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Indentation Versus Tensile Measurements of Young's Modulus for Soft Biological Tissues

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In this review, we compare the reported values of Young's modulus (YM) obtained from indentation and tensile deformations of soft biological tissues. When the method of deformation is ignored, YM values for any given tissue typically span several orders of magnitude. If the method of deformation is considered, then a consistent and less ambiguous result emerges. On average, YM values for soft tissues are consistently lower when obtained by indentation deformations. We discuss the implications and potential impact of this finding.

Introduction

The rigid skeletal system of vertebrates provides support and protection for soft tissues that reside within and on the skeletal frame. These soft tissues range from large organs to small connective tissues with cells and extracellular matrices. They are continually stressed by a multitude of macro-, micro-, and nanoscopic forces, both internally and externally, and must be resilient enough to deform reversibly without damage and still maintain function. For decades it has been understood that changes in the macroscopic stiffness of tissues can indicate internal disease or injury. We now understand that changes in the bulk compliance of soft tissues can indicate the onset of conditions such as breast cancer,[1–3] atherosclerosis,[4–11] fibrosis,[12] or glaucoma[13] at a macroscopic level. At the micro- to nanoscopic level, bulk and local compliance influence a wide menu of fundamental cell behaviors, including cell morphology,[14–17] proliferation,[16,18–20] motility,[15,21–25] differentiation,[18,26–29] and response to therapeutic agents.[30] It is therefore important to properly characterize the compliant biophysical state of tissues to understand how this biophysical attribute relates to proper function. However, a cursory review of the literature quickly reveals significant differences in reported values of the compliance of soft tissues. These variations can influence the understanding of tissue function and/or failure, interpretation of cellular responses to biophysical stimuli, or the rational design and use of biological simulants.[31–35] The aim of this review is to determine the origins of these variations. We do not explore every confounding variable and material property for a given tissue; rather, the focus of this review is to delineate the extent to which the experimental method for measuring modulus affects the interpretation of a commonly quantified compliant descriptor, Young's modulus (YM), by specifically comparing probe indentation to tensile stretching measurements of soft biological tissues.

YM describes the ability of an elastic material to resist deformation to an applied stress. Unfortunately, reported values of YM for a given tissue can span several orders of magnitude. The human cornea is a good example, with reported modulus values ranging from 2.9 kPa[36] to 19 MPa[37] when measured by atomic force microscopy (AFM)[38] tensile stretching,[39,40] tonometry,[41–43] or inflation/bulge testing,[36,37,44–47] This wide variation in reported YM values is not limited to the cornea. Part of the variation in reported YM values stems from variation in controllable experimental variables. Examples include *in vivo* versus *ex vivo* measurements, tissue hydration state, time from death/tissue excision, temperature, storage medium, and the experimental method used. These experimental differences make direct comparison of results between studies difficult. Direct comparisons are also complicated by the various material properties that can be used to describe compliance. Again, the eye is an excellent example; intermixed with reports on intrinsic material properties such as bulk, shear, or YM are reports on properties such as ocular or scleral rigidity.[48–50] Reliance on empirical values, such as ocular rigidity, can potentially complicate understanding and diagnosis of disease and should therefore be used with caution.

Variations in reported YM values for soft tissues may also stem from the application of elastic models to describe viscoelastic responses. YM is commonly used to try and quantify an intrinsic elastic property of soft, viscoelastic biomaterials. Strictly speaking, however, YM quantifies the response of a perfectly elastic material, limiting its use to metals and crystalline solids or to materials that possess significant regions of linear stress–strain behavior. These

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complications have been discussed in detail[51] and contribute to confusion and variability on reported mechanical properties of soft tissues. As noted by Fung,[51] YM values obtained by tensile measurements must be accompanied by a statement of the levels of stress and strain applied to the tissue to be of any quantitative value. The nonlinear response of soft tissues and the inherent difficulty in obtaining YM values by tensile deformation has also resulted in the formulation of nonlinear stress–strain models[39,51,52] that do not attempt to define YM as the quantitative descriptive parameter that it is. However, given the aim of this review, which attempts to address the effect of experimental method on reported values of elastic modulus, its inclusion in this review is warranted.

