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From mechanical resilience to active material properties in biopolymer networks

Federica Burla[1,2], *Yuval Mulla*[1,2], *Bart E. Vos*[1], *Anders Aufderhorst-Roberts*[1] *and Gijsje H. Koenderink*[1]*

Abstract | The cells and tissues that make up our body manage contradictory mechanical demands. It is crucial for their survival to be able to withstand large mechanical loads, but it is equally crucial for them to produce forces and actively change shape during biological processes such as tissue growth and repair. The mechanics of cells and tissues is determined by scaffolds of protein polymers known as the cytoskeleton and the extracellular matrix, respectively. Experiments on model systems reconstituted from purified components combined with polymer physics concepts have already uncovered some of the mechanisms that underlie the paradoxical mechanics of living matter. Initial work focused on explaining universal features, such as the nonlinear elasticity of cells and tissues, in terms of polymer network models. However, there is a growing recognition that living matter exhibits many advanced mechanical functionalities that are not captured by these coarse-grained theories. Here, we review recent experimental and theoretical insights that reveal how the porous structure, structural hierarchy, transient crosslinking and mechanochemical activity of biopolymers confer resilience combined with the ability to adapt and self-heal. These physical concepts increase our understanding of cell and tissue biology and provide inspiration for advanced synthetic materials.

From a physicist's perspective, cells and tissues are fascinating materials because they combine an extraordinary mechanical strength with the ability to grow, reshape and adapt to environmental conditions. This paradoxical combination of strength and dynamics is essential for supporting life. Mechanical strength is crucial because cells and tissues constantly experience large mechanical loads[1]. With every breath we take, endothelial cells lining blood vessels and epithelial cells in the lung, for instance, experience large tensile stresses. With every step we take, muscles, tendons and skin stretch while cartilage compresses. Cells and tissues are able to cope with these mechanical challenges because they are supported by filamentous protein networks that provide an efficient means of mechanical scaffolding. Unlike man-made polymers, however, biopolymer networks not only provide mechanical support but also actively reconfigure themselves. Cells are able to actively adjust their stiffness in response to environmental conditions and produce forces that drive cell division and motility. At the tissue level, cellular force generation drives the formation of tissues and organs in developing embryos and the regeneration of tissues in adult organisms.

Cells are mechanically supported by the cytoskeleton, a composite network of three types of protein filaments: actin filaments, microtubules and intermediate filaments[2] (FIG. 1a). It is generally believed that intermediate filaments are particularly important for the protection of cells against large deformations, as they form resilient and long-lived elastic networks. By contrast, actin filaments and microtubules form dynamic networks that actively generate forces with the aid of motor proteins and proteins that regulate filament polymerization and depolymerization. Connective tissues such as skin and arteries are supported by the extracellular matrix, which is also a composite network that comprises polymers (FIG. 1b) with complementary physical properties[3]. Collagen forms a rigid fibrillar network that endows tissues with a high tensile strength, whereas proteoglycans and glycosaminoglycans form a soft hydrogel that holds water and confers resistance against compressive loads. In addition, connective tissues contain varying amounts of the elastomeric protein elastin and other fibrous proteins, such as fibronectin and laminin, which regulate cellular functions.

Cells adhere to the extracellular matrix (FIG. 1c) through transmembrane proteins known as integrins,

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Key points

- Cells and tissues are supported by biopolymer scaffolds that are mechanically resilient yet dynamic. There is a growing realization that biopolymer networks acquire these unique features from their hierarchical structure combined with internal mechanochemical activity.
- Biopolymer networks are embedded in water and therefore experience a strong coupling with the solvent, resulting in poroelastic effects.
- Fibrous networks respond to cyclic mechanical loading with plastic effects, self-healing and fracture. These responses originate from all structural levels — from molecule to fibre to network.
- Non-equilibrium activity causes biopolymer networks to undergo active stiffening, fluidization or self-driven flow, enabling a cell to deform.
- Composite biopolymer systems, in which all these mechanisms act together, endow cells and tissues with their adaptive mechanical properties.

which directly bind components of the extracellular matrix (such as collagen and fibronectin) and indirectly couple to the actin and intermediate filaments of the cytoskeleton through accessory proteins[4]. Through these adhesion complexes, contractile forces generated by the actin cytoskeleton are transferred to the extracellular matrix. Cells thereby actively remodel and tense the extracellular matrix, a process that is essential for tissue formation and wound healing. Conversely, the architecture and mechanical properties of the matrix strongly influence cell behaviour. Cells probe the physical properties of the matrix through the contractile forces they apply at integrin adhesions (a process known as mechanosensing), and they convert this mechanical information into biochemical signals that elicit a cellular decision such as cell growth and differentiation (which is known as mechanotransduction). In the past decade, it has become well established that mechanical forces steer many essential biological processes, including wound healing and embryonic development[5], as well as pathological processes such as cancer metastasis[6]. This realization has driven the emergence of mechanobiology as a new research field[7–11]. To resolve the molecular mechanisms involved in cellular mechanosensing and mechanotransduction, we need to understand the mechanical response of the individual networks involved in the communication processes between the cell and its environment, that is, between the cytoskeleton and the extracellular matrix.

Two fundamentally different approaches can be taken to investigate the physical basis of cell and tissue mechanics. The first approach is top down and involves mechanical measurements and phenomenological modelling of whole cells or tissues. Such measurements have revealed that living matter exhibits surprisingly universal mechanics. First, cells behave as viscoelastic materials with a power-law dependence of the elastic and viscous shear moduli on the deformation frequency, which suggests that they dissipate elastic stresses with a broad range of relaxation times[12]. Note that the elastic and viscous shear moduli are generally referred to as storage and loss moduli, respectively, in the rheology and polymer physics literature. Second, cells and tissues exhibit a nonlinear elastic response to mechanical loading. Cells and tissues often strain-stiffen but, depending on the rate, amplitude and type of loading (that is, compression, shear or tension), can also soften[13–15]. Third, cells and tissues are usually under substantial internal stress. The contractile activity of cells generates stress in the cytoskeleton, which is transferred to the extracellular matrix through integrin adhesions[16,17]. Owing to their charged nature, proteoglycans in the extracellular matrix can generate additional mechanical stress[18]. Unfortunately, elucidating the physical mechanisms that underlie these intriguing collective mechanical properties is extremely challenging owing to the molecular and structural complexity of living systems and the presence of mechanochemical feedback. Furthermore, cytoskeletal biopolymers are crosslinked by specialized crosslinker molecules that are at least partially redundant, and the depletion of one protein can lead to upregulation of another, making it difficult to disentangle their functions[19]. This complexity has motivated a second, bottom-up approach to cell and tissue physics. In this approach, components of the cytoskeleton and/or the extracellular matrix are purified and studied in isolation or together with a limited set of regulatory proteins. This reductionist approach has successfully driven the development of quantitative theoretical frameworks to describe cell and tissue mechanics and biological processes such as cell migration[20,21]. Owing to their large size (~10–100 nm in diameter) and large bending rigidities compared with those of typical synthetic polymers, biopolymers are usually modelled as elastic beams or semiflexible polymers. However, there is a growing realization that biopolymers exhibit many material properties that are not captured by these simple models.

Here, we review recent insights into the physical basis of cell and tissue mechanics, with a focus on bottom-up experimental studies coupled with theoretical modelling. We begin by discussing the elastic properties of biopolymer networks, for which coarse-grained polymer models explain some aspects of cell and tissue mechanics, such as their strain-stiffