

Strain-stiffening universality in composite hydrogels and soft tissues

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Soft biological tissues exhibit mechanical properties that reflect their composite structure of cells embedded within a biopolymer matrix. However, the microscopic mechanisms underlying their unique nonlinear mechanical response—characterized by strain stiffening in compression, but strain softening in shear or tension—remain poorly understood. Here we show that strain softening in composite systems can arise due to plastic dissipation, which is mediated by filler–polymer interactions. We characterize the nonlinear elasticity of composite hydrogels and soft tissues in isolation from these plastic effects, and show that their nonlinear elastic strain stiffening is driven by the stretching of the underlying biopolymer matrix. We thus show that strain stiffening in composite hydrogels and tissues is mediated by strain amplification factors that are universal in compression and shear. In doing so, we demonstrate the importance of fundamental composite properties such as filler concentration and filler–polymer interaction strength in mediating strain stiffening in composite systems. These findings highlight key structure–property relationships that underlie the nonlinear mechanics of biologically relevant soft solids such as composite gels and tissues.

Soft biological tissues exhibit remarkable resilience to large mechanical loads, which facilitates many of their physiological functions, and serves as a design consideration for tissue engineering and tissue-interfacing biomaterials. Although these mechanical properties are associated with the nonlinear response of the biopolymer networks that structurally support the tissues—which stiffen in shear and tension but not in compression^{1,2}—many tissues and blood clots exhibit a rather different nonlinear response, which is characterized by a dominant stiffening response in compression rather than in shear and tension³. This paradoxical mechanical response has been attributed to the composite nature of tissues and, indeed, the introduction of cells or fillers into biopolymer networks give rise to mechanical responses that mimic the compression stiffening and shear or tension softening observed in soft tissues^{3,4}. A systematic understanding of this behaviour could

enable the rational design of tissue-mimetic biomaterials and devices, and provide a deeper insight into the mechanical microenvironment of cells in vivo, especially in tissues under physiological mechanical loads. However, the microscopic mechanisms underlying these nonlinear mechanical properties of soft tissues, and composite systems more generally, remains poorly understood.

Here we address this problem through a systematic experimental study of the nonlinear mechanical response of composite hydrogels as well as soft biological tissues. We first study the compression stiffening of model composite hydrogel systems that consist of Ca²⁺-cross-linked pectin hydrogels filled with large cell-scale dextran particles, as a minimal structural mimic of soft biological tissues consisting of cells embedded in a biopolymer network. Prior work has demonstrated this concept with a composite system consisting of fibrin and dextran,

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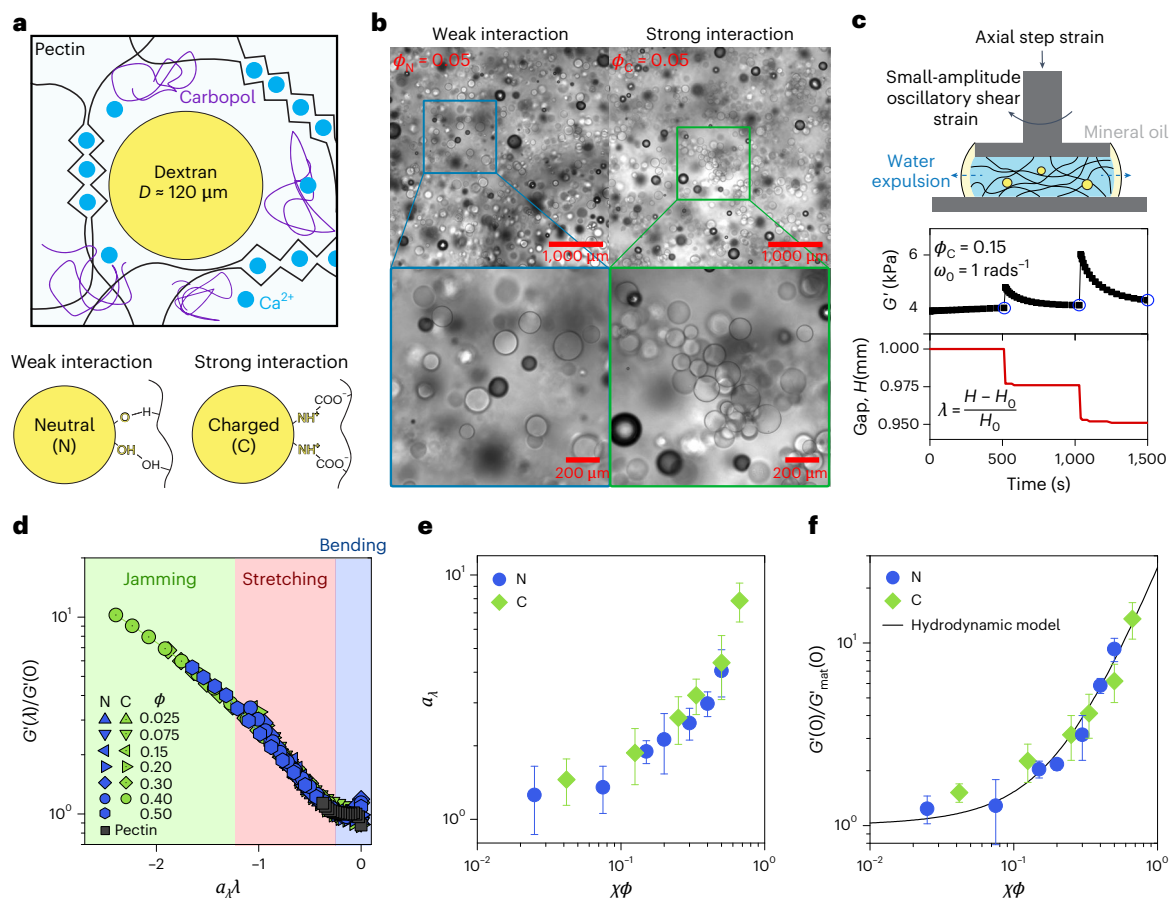


Fig. 1 | Compression-stiffening universality in composite hydrogels.

a, Illustration of the composite hydrogel microstructure. The system consists of low-methoxy pectin cross-linked by Ca^{2+} ions, Carboxypol, and neutral (N) or charged (C) dextran particles that undergo weak (hydrogen bonding) or strong (ionic) interactions with anionic pectin chains. Image analysis of the diameter D of the particles is shown in Supplementary Fig. 2. **b**, Representative bright-field confocal images of composite hydrogels with weak and strong interactions at $\phi = 0.05$ (Supplementary Fig. 3 shows data for $\phi = 0.20$). The bottom panel highlights the formation of aggregates in the latter case. **c**, Illustration of the experimental protocol, which was adapted from ref. 3. Time-dependent small-amplitude oscillatory shear (measured at an angular frequency of $\omega_0 = 1 \text{ rad s}^{-1}$) is superposed on a step axial strain $\lambda = (H - H_0)/H_0$, where H and H_0 are the final and initial gap sizes, respectively. The resulting storage modulus G' is monitored for 500 s (to allow for poroelastic equilibration⁶⁹), and the last data points at each step (blue circle) are reported. The hydrogels are fully compressible under these conditions (Supplementary Fig. 8).

d, Representative master curve of normalized G' (relative to the modulus at zero compressive strain $G'(0)$) as a function of λ rescaled by the compressive strain amplification factor a_λ , for the composite hydrogel system as a function of ϕ and ε (Supplementary Fig. 10 shows a repeat set of data). The corresponding regimes of bending (softening in G' with λ), stretching (sharp increase in G' with λ) and jamming (decrease in slope of G' with λ), as proposed in ref. 8, are highlighted. **e**, Compressive strain amplification factor a_λ as a function of $\chi\phi$, where $\chi(C) = \phi_{\text{jam}}(N)/\phi_{\text{jam}}(C) = 1.67$ and $\chi(N) = 1$. Data shown are mean values $\pm$ standard deviations from $n = 4$, obtained by analysing a_λ across two sets of experiments (Fig. 1d and Supplementary Fig. 10). **f**, Linear elasticity of the composite hydrogels at no axial strain $G'(0)$ normalized to that of the pectin hydrogel matrix $G'_{\text{mat}}(0)$, as a function of $\chi\phi$. Data shown are mean values $\pm$ standard deviations from $n = 4$. The data are fitted to the Guth–Gold ($c_2 = 14.1$) hydrodynamic model in equation (1) (Supplementary Fig. 13 shows fits to the Batchelor–Green ($c_2 = 5.2$) model).

in which fibrin mimics the fibrous components of the extracellular matrix (ECM)³. In this sense, our model system with pectin provides a complementary insight into the mechanical role of the polysaccharide components of the ECM⁵, and serves as a potential candidate for a cost-effective food-grade tissue-mimetic soft material. Gelation of the Ca^{2+} -cross-linked pectin occurs over $\sim 3 \text{ h}$ (Supplementary Fig. 1a), during which the dextran particles can sediment readily in the solution due to their large size (diameter, $\sim 120 \mu\text{m}$; Supplementary Fig. 2) and high stiffness relative to the polymer matrix ($\sim 10^6 \text{ Pa}$ (ref. 6)). To address this issue, we incorporate Carboxypol into all of our composite hydrogels, which prevents sedimentation through yield stress stabilization of the particles (Supplementary Fig. 4), whereas only slightly weakening the pectin– Ca^{2+} network (Supplementary Fig. 1b).

With this model system, we systematically vary the particle volume fraction ϕ , and study two different types of dextran particle—neutral (N) and positively charged (C)—to investigate the effect of

particle–polymer interaction strengths ε on composite mechanics (Fig. 1a). Confocal imaging shows that composite hydrogels with N and C particles exhibit different microstructures, in which the N particles remain well dispersed whereas the C particles self-assemble into aggregates (Fig. 1b). This can be attributed to the higher ε value of the C particles, which causes interparticle interactions via bridging interactions. Binding assays to measure the pectin adsorption capacity of the two particle systems indeed confirm the higher interaction strength of C particles compared with N particles (Supplementary Fig. 5). Interestingly, confocal imaging using fluorescent pectin reveals that this adsorption occurs inside the particles, which can arise due to the relative permeability of dextran (a high-stiffness microgel), the flexibility of pectin chains and the broad molecular weight distribution of pectin⁷, which facilitates the diffusion of shorter polymer chains into the dextran particles (Supplementary Fig. 6). Due to the combination of increased polymer incorporation

into the particles and the bridging-induced particle interactions, we observe greater bulk contractions in hydrogels with C particles compared with N particles from normal force measurements during gelation (Supplementary Fig. 7).

We perform quasi-static compression experiments on the composite hydrogels (Fig. 1c); our systems exhibit robust sample-geometry adhesion and are completely compressible under these conditions, as revealed by the near-zero Poisson's ratio ν during compression (Supplementary Fig. 8). The experiments show that the pectin hydrogels undergo an increase in the linear viscoelastic shear modulus with increasing ϕ , as well as exhibit greater nonlinear stiffening in $G'(\lambda)$ —the storage modulus as a function of axial strain λ (Supplementary Fig. 9a). This compression-stiffening effect is amplified in composite hydrogels with C particles compared with N particles (Supplementary Fig. 9b), an effect that is independent of both filler concentration and linear modulus of the hydrogel (Supplementary Fig. 9c). We find that the normalized compression-stiffening curves as a function of ϕ and ε can be shifted onto a universal master curve (Fig. 1d), with the associated compressive strain amplification factor a_1 systematically increasing with ϕ and ε (Fig. 1e).

These findings can be contextualized with some of the recently proposed models for understanding compression stiffening in tissues and composite hydrogels. One such model suggests that incompressible fillers can cause amplified stretching of the polymers under compression by restricting interstitial space³. Another model suggests that compression can cause the non-affine displacement of rigid fillers, which then causes the stretching of bonded polymers in a percolated filler–polymer network, eventually jamming the fillers themselves⁸. Our results provide experimental support to the latter model as we observe clearly demarcated regimes of bending, stretching and jamming in our master curve (Fig. 1d), which are predicted in their simulations.

The agreement with the computational predictions of the model in ref. 8 provides an appropriate context to understand the higher compressive strain amplification factor a_1 observed in composite hydrogels with higher ε . The onset of the steep increase in $G'(\lambda)$ at low λ and the reduction in slope at high λ can be attributed to compression-induced structural transitions of the filler network, namely, percolation and jamming, respectively. Although the percolation threshold is difficult to isolate in the system as the polymer network alone also exhibits stiffening at large λ , the jamming threshold can be isolated from the inflection point of the compression curve at which the $G'(\lambda)$ slope flattens (approximately at $\lambda_{\text{jam}} = 1.25/a_1$; Fig. 1d). A quantitative evaluation of this inflection point over two sets of experiments allows us to characterize the particle-jamming threshold $\phi_{\text{jam}} = \phi(1 - \lambda_{\text{jam}})$, yielding $\phi_{\text{jam}}(\text{C}) = 0.49$ and $\phi_{\text{jam}}(\text{N}) = 0.82$ (Supplementary Fig. 11). Given the size and stiffness invariance of the two particle systems, we interpret the C particles as exhibiting larger effective volume fractions ϕ_{eff} in the hydrogels relative to the N particles, based on the proximity of their volume fractions to the jamming threshold. Accordingly, we define an effective volume fraction $\phi_{\text{eff}} = \chi\phi$, where χ quantifies the relative volume of the C fillers relative to the N fillers based on distances to the jamming thresholds (that is, $\chi(\text{C}) = \phi_{\text{jam}}(\text{N})/\phi_{\text{jam}}(\text{C}) = 1.67$ and $\chi(\text{N}) = 1$). We find that the compressive strain amplification factor a_1 shows a good collapse when plotted as a function of ϕ_{eff} (Fig. 1e), suggesting that the difference in effective filler volume can adequately explain the strain amplification observed in composite hydrogels with different filler–polymer interaction strengths ε .

These findings are corroborated by comparing the linear viscoelastic shear modulus of the hydrogels at zero axial strain $G'(0)$, which are systematically higher for hydrogels with C fillers than N fillers at all ϕ , and importantly, can also be collapsed when plotted as a function of ϕ_{eff} (Fig. 1f). We find the filler-induced reinforcement effect in our hydrogels to be substantially greater than that expected from the phantom network theory⁹, and fit our rescaled linear viscoelastic shear

moduli results to a modification of the more classical reinforcement model of the form

$$\lim_{\gamma \rightarrow 0} \frac{G'(\gamma)}{G'_{\text{mat}}(0)} = 1 + 2.5(\alpha\phi_{\text{eff}}) + c_2(\alpha\phi_{\text{eff}})^2, \quad (1)$$

where $G'_{\text{mat}}(0)$ is the modulus of the pectin matrix and c_2 is the second virial coefficient, which varies in the literature from 2.5 to 15.6 (ref. 10). This model is often referred to as the hydrodynamic model as it is analogous to the expression used to describe the hydrodynamics of particle suspensions¹¹. Using the classical Guth–Gold solution of $c_2 = 14.1$ (ref. 12), we find good agreement with our $G'(0)$ data with an empirical factor of $\alpha = 1.50$ (Fig. 1f); similarly, using the Batchelor–Green solution of $c_2 = 5.2$ (ref. 13) results in a good fit with $\alpha = 2.32$ (Supplementary Fig. 13). The increased reinforcement effects (indicated by $\alpha > 1$) could arise from other contributing effects such as polymer adsorption^{14,15}, filler aggregate shape¹², percolation of the particle network^{16–18} and higher-order hydrodynamic contributions¹⁹; it is possible that using models that consider such effects more explicitly may result in improved fits to our data. Nonetheless, our data show that the linear elasticity of our composite hydrogels can be reasonably described by a simple hydrodynamic model, and that the linear viscoelastic properties of the composite hydrogels with different particles can also be described by a difference in ϕ_{eff} .

This increased ϕ_{eff} of the C particles can be attributed to the bridging-mediated interparticle interactions, which can cause particles to cluster (Fig. 1b and Supplementary Fig. 2) and form system-spanning clusters at lower volume fractions, thereby decreasing the jamming thresholds (Supplementary Fig. 11)^{20,21}. We note the possibility that the different jamming thresholds of the particles can also be mediated by a difference in the polymer adsorption on the surface of individual particles, which can result in the formation of a rigid shell-like interphase layer that increases the effective size of the particles^{22–24}. However, our confocal images do not show the formation of such adsorbed layers in our system, but instead reveal increased adsorption and occlusion of polymers within the high- ε particles (Supplementary Fig. 6). Although this could affect ϕ_{eff} by reducing the total volume of the matrix relative to the fillers, an estimation of this effect based on the fluorescence intensities of the polymers in the particles yields only a modest increase of ~10% in ϕ_{eff} of the C particles compared with the N particles, which is not sufficient to explain the different behaviours of our two particle systems (Supplementary Fig. 12). Thus, our results suggest that the ε -dependent mechanical properties of the composite hydrogels may be explained by an increased particle-clustering behaviour at high ε , which increases the effective volume fraction of the particles.

In contrast to the strain stiffening observed in compression shown thus far, a strain-softening behaviour is often observed in composite hydrogels and soft tissues in tension and shear^{3,25}. This behaviour appears paradoxical given the dramatic tension and shear stiffening exhibited by their constituent biopolymer matrix^{1,2}. This tension- and shear-softening behaviour has been rationalized³ through a mechanism in which incompressible fillers shield the polymer matrix from strain. However, this form of tension- and shear-softening effect has also been observed more generally in polymeric composites for many decades, and is canonically described as the Payne effect²⁶ (in which the storage modulus G' decreases with increasing strain amplitude γ_0 and in which the loss modulus G'' exhibits an overshoot at the yield point) or the Mullins effect²⁷ (in which stress softening occurs with increasing cyclic strain) depending on the experimental protocol. We tested the non-linear shear properties of our composite hydrogel via large-amplitude oscillatory shear (LAOS), and indeed observed a strain-softening response that is consistent with these past observations. Although pectin hydrogels without fillers exhibit strain stiffening (as evidenced by an increase in G' with increasing γ_0), pectin hydrogels filled with C and N particles do not exhibit any form of stiffening in shear (Fig. 2a).