For perfectly elastic materials, YM is defined as the ratio of applied stress to resultant strain and has units of measurement in N/m^2.

$$E_{(Pascals)} = \frac{\sigma}{\varepsilon} = \frac{stress}{strain} \tag{1}$$

Stress is the force divided by the area over which it is applied. Strain is a dimensionless quantity defined by the stress-induced change in length of a material divided by its unstressed length ($\Delta L/L$). Soft tissues are not perfectly elastic materials or homogeneous, and they typically display both viscous and elastic properties that are dependent on time and typically display nonlinear stress–strain functions (nevertheless, nonlinear functions do not necessarily define a material as viscoelastic). For perfectly elastic materials a single YM value defines the response of material to deformation. For soft biological tissues, the resistance to deformation typically increases as the applied stress increases. Therefore, YM, defined by Equation 1, is not constant and depends on the specific applied stress, which is particularly important as tissues *in vivo* typically exist in a prestressed state.[53] As the solution to Equation 1 is dependent on the applied stress, multiple YM values could be obtained for soft biological tissues. To avert this problem, soft biological tissues are typically assumed to behave as elastic solids if a significant linear regime of stress-to-strain exists in the limit of small strain response to applied stress.

Two methods are commonly used to deform soft tissues: probe indentation and tensile stretching. Both methods are employed to describe the compliant response of a material to an applied stress. They are, however, distinct. Indentation deformations are maximized at the point of indenter contact and radially diminish to zero with increasing distance from the probe, whereas tensile-induced strains span the bulk of the sample being stressed. If a tissue is not homogeneous from nanoscopic to macroscopic length scales, the measured compliance may depend on the experimental technique employed. This review summarizes previously published results and documents that differences in methods used are major contributors to the wide range of values reported for soft biological tissues. The results highlight that knowledge of the method used to measure YM is essential for correct interpretation of the data.

In this review, only those publications that report elastic modulus values are presented. The nonelastic properties of these viscoelastic tissues are not included here, as these descriptions of tissue mechanics would make the stated purpose of this review impossible. However, the time dependent, viscous properties of biological tissues are important and we direct readers to the following reviews and articles, which discuss these properties for many of the tissues reviewed here.[54–62] In addition, values, such as the tangent modulus, obtained from regions of stress–strain curves that are outside of the elastic regime are not included in this review. Where possible, we compare YM values obtained when both values (by indentation and tensile stretching) for a given tissue have been reported (not all tissues we reviewed have reported values for both methods).

Indentation

There are a variety of instruments used to indent samples,[63–66] ranging from AFMs, capable of applying piconewton loads, to nanoindentors, which can resolve nanonewton loads, to larger industrial indenters capable of applying micro to meganewton loads. No single indenter is ideally suited for every tissue type and ultimately the specific research objective will dictate which instrument is deemed appropriate. However, accurate determination of YM requires the specific apparatus be capable of sensitive detection of the initial point of contact between the indenter and the sample. The instrument must also have high resolution in the subsequent changes in load and indentation depth and fine control of the indentation velocity.

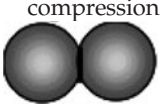
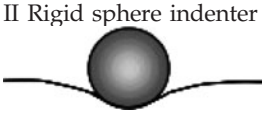
Under the assumption of elastic deformation, YM of a given biological material is typically determined by fitting the measured indentation depth as a function of indenter load during approach.[67–70] YM values can also be obtained from the interpretation of unloading curves when the indenter is being withdrawn from the sample.[71] The models used to fit these indentation curves are indenter geometry specific; therefore, indenters are objects that are, or can be, approximated as spheres, cones, or flat cylinders, as the contact mechanics for these geometries are well established.[72–78] Table 1 lists the elastic model solutions for the common geometries used to determine YM. A full description of the elastic and viscous properties of a tissue would require additional measurement of its frequency-dependent response.[79] It is instructive to generalize the equations listed in Table 1 to the following equation:

