229,230
Ultrasonic shear wave elastography	Ultrasonic pulses produce shear waves through the tissue; the velocity of these waves is measured and used to derive the tissue's Young's modulus	E (elastic)	In vivo tissue	Macroscale (millimetre)	NA	231

NA, not available. [a]Additional test methods are suggested by ASTM F2150-13 where applicable; sample preparation and conditioning guidelines are provided by ASTM F1634 and ASTM F163.

Timescale

Some materials exhibit different responses when deformed at different speeds. For example, the toy Silly Putty is made of a silicone blend that exhibits a strong elastic response at high rates of deformation and a strong viscous or liquid-like response at low rates of deformation[15]. Mechanical tests need to take into consideration the potential for time dependency. A frequency sweep, for example, is commonly performed in dynamic tests to evaluate the response of the sample to different speeds of deformation. During these tests, the storage modulus typically increases with rising deformation frequency; that is, the elastic response of these materials increases with the speed of deformation. Thus, deformation speed can play a crucial part in defining the response of a material under load[16,17]. Particularly slow speeds of deformation can approximate a static system. Some mechanical tests can be performed under either such quasi-static conditions or dynamic conditions[18].

Three important consequences can be derived from these considerations: first, that under quasi-static conditions, the time-dependent behaviour (viscoelasticity) of materials is simplified because the behaviour of the material corresponds to its ‘associated’ elasticity[19,20]; second, that quasistatic testing cannot provide information about the viscous and/or elastic responses that occur under dynamic loads — namely, those resembling physiological deformations; and third, that substantial differences can be expected to occur at distinct load timescales, and as such, direct comparisons between scales are inadvisable (BOX 1).

The stiffness range of living tissues

Not surprisingly, a system as complex as a human organism comprises tissues that span a remarkable spectrum of stiffness; elastic moduli range from the 11 Pa of intestinal mucus[103] to the 20 GPa of cortical bone[81]. Between these extremes, almost all orders of magnitude are represented by a distinct tissue (FIG. 2).

Neural tissues are among the softest of the human body[104], which should be expected considering their anatomical protection and how easily they can be damaged. These are followed by most abdominal organs (such as the pancreas, spleen and liver) and muscles, and finally by supportive structures such as cartilage, tendons, ligaments and eventually bone. From the analysis of the

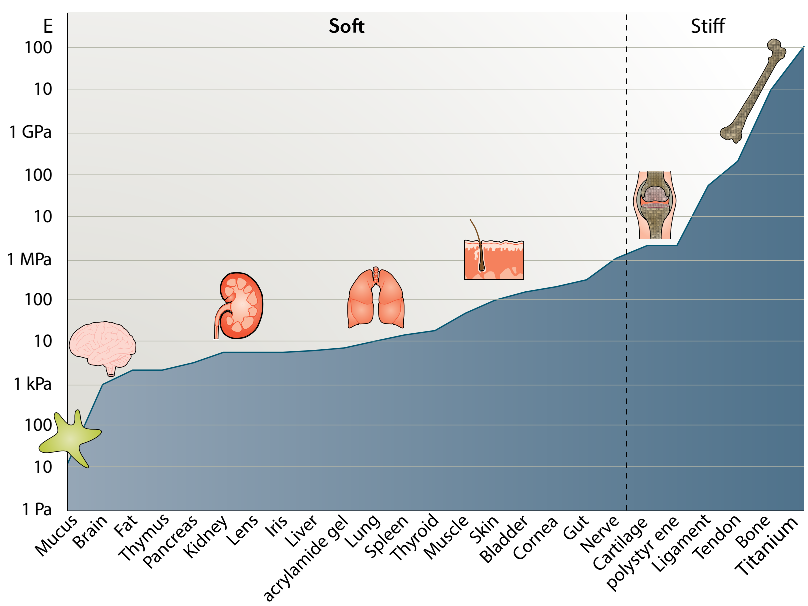

Fig. 2 | The stiffness of living tissues spans a full pascal- to-gigapascal range. The elastic moduli (E) of different tissues as described in the literature are reported on the left (logarithmic) scale. Tissues are organized by increasing crescent moduli. Central nervous system tissues, as well as most abdominal organs and skin, have moduli on the submegapascal level and as such are generally characterized as soft tissues (in biological terms). Cartilage, ligament, tendon and bone are the stiffest tissues of the human body. For comparison purposes, the moduli of some common tissue engineering materials are included in the graph: 8% acrylamide gel[217], tissue culture polystyrene[218] and titanium (used for dental and bone implants)[219]. Tissue culture polystyrene, the standard cell-culture substrate, is clearly stiffer than almost all biological tissues, and this substrate stiffness is an important source of error in mechanical analyses derived from in vitro studies. The reported moduli are derived from studies across different animal models and types of deformation, although we have prioritized studies using human tissues and physiological-like deformations (mostly at macroscale dimensions).