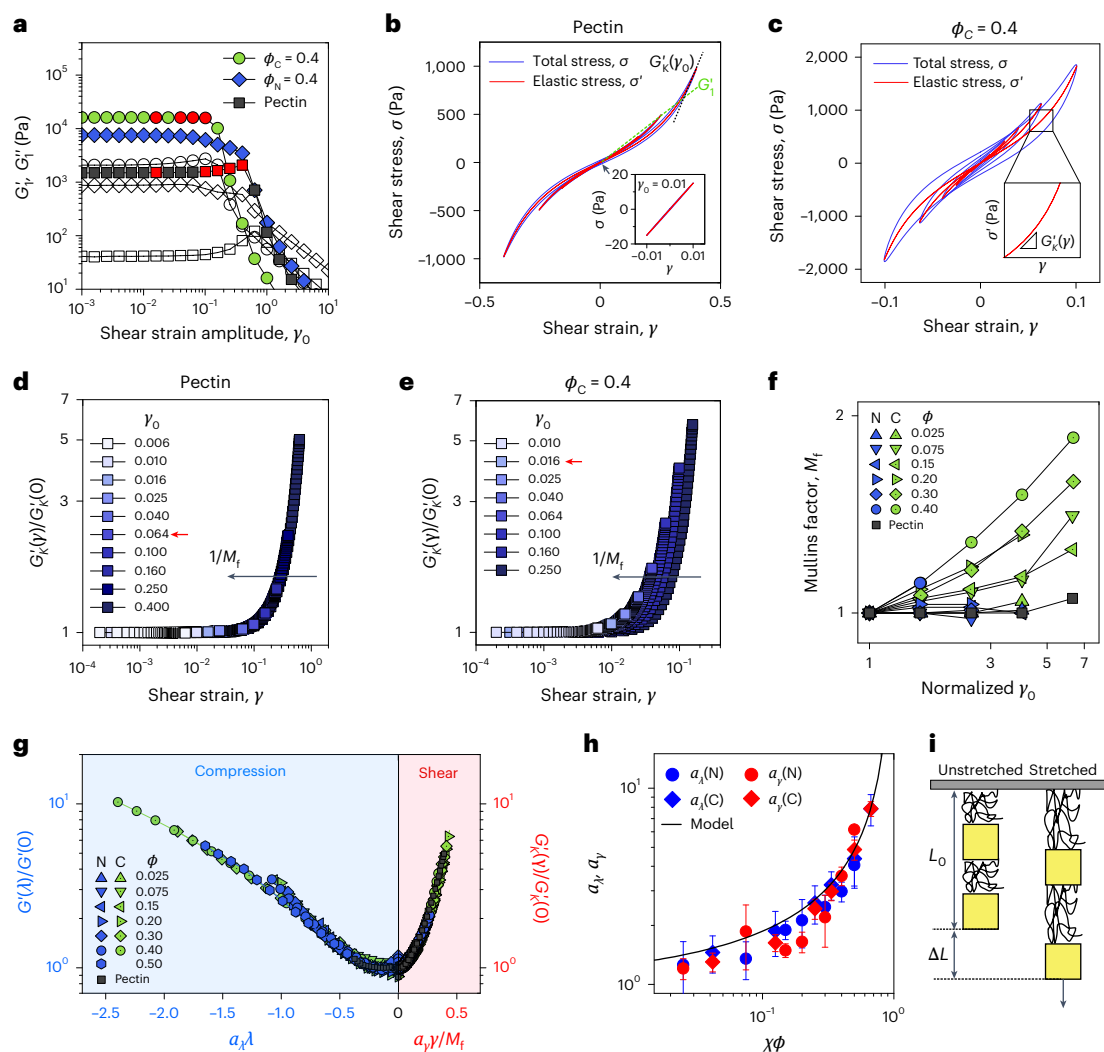


Fig. 2 | Shear-stiffening universality in composite hydrogels. **a**, First-harmonic storage modulus G'_1 and loss modulus G''_1 as a function of oscillatory shear strain amplitude γ_0 for pectin and the representative composite hydrogels. **b,c**, Representative Lissajous curves of pectin and $\phi_c = 0.4$ hydrogels at γ_0 corresponding to those highlighted by the red symbols in **a**. The Lissajous curves are ellipsoidal in strain at low γ (**b**, inset), but become distorted in the nonlinear regime. The first-harmonic storage modulus G'_1 is computed from a linear fit from the origin (green dashed line), whereas the differential storage modulus $G'_k(\gamma_0)$ is computed from the tangent slope at γ_0 (black dotted line), both illustrated in **b**. Our protocol for calculating $G'_k(\gamma)$ is highlighted in panel **c**, inset. **d,e**, $G'_k(\gamma)$ of the pectin and $\phi_c = 0.4$ gels, normalized by $G'_k(\gamma = 0)$. All data are subsequently shifted to a reference curve measured at γ_0 at which the $G'_k(\gamma)/G'_k(0)$ value exhibits a 5% increase (marked by red arrow) and shows an exact overlay

(see Supplementary Fig. 18). **f**, Mullins factor (M_f) values for the pectin and composite hydrogels as a function of γ_0 (normalized to the reference γ_0) for each system. **g,h**, Universal master curve for the composite hydrogels in compression and in shear (**g**) and the corresponding strain amplification factors a_λ and a_γ as a function of $\chi\phi$ (**h**), where $\chi(C) = \phi_{\text{jam}}(N)/\phi_{\text{jam}}(C) = 1.67$ and $\chi(N) = 1$. Data shown are mean values $\pm$ standard deviations from $n = 4$; statistics for the shear experiments are obtained from two sets of experiments (master curve and M_f values of repeat experiments are shown in Supplementary Fig. 19). **i**, Illustration of the one-dimensional affine strain amplification model³⁶ highlighted in **h**. Total strain is defined as $\lambda_{\text{tot}} = \Delta L/L_0$, but the polymeric components of the system experience a strain of $\lambda_{\text{pol}} = \Delta L/L_0(1 - \phi^{1/3})$. Thus, strain is amplified by a factor $a = \lambda_{\text{pol}}/\lambda_{\text{tot}} = 1/(1 - \phi^{1/3})$ (equation (2)).

However, since the raw stress waveform becomes distorted in LAOS experiments, the G' and G'' computed by the rheometer (which considers just the first-harmonic response of the stress and strain, and are denoted by G'_1 and G''_1 , respectively) provide an oversimplified description of the nonlinear mechanics of both systems (Fig. 2b,c)²⁸. An alternative method of analysis is clearly needed to extract nonlinear elasticity in these systems.

One accepted method is to compute the differential storage modulus at the maximum strain of each cycle: $G'_k(\gamma_0) = (d\sigma'/d\gamma)|_{\gamma_0}$, where σ' is the elastic component of the total stress (Fig. 2b)²⁹. This quantity is the LAOS-equivalent of the differential storage modulus $K'(\sigma_0)$ obtained by superimposing a small-amplitude oscillatory shear on a step stress σ_0 , a protocol frequently used to characterize the nonlinear elasticity

of biopolymer networks^{5,30}. Analysis of $G'_k(\gamma_0)$ for our systems reveals shear strain stiffening in the composite hydrogels, but to a lower extent than that exhibited by the filler-free pectin hydrogels (Supplementary Fig. 14). The raw stress-strain Lissajous curves of the pectin hydrogels and composite hydrogels provide clues on why composite hydrogels exhibit lower $G'_k(\gamma_0)$ values than pectin hydrogels: although the $\sigma'(\gamma)$ curves of the pectin hydrogels are superposed on each other at all γ_0 until reaching the yield strain (the point of visible decrease in G'_1 with γ_0 ; Fig. 2a), the $\sigma'(\gamma)$ curves of the composite hydrogels are horizontally translated at input strains below the yield strain (Fig. 2b,c). These results show that inelastic dissipation occurs prominently in the composite hydrogels before yielding, thereby obfuscating the measurements of nonlinear elasticity in these systems.