$$F = \alpha\delta^m \tag{2}$$

where F is the force applied by the indenter, δ is the indentation depth, α and m are constants where the geometry of the indenter determines the value of m. The value of m is 1 for flat cylinders, 1.5 for spheres, and 2 for cones. Equation 2 is easily linearized (log–log plot), which allows for a quick and easy check to ensure that experimental data fits the correct power law for the indenter geometry used.

Using force versus indentation curves to determine YM can be complicated due to the viscoelastic nature of a given biological sample. It is at times difficult to determine the correct indentation depth over which the sample behaves as an elastic solid. This region must be defined so that YM can be accurately determined. For a perfectly elastic material, no energy is lost to the sample during indentation and both the loading and unloading curves will be coincident. The elastic regime of a viscoelastic material can therefore be experimentally determined by controlling the indentation velocity and depth to produce loading and unloading curves that fall

Table 1. Review of Theoretical Models

Model	*Theoretical* F *versus* δ	*Reference*
I Purely elastic sphere compression 	$F=\left[\frac{16E^{*2}\delta^3}{9\pi}\frac{R_1R_2}{R_1+R_2}\right]^{1/2}$	72,88,110
II Rigid sphere indenter 	$F=\frac{4}{3}\frac{E}{1-v^2}\delta^{3/2}R^{1/2}$	75
III Rigid, flat-ended cylinder indenter 	$F=\frac{2ER\delta}{1-v^2}$	75
IV Rigid cone indenter 	$F=\frac{2}{\pi}\tan\alpha\frac{E}{1-v^2}\delta^2$	74,75
V Rigid cone indenter 	$F=\frac{2}{\pi}\cot\alpha\frac{E}{1-v^2}\delta^2$ INCORRECT	78

Note that the reduced modulus (E^*) is used in Equation I and not in II–V, where it is assumed that the indenter is infinitely rigid: $\frac{1}{E^*}=\frac{1-v_1^2}{E_1}+\frac{1-v_2^2}{E^2}$.

E, Young's modulus; δ, indentation depth; R, radius; ν, Poisson's ratio; α, half angle opening.

on one another. This result is not always possible as adhesions between the probe and sample can occur that exceed either the load capacity or drive range of a given apparatus. This is particularly problematic when using AFMs, which use very weak springs and piezoelectric crystals with small drive ranges to move the indenter into a sample. A more quantitative solution to determine the elastic regime of a viscoelastic material can be obtained by noting that the models in Table 1 predict a constant value of E for any indentation depth. Therefore, the ratio of experimental values of force and indentation can be used to determine the range over which a specific model applies. For example, Figure 1A is a plot of indentation force versus depth on a polyacrylamide hydrogel prepared in our laboratory and indented using an AFM cantilever with an incorporated square pyramid tip. Figure 1B is a plot of $\frac{\pi F(1-v^2)}{2\tan(\alpha)\delta^2}=E$ versus indentation depth, where F is the force, v is Poisson's ratio, α is the half angle opening of the AFM tip and δ is the depth of penetration. In using this equation, we have assumed the square pyramid is a cone. This plot shows that the hydrogel behaved as an elastic solid with a constant E to $\sim$80 nm of indentation. Beyond 80 nm, E is no longer constant, indicating the material is no longer behaving as an elastic solid. When using an AFM, care must be taken to ensure that these deviations from linearity are not due to the common and often ignored, nonlinear response of cantilever deflection versus load for increasing loading conditions.[80]

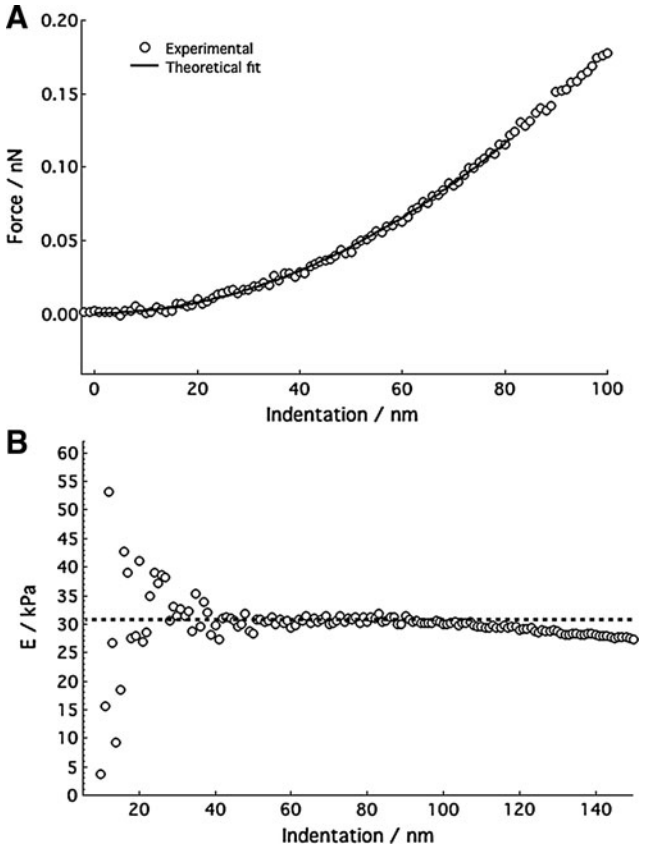


FIG. 1. **(A)** Indentation force versus indentation depth of a polyacrylamide hydrogel with an overlay of the theoretic force based on Equation IV in Table 1. The appropriate indentation depth over which the fit was applied was determined from a plot of $\frac{\pi F(1-v^2)}{2\tan(\alpha)\delta^2}=E$ versus indentation depth, **(B)**, which demonstrates that the gel behaves as an elastic solid up to $\sim$80 nm of indentation.

With very small indentations, the working end of the pyramid has been modeled as a sphere[81] especially when the sharp tip has been made blunt.[82–84] More typically, pyramidal indenters are assumed to be cones, especially in the case of cell indentation studies using AFMs.[67,85] We emphasize the pyramidal indenter here, due to a citation error, which has occurred when referencing the rigid cone solution. The solution of the pyramidal indenter as a rigid cone has been presented in numerous publications. Frequently, Sneddon's 1965 publication[78] is cited for the solution of a rigid cone indenter. However, the rigid cone solution published in Sneddon's 1965 publication is not consistent with his previous publications[75,77] or the solution that preceded it.[74] Although this error has been noted before,[86] research articles continue to improperly cite Sneddon's 1965 article. This obviously becomes a problem when authors refer to Sneddon's 1965 article without also including the equation they used. We therefore recommend that Love[74] or Sneddon[75] be cited when referencing the use of the rigid cone solution.

Tensile stretching

Methods that involve tensile stretching offer a more direct and more economical approach for obtaining the material

properties of a sample. Tests can be as simple as measuring the change in length (strain) of a sample when a mass is suspended from it (stress). Tensile measurements directly quantify the strain that is induced by a given stress and under the assumption of a linear elastic response, YM is determined from the slope of the stress–strain curve (Equation 1).