These inelastic effects can be attributed to plastic deformations that occur in the gels before macroscopic yielding. Evidence for these effects can be seen in the overshoot in G'' in the composite hydrogels before yielding, which is a hallmark property of the Payne effect²⁶ and a measure of the irrecoverable plastic deformation that occurs in the microstructure in the nonlinear regime³¹. Further evidence for these effects is seen from cyclic compression and transient LAOS experiments on the composite hydrogels, which show that subsequent loading curves memorize and trace the previous unloading curves, with increasing irreversible plastic deformations per loading cycle (Supplementary Fig. 15)—a hallmark property of the Mullins effect²⁷. The Mullins effect leads to a horizontal translation of $\sigma'(\gamma)$, resulting in a softening effect that is convolved in the measurements of G'_i (Fig. 2a) and $G'_k(\gamma_0)$ (Supplementary Fig. 14). In the absence of such plastic effects, we may expect the nonlinear elasticity of the pectin hydrogels and composite hydrogels to be coincident. This would correspond to the physical scenario studied in ref. 8, in which tension is predicted to cause stiffening of composite gels via the stretching of the bridging polymers, under plasticity-free conditions where polymers are permanently bound to the particles.

We show that the Mullins effect observed in LAOS (Fig. 2c and Supplementary Fig. 15) can be eliminated to obtain the true nonlinear elastic response of the system by computing the strain-dependent differential storage modulus $G'_k(\gamma) = d\sigma'/d\gamma$ (Fig. 2c, inset). When represented as a function of the input oscillation amplitude γ_0 , the $G'_k(\gamma)$ curves are self-similar for both pectin and composite hydrogels. However, the latter results become horizontally offset with increasing γ_0 due to the Mullins effect (Fig. 2d,e). The translated $G'_k(\gamma)$ curves can, thus, be horizontally shifted onto a reference $G'_k(\gamma)$ curve in the linear regime to construct a master curve for each system^{32,33}, via a shift factor that characterizes the strain-dependent plasticity in our system due to the Mullins effect, which we refer to as the Mullins factor M_f . The M_f values are near unity for the pectin hydrogels at all γ_0 (minimal plastic effects), but they increase in composite hydrogels with increasing ϕ and ε (Fig. 2f). The observation of greater M_f in attractive hydrogels with C particles compared with N particles is also supported by the differences in the Mullins effect observed in cyclic compression experiments for these two systems (Supplementary Fig. 15b–e). Overall, these results suggest that a stronger filler–polymer interaction strength ε results in greater plastic dissipation in composite hydrogels. This can be rationalized by the greater degree of immobilization of polymers on particles with increasing ε (Supplementary Fig. 6)^{23,24}, as the Mullins and Payne effects can be attributed to the strain-induced detachment of the immobilized polymers on the particle surface that bridge the particles^{16,34}. It should be noted that the shift factor data for composite hydrogels with N particles are truncated at high γ_0 due to the system exhibiting clear signatures of slip before yielding occurs. This is identified by a gradual drop in G'_i and G'_l before yielding (Fig. 2a and Supplementary Fig. 17a)³⁵ and a more softened response in the $G'_k(\gamma)$ curve relative to those in systems without slip (Supplementary Fig. 17b,g). We find neither the pectin hydrogels nor the composite hydrogels with C fillers affected by these slip-related artefacts during measurement.

The shifted $G'_k(\gamma)$ curves provide an unadulterated characterization of the nonlinear elasticity of the different composite systems (Supplementary Fig. 18a). The shifted $G'_k(\gamma)$ curves for pectin and composite hydrogels as a function of ϕ and ε are self-similar, and can be shifted onto a single master curve using a shear strain amplification factor a_γ —in a similar vein as the results from the compression experiments in Fig. 1d (Supplementary Fig. 18b). In fact, we find that both compression and shear stiffening of composite hydrogels as a function of ϕ and ε can be described by a master curve (Fig. 2g), with universal values for both a_λ and a_γ (Fig. 2h). These results demonstrate the universal nonlinear elastic response of composite hydrogels in compression and shear, which is governed by the stretching of the matrix polymer chains. This observation is consistent with earlier simulation

results in which a similar universality is observed in compression and tension in composite systems without plastic effects⁸. Furthermore, we find that the strain amplification factors a_λ and a_γ can be reasonably described in a parameter-free manner with a simple one-dimensional filler-induced affine strain amplification model (Fig. 2i)^{36,37}:

$$a_i/a_i(0) = 1/(1 - \phi_{\text{eff}}^{1/3}), \quad (2)$$

where $i = \lambda$ or $i = \gamma$. This model suggests that fillers effectively enhance the strain experienced by the bridging polymer matrix^{8,38}, an effect that is amplified with increasing filler–polymer interaction strengths due to the increase in the effective filler volume ϕ_{eff} in the system (Fig. 1f)²². Overall, these results demonstrate the critical roles played by filler volume and filler–polymer interactions, which are fundamental parameters in biological tissues and technological composites alike, in amplifying the nonlinear mechanical responses of the system.

It is interesting to compare the magnitude of compressive and shear strain amplification factors with the magnitude of reinforcement in the linear viscoelastic shear modulus of the composite hydrogels with increasing filler concentrations (Fig. 1e). We find that a_λ and a_γ trace the reinforced linear shear modulus G'/G'_{mat} at $\phi \leq 0.2$, suggesting that strain amplification can provide an empirical description of the increased elasticity of composite systems at low filler concentrations (Supplementary Fig. 20), even if it does not account for some of the pertinent physics arising from filler incorporation such as the hydrodynamic reinforcement effect^{10,39,40}. However, beyond $\phi > 0.2$, we find that G'/G'_{mat} exhibits a steep increase and deviates from a_λ and a_γ . We rationalize this steep increase in G'/G'_{mat} by the emergent elasticity of a percolated particle–polymer network^{16–18}, the presence of which is evidenced in the hallmark behaviours of our composite hydrogels such as compression stiffening⁸ and Mullins plastic dissipation at large γ_0 values³⁴.

Our experimental results and the simulation results from ref. 8 demonstrate a strain-stiffening universality in composite hydrogels in which stiffening is equally amplified in shear (or tension) and in compression by essential parameters such as ϕ and ε . However, both our work and ref. 8 demonstrate this effect on model composites that are structurally different to tissues. The model from the latter work utilizes fibre networks with rigid inclusions, whereas our model utilizes polysaccharides with relatively stiff inclusions ($\sim 10^6$ Pa)⁶. These model systems are not equivalent to soft tissues, which are multipolymer composites of flexible proteoglycans and fibrous polymers embedded with cells, which have low stiffness ranging from $\sim 10^2$ Pa to 10^5 Pa (ref. 41) and that mechanically interact with the ECM⁴². Although tissues have been shown to exhibit strain softening under tension and shear^{3,25}, in a similar vein to the composite hydrogels studied here, the prevalence of compression- and shear-stiffening universalities in tissues remains unclear.