Typical stress–strain relationships observed for tensile measurements of soft biological tissues demonstrate that the resistance to deformation of the tissue increases with increasing stress. This nonlinear response means that the gradient of stress to strain is always increasing. Thus, the solution for E obtained by Equation 1 is always increasing. As mentioned earlier, YM values for soft tissues are typically based on the initial linear response. Measurement of tensile stretch will certainly lead to variation in reported values, as YM will be dependent on the stress that is applied. More importantly though, because the functional form of the stress–strain curve is nonlinear and demonstrates increasing resistance to deformation, the gradient of linear fits (E) to the initial response will always increase with increasing range of fit. This leads one to conclude that YM values measured by linear model fits using tensile measurements for soft biological tissues arguably represent an over estimate of the actual value. As noted by Fung,[51] YM values, obtained by tensile measurements, must be accompanied by a statement on the levels of stress and strain applied to the tissue to be of any quantitative value.

Article inclusion criteria

The following are our inclusion criteria for values tabulated in Tables 2 and 3: (1) Cited articles stated they measured the elastic response of a tissue. (2) The cited articles used established models for defining the elastic modulus, which is dependent on tensile or indentation measurements. (3) To the best of our ability, we confirmed that the reported values corresponded with an elastic response. In the case of tensile measurements, this was primarily determined by confirming that the reported data displayed a linear stress–strain response (although a number of articles also used nonlinear models). If two elastic modulus values were reported, based on two separate linear regimes, we used the smaller elastic modulus value obtained at low strain. With the exception of "spinal cord and gray matter," if tensile articles presented both instantaneous and relaxed elastic modulus values, we included the lower, relaxed elastic modulus value. For "spinal cord and gray matter," all of the cited articles reported an instantaneous modulus using a model solution for hyperelastic materials.[87] For indentation measurements, if multiple YM values were reported as a function of indentation depth, value inclusion was limited to data reported from the initial response of the sample. Review of indentation measurements has an additional complication in that the functional form of force versus indentation curves for elastic materials is always nonlinear. We therefore relied on representations of theoretical fits to the raw data if it was presented. Not all articles included the raw data that was used to define the reported value of YM, so we also relied on the written wording of the article and criteria 1 and 2. (4) When possible, we ensured that for each group of tissues, the cited articles were self-consistent in their measurement. For example, in the methods sections of the tensile reports on "tendon," the authors described, in similar fashion, that the gradient of stress–strain curves were measured directly after the "toe" region, in the "elastic phase" or "linear region," which they termed the elastic or YM of the sample. This task was not always possible though, especially for indentation measurements, as the model predictions are highly specific to the indentation method used, in which case we relied on criteria 1, 2, and 3. (5) We did not include YM values from diseased tissues.

Table 2. Young's Modulus of Soft Tissues, Measured by Indentation

	Indentation data		
Tissue	*Range (kPa)*	*Average Young's modulus (kPa)*	*Reference*
L&K	0.6–4000	~950	111–115
L&K (outlier)	4000		111
L&K (without outlier)	0.6–760	~190	112–115
A&V	6.5–21,000	~3600	109,116–120
A&V (outlier)	21,000		109
A&V (without outlier)	6.5–560	~125	116–120
Skin	6–50,000	~7700	112,121–127
Skin (outliers)	50,000–11,100		121,122
Skin (without outlier)	6–222	~85	112,123–127
Cornea anterior base & Descemet's membrane	7.5–50	~29	38
Sclera	No values		
Breast tissue	0.167–29	~8	1,3,128,129
Muscle	2–12	~7	120,130
Spinal cord & gray matter	0.2–7	~3	66,131
Tendon	No values		

L&K, liver & kidney; A&V, artery & vein.

Comparison of Indentation and Tensile Stretching Modulus Values

Tables 2 and 3 list YM values compiled from a number of soft biological tissues measured by indentation and tensile stretching, respectively. Tables 2 and 3 are arranged from

Table 3. Young's Modulus of Soft Tissues, Measured by Tensile Stretching

	Tensile data		
Tissue	*Range (MPa)*	*Average Young's modulus (MPa)*	*Reference*
Tendon	43–1660	~560	53,132–136
Muscle	480	480	137
Skin	21–39	~30	135,138
L&K	1–15	~10	139–141
Cornea	0.1–11.1	~3.0	40,142–145
Sclera	0.6–4.9	~2.7	146–150
Spinal cord & gray matter	0.4–3.6	~2	151–154
A&V	0.6–3.5	~2	155,156
Breast tissue	No values		

largest to smallest average modulus. YM values for indentation have a range from ~190 kPa for the organs located in the abdominal cavity, to around 3 kPa for spinal cord and gray matter. Tensile modulus values range from about 560 MPa for tendons to ~2.0 MPa for spinal cord and gray matter. Some reported modulus values appeared to be clearly outside the median range. We suspect some of the values are outliers and have tabulated averages for both these outlier values included and excluded. For example, the indentation of arteries and veins had reported YM values that ranged from 6.5 to 21,000 kPa, giving an average YM around 3600 kPa. However, the single reported value of 21,000 kPa increases the average over 28-fold if included with the other values, potentially complicating interpretation of results. Table 4 compares the averaged result for the two methods. Comparisons between indentation and tensile measurements in Table 4 do no include the suspected outliers noted in Tables 2 and 3.

To put these values in context, YM of a 25% aqueous solution of gelatin is reported to be ~30 kPa[88] (sphere–sphere compression), polydimethylsiloxane (PDMS) silicone rubber ~800 kPa[89] (rheometry), and tissue culture polystyrene to be ~3 GPa[90] (indentation). In assembling this review, there were some tissues that could not be directly compared due to a lack of published YM values in either tensile or indentation measurement. We have included these tissues to highlight possible research areas of interest. Other materials of interest to biological systems include polymeric hydrogels.[91–95] In general, hydrogels can be formulated from a variety of polymers with modulus values that span from very compliant to extremely rigid. A particularly interesting biological simulant, Matrigel™,[31] initially derived from a mouse tumor cell line is used as a three dimensional platform for tissue cultured cells and has a reported YM of around 1 kPa.[35]

Table 4. Comparison of Indentation and Tensile Measurements of Young's Modulus

Indentation versus tensile		
Tissue	*Indentation (kPa)*	*Tensile (MPa)*
Skin	~85	~30
L&K	~190	~10
Spinal cord & gray matter	~3	~2
Muscle	~7	~480
Tendon	No values	~560
Breast tissue	~8	No values
A&V	~125	~2
Sclera	No values	~2.7
Cornea	~29	~3.0

The values are the averages without the suspected outliers. Of the tabled citations on tissue mechanics, eight reports did not clearly state the tissue hydration condition, and the rest were measured in "wet" or "hydrated" (such as skin) conditions. Of those eight, we considered only one as an indentation outlier.[109] The results in this table are therefore not changed by consideration of this variable.

Discussion

One can conclude from the information and data presented that the values of YM for soft biological tissues depends on the method by which it is obtained. Additionally, tensile measurements consistently result in larger YM values compared with indentation measurements. The difference in YM between these two methods is an experimental confirmation that soft biological tissues are not homogeneous over all length scales. Indentation measurements are localized to the region of indenter contact in the order of millimeters to nanometers depending on probe size/geometry. Tensile measurements induce macroscopic deformations that span the bulk of a tissue with the entire specimen stretched. Understanding the differences is straightforward with muscles or ligaments. These tissues are better suited to resist deformation from a given tensile stress in the direction of fiber orientation as compared to a localized indentation that might be perpendicular to the fiber orientation. It is less clear, however, why tensile measurements consistently result in larger YM values for every soft tissue reviewed here. As discussed earlier, the discrepancy may be due, in small part, to the application of Equation 1 to *tensile* measurements of tissues that display increasing resistance to deformation with increasing stress, resulting in modulus values that are greater than the actual YM. This may not account for the entire difference, as tensile measurements of YM can be several orders of magnitude greater than the indentation measurement. One hypothesis that might contribute to the difference is due to the combined response of extracellular matrices, individual cells, longer-ranged protein polymers like collagen, actin and elastin, and the effect of constrained water, which lead to an increased resistance to deformation for macroscopic tensile measurements. These effects would not be observed in indentation measurements as the indenter induces sample deformation on the local environment only, both in terms of the indenter geometry and the depth of penetration. This is particularly relevant in relation to the water constrained within the interstitial spaces of the tissue. Indentations, especially in the case of AFMs or nanoindenters, typically perturb the tissue on the same scale as the constituents that make up the tissue. The local volume of water around the indenter therefore contributes very little to the resistance to deformation, as the tissue surrounding the indenter is under very little stress and is therefore capable of accommodating these small fluctuations in water content. Tensile measurements, on the other hand, stress the bulk of all the constituents of the tissue. Trapped water, which is incompressible, will significantly increase the tissue's resistance to deformation during an applied tensile stress. The significant differences in reported YM suggest that indentation and tensile measurements are inherently measuring different properties of the same tissue and that the scale of tissue perturbation is the dominant factor. For example, a YM value that includes the effect of constrained water may best represent the behavior of a tissue, *in vivo,* to a macroscopic load.