To explore this, we perform compression-stiffening experiments on mouse tissues: lung, heart, adipose, liver and partially decellularized (PD) liver (liver treated in Triton X to dissolve the cell membrane⁴³) (Fig. 3a and Supplementary Fig. 26c); all the samples are loaded ex situ with sandpaper to improve grip. We find the tissue samples to be more incompressible than the composite hydrogels, with ν ranging from 0.2 to 0.4, which is indicative of their low poroelasticity relative to the pectin hydrogels (Supplementary Fig. 22)⁴⁴. All the measured $G'(\lambda)$ data can be shifted onto the lung data, which shows the weakest compression stiffening (Fig. 3b). We attribute the deviations observed in the heart sample to the round shape of the excised heart tissue (Supplementary Fig. 21a) and the nonlinear dependence of radial strain on axial strain (Supplementary Fig. 22d). The first-harmonic storage modulus G'_l collected from LAOS experiments on the tissues show an even more pronounced Payne effect (Fig. 3c) compared with the composite hydrogels (Fig. 2a). Correspondingly, we observe significant plastic dissipation occurring at large strains, as indicated by the horizontal

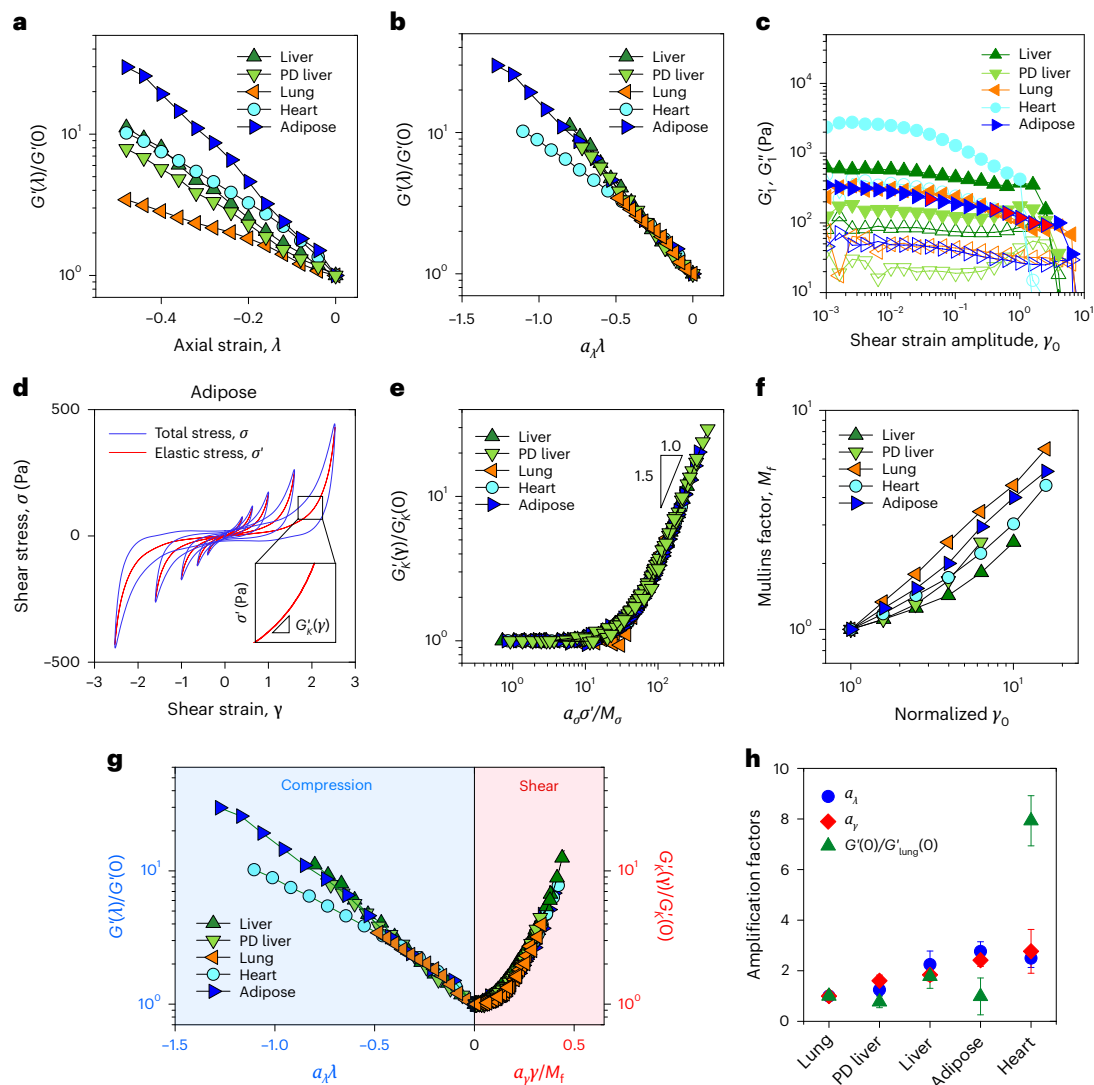


Fig. 3 | Compression- and shear-stiffening universalities in soft biological tissues. **a, b**, Compression stiffening (**a**) of normal liver, partially-decellularized (PD) liver, lung, heart and adipose tissues excised from mice (representative pictures are shown in Supplementary Fig. 21), which is shifted to the (least strain-stiffening) lung sample using the compressive strain amplification factor a_λ (**b**). The heart data show an inflection in the curve with λ , which can be attributed to the round shape of the sample (Supplementary Fig. 21a) that causes bulging resistance at the initial stages of compression (Supplementary Fig. 22d). **c**, First-harmonic storage modulus G'_1 and loss modulus G''_1 as a function of oscillatory shear strain amplitude γ_0 for the tissue samples. **d**, Representative

Lissajous curves of adipose tissue at γ_0 corresponding to the red symbols in **c**. The inset illustrates the calculation protocol for $G'_k(\gamma)$. **e**, Master curve of $G'_k(\gamma)$ for different tissues as a function of elastic stress σ' (Supplementary Fig. 24 shows the related shift factors a_σ and M_σ). **f**, Mullins factor (M_f) for the different tissue samples (Supplementary Fig. 26b shows repeat data). **g, h**, Universal master curve for the tissue samples in compression and in shear (**g**), and the corresponding strain amplification factors a_λ and a_γ (**h**), plotted alongside $G'(0)/G'_{lung}(0)$ ($n = 4$). All curves are shifted to the lung reference curve. Data shown are mean values $\pm$ standard deviations from $n = 4$; statistics for the shift factors are collected across two sets of experiments for compression and shear.

translations in the Lissajous curves (Fig. 3d). The Lissajous curves also show significant strain-stiffening behaviour in $\sigma'(\gamma)$, which can now be extracted via our $G'_k(\gamma)$ protocol (Supplementary Fig. 24).