The strong dependence of YM on experimental method can significantly affect our interpretation of tissue function, disease status, or how cells respond to biophysical cues. A clear distinction exists between macroscopic, microscopic, and sub-microscopic responses to external stimuli. For example, it has been shown that cellular behavior is influenced by the compliance of the substrate on which the cell resides.[15,16,18,20,23,29,96–101] To accurately relate these laboratory observations back to *in vivo* cell function, compliance of the tissue and the matrix with which a cell interacts must be characterized. These values can be obtained with the AFM or

possibly nanoindenters, but certainly not by macroscopic tensile measurements. The design of larger implants such as artificial joints or cartilage, however, may be better served by macroscopic tensile measurements. The results of this review clearly demonstrate that the rational design of engineered tissues and biological simulants for use in the laboratory or clinic must reflect the heterogeneous physical properties of a soft tissue. Therefore, measurements of YM obtained by both tensile and indentation methods are relevant in the design and fabrication of such devices.

Of considerable interest is the modeling of tissue mechanics through mathematical models such as finite element analysis, which is used to better understand how tissues may respond to a given stress.[102–108] These models are complex but can be understood generally by assuming that tissues behave as perfectly elastic bodies and incorporate experimentally obtained material properties, such as YM, to describe tissue function. As noted in the introduction, YM values of soft tissues can be obtained only if one assumes that the tissue behaves as an elastic body under small stress conditions. However, the tabled data in this review demonstrate that this assumption does not lead to a single YM value independent of experimental method. At least two values obtained from the different measurement techniques exist for each soft tissue reviewed here (highlighting our previous argument that the term YM must be very clearly defined when describing the material property of a soft, viscoelastic biological tissue). Therefore, research articles using theoretical models must state how YM was determined, justify why a single value was used, or explicitly account for the heterogeneous nature of soft tissues. For example, it would not be appropriate to use tensile measures of YM in mathematical models designed to understand tissue mechanics at the micro- through nanoscopic scale.

The successful design of tissues engineered for improved function or replacement of native tissues requires the combined knowledge of biology, chemistry, and materials science, as they must interface with complex biochemical and biophysical environments *in vivo*. Increasingly, we understand that the biophysical environment plays a crucial role in the success of these engineered tissues. This review highlights and re-enforces an important attribute of soft tissues that must be considered when trying to model or engineer replacements; the response of soft tissues to an applied stress should not be considered independent of experimental method and that engineered tissues must reflect the heterogeneous material properties of native tissues.

In summary, this review has shown that soft biological tissues do not have a single YM value independent of experimental method, and that modulus values for a single tissue can span several orders of magnitude. If the method of deformation is delineated then the data provided can be better interpreted in context. On average, YM values of soft tissues are consistently lower when obtained by local indentation as compared to bulk tensile deformations. The scientific objective of a given research proposal therefore dictates which method is most appropriate.

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