Having isolated the nonlinear elasticity of biological tissues through $G'_k(\gamma)$, we now analyse $G'_k(\gamma)$ as a function of elastic stress σ' , from which we can gain molecular insights into the origin of nonlinear elasticity in tissues. There is extensive literature on the origins of the scaling of the differential modulus with stress, that is, $G'_k(\gamma) \sim \sigma'^z$. The most common interpretation is that this scaling arises due to the force-displacement response of the individual polymer strands^{2,30,45}. In this case, z follows the force-extension (F-L) relationship of individual polymer or filament strands (which can be characterized by atomic force microscopy experiments), with $dF/dL \sim F^z$ (ref. 30). The most common value of z in biopolymer systems is the case in which the constituents obey entropic elasticity predicted by the worm-like chain

model⁴⁶, where $z = 1.5$. However, this number can be lower in biopolymer systems such as polysaccharides, which are more extensible or have enthalpic components to elasticity⁴⁷. One such example is pectin, and an analysis of the F-L relationship of pectin strands⁴⁸ reveals a scaling of $dF/dL \sim F^{1.1}$ (Supplementary Fig. 23a), which is in excellent agreement with the scaling of $G'_k(\gamma) \sim \sigma'^{1.1}$ in our composite systems (Supplementary Fig. 23b). This shows that polymer chain stretching underlies the nonlinear elasticity of pectin hydrogels and pectin hydrogel composites. This analysis also suggests that a similar mechanism might be responsible for the $z < 1.5$ scaling of the differential modulus observed in other polysaccharide gels in the literature, for example, agarose⁴⁹ and chitosan⁵⁰.

In tissues, we may expect a scaling of $G'_k(\gamma) \sim \sigma'^{1.5}$ because most of the biopolymer constituents including actin⁵¹, collagen molecules⁵² and proteoglycans⁵³ follow the worm-like chain model. By contrast,

tissue mechanics are often assumed to be dominated by a fibrillar collagen network in the connective tissue ECM scaffold. Collagen fibres are hierarchical assemblies of collagen molecules, have persistence lengths on the order of centimetres⁵⁴ and do not follow a worm-like chain behaviour⁵⁵. Reconstituted collagen fibre networks show a scaling of $z < 1.5$ in the differential modulus, which is often attributed to the stress-induced stabilization of the sub-isostatic fibre network^{56,57}. We find that all our tissues—both whole organs and the connective tissue adipose—follow a scaling of $G'_k(\gamma) \sim \sigma^{1.5}$ (Fig. 3e). This suggests that nonlinear shear strain induces the stretching of polymer chains and filaments in the tissues, rather than directly engaging the nonlinear response of the collagen fibre network in the ECM. Prior studies of tension experiments on rat tendon have shown that the total strain of the whole tendon is greater than those experienced by the collagen fibres inside the tendon, thereby suggesting that the strain may primarily occur in the proteoglycan-rich matrix^{55,58}. Our results support this observation across different whole organs and connective tissues. The observation of $G'_k(\gamma) \sim \sigma^{1.5}$ also allows us to rule out the effect of slip on the nonlinear elasticity of tissues, as $z = 1.5$ corresponds to the upper limit of the theoretical expectations, and sample slip has been shown to cause a reduction in z (Supplementary Fig. 17b,g).

We also find signatures of substantial plastic dissipation in the characterized tissues, evidenced by the Payne effect (Fig. 3c) and the horizontal translation of the Lissajous curves with γ_0 (Fig. 3d). However, the resulting Mullins factor (M_f) of the tissue samples are noticeably higher than that observed in the composite hydrogels (Fig. 3f and Supplementary Fig. 26b), with no apparent correlation with the onset strain for strain stiffening as was previously observed in composite hydrogels (as evidenced by the lung exhibiting the greatest value of M_f). This indicates the presence of dissipative mechanisms in tissues beyond those governing our composite hydrogel systems. The whole tissues studied here are architecturally complex, and could exhibit other dissipative mechanisms, for instance, those arising from the scission of covalent bonds in the ECM, or detachment of cell–cell junctions in epithelial tissues. We also cannot rule out dissipative contributions from slip as the tissue samples are loaded *ex situ*; however, we note that we do not find signatures of slip from the observed nonlinear elasticity $G'_k(\gamma)$ of the tissues, and that similar extents of horizontal translation in the Lissajous curves at large strains have been reported in tissue samples rigidly bound with cyanoacrylate glues^{59,60} (which itself could introduce additional dissipative mechanisms).

We now compare the shear measurements of tissues with compression measurements. We find that the onset strain for shear stiffening are in agreement with the onset strain for compression stiffening (Supplementary Fig. 25a). We also find that the $G'_k(\gamma)$ data can be shifted onto a master curve with a_γ (Supplementary Fig. 25b), in a similar vein as the compression data (Fig. 3b). Thus, similar to our findings in composite hydrogels (Fig. 2g), we observe a universal master curve for various tissues as a function of compression and shear (Fig. 3g). Furthermore, we observe that the strain amplification factors a_γ and a_γ (shifted with reference to the lung data) are practically equivalent for all tissues, and that they also deviate from $G'(0)/G'_{lung}(0)$ (Fig. 3h)—both findings are in agreement with observations in our composite hydrogels.

In total, these results demonstrate that the strain-stiffening responses of composite hydrogels (Fig. 2g) and tissues (Fig. 3g) are mediated by the stretching of the constituent polymer matrix, and can be rescaled by universal strain amplification factors in compression and shear (Figs. 2h and 3h). These results also show that the nonlinear mechanics of tissues, especially the nonlinear elastic stiffening and plastic dissipation, can be recreated using a composite hydrogel consisting of strain-stiffening polymers and attractive fillers. In this context, we have demonstrated the importance of key composite variables such as filler volume ϕ and filler–polymer interaction strength ε in mediating the nonlinear mechanics of composite hydrogels,

particularly through their roles in filler-induced strain amplification and the Mullins effect. These findings suggest that equivalent tissue variables such as cell density and cell–ECM interactions may play key roles in dictating the nonlinear mechanics of living tissues. Living tissues also exhibit additional complexities not found in composite hydrogels that could contribute to their nonlinear mechanics, such as cell-induced contractile alignment of the ECM^{42,61–63}, softness⁴¹ and poroelasticity⁶⁴ of the cells, interpenetration of fibrous and non-fibrous biopolymers in the ECM⁶⁵, and cell–cell adhesions and aggregates as found in epithelial tissues and tumours. Deciphering the roles played by these different mechanisms on the nonlinear response of tissues could provide important insights into cell mechanobiology under external mechanical forces, especially in the context of development⁶⁶ and disease^{67,68}, and offer a rigorous guideline for designing tissue-mimetic soft materials.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41567-025-02869-x>.

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Methods

Composite hydrogel

The hydrogels were prepared using a similar protocol previously reported for alginate⁷⁰, by mixing aqueous solutions of low-methoxy pectin (Unipectin 700, Cargill) with CaCO_3 at a stoichiometric ratio of 1 Ca^{2+} :2 COO^- present in the galacturonic acid groups. Carbopol (940, Lubrizol) and dextran (Sephadex A-25 and G-25, Cytiva) particles were then added to the system. Last, glucono-D-lactone was added at a stoichiometric ratio of 1 Ca^{2+} :3 glucono-D-lactone to induce Ca^{2+} dissolution and gelation. The composite hydrogels had a final composition of 1 wt% pectin, 0.5 wt% Carbopol (Supplementary Fig. 4 shows the flow curve) and varying the percentage by weight of dextran to meet a specified target volume fraction of their swollen state ϕ (Supplementary Fig. 2).

Confocal imaging

Composite hydrogel samples were made in well plates with #1.5 cover glasses, and were imaged using a Nikon ECLIPSE Ti2 inverted microscope with a $\times 20$ objective (numerical aperture, 0.75) at room temperature, using bright-field (Fig. 1b and Supplementary Fig. 3) and 488 nm (Supplementary Fig. 6) excitations. The large images shown in Fig. 1b and Supplementary Fig. 3 were acquired by stitching a 4×4 field in the x - y dimensions with 25% overlap configurations in the NIS-Elements software (v.5.21.03).

Fluorescent pectin

Pectin polymers conjugated with 6-aminofluorescein (Sigma) were synthesized following the adaptations of published protocol on alginate⁷¹. Pectin was first dissolved in 0.1 M of 2-(*N*-morpholino)ethanesulfonic acid (Sigma) buffer, pH 6.5, at 1 wt%. Then, *N*-hydroxysulfosuccinimide (Thermo Fisher) and 1-ethyl-3-(dimethylaminopropyl) carbodiimide (Sigma) were added at a 1/10 molar ratio to the galacturonic acid groups in the pectin. After *N*-hydroxysulfosuccinimide and 1-ethyl-3-(dimethylaminopropyl) carbodiimide were dissolved in the solution, 6-aminofluorescein, which was previously dissolved in dimethyl sulfoxide (Sigma), was added to the mixture, at a final concentration of 500 μM in a 1 wt% solution of pectin. The reaction was allowed to proceed overnight, after which the polymer was dialysed (molecular weight cut-off of 3,500 Da) against deionized water for 3–4 days and lyophilized before use for making composite hydrogels.

Tissues

All animal experiments were approved by the Massachusetts Institute of Technology Committee on Animal Care, protocol no. 0720-048-23. Tissues were collected from 20-week-old outbred CD-1 mice (Charles River Laboratory) that were euthanized by CO_2 asphyxiation and confirmed via a cervical dislocation. Lung, heart, liver and omentum adipose tissue were collected from the mice, stored in phosphate-buffered saline solution on top of ice and analysed within 6 h of euthanasia and collection (sample pictures are shown in Supplementary Fig. 21). PD liver was prepared by immersing a liver sample in 2% Triton X solution for 24 h to wash the cell membrane from the tissue, following adaptations of literature protocol³.

Rheology

Compression and shear experiments were conducted using Anton Paar MCR-302 and TA DHR-3 rheometers. Compression experiments were done by performing a $\lambda = 0.025$ compressive step strain every 500 s, superposed by a small-amplitude oscillatory shear with an imposed shear strain of $\gamma_0 = 0.005$. LAOS experiments were performed by collecting the steady-state waveforms of the stress–strain response at each γ_0 . For composite hydrogels, pre-gel mixtures were loaded onto MCR-302 (25-mm parallel plate) or DHR-3 (20-mm parallel plate), and allowed to undergo gelation for 3 h before compression or shear characterization (the gelation profile is shown in Supplementary Fig. 1). Regular stainless

steel plates were used for characterizing the composite hydrogels as we observed minimal differences when using sandpaper (Supplementary Fig. 16). Tissue samples were loaded onto a MCR-302 (10-mm parallel plate) or DHR-3 (8-mm parallel plate) rheometer, which was coated with sandpaper (Trizact A10, 3M) to minimize slip effects. Tissue samples were loaded until a normal force of 0.1 N was reached, and trimmed around the rim of the plate before proceeding with the analysis. The outermost rim of the composite hydrogel and tissue samples were then sealed with mineral oil to prevent dehydration during testing.

Calculations of elastic stress

The elastic stress $\sigma'(t)$ and viscous stress $\sigma''(t)$ contributions to the total stress $\sigma(t)$ response of an oscillatory strain waveform $\gamma(t) = \gamma_0 \sin(\omega t)$ from LAOS experiments were isolated using protocols within the MIT-laos package⁷². In brief, the stress contributions are isolated through a Fourier decomposition of the odd harmonics of the sine and cosine components of $\sigma(t)$, that is,

$$\begin{aligned}\sigma'(t) &\equiv \gamma_0 \sum_{n(\text{odd})} G'_n(\omega, \gamma_0) \sin n\omega t \\ \sigma''(t) &\equiv \gamma_0 \sum_{n(\text{odd})} G''_n(\omega, \gamma_0) \cos n\omega t\end{aligned}\quad (3)$$

Data availability

Additional data regarding the study are available from the corresponding authors upon request.

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Author contributions

J.S. conceived the project. J.S. and G.H.M. designed the study. J.S. and S.Y. performed the experiments. E.D.-Y. prepared the tissue specimens for the study. G.H.M. supervised the study. J.S. and G.H.M. analysed the data and wrote the manuscript with input from all authors.

Competing interests

The authors declare no competing interests.

Additional information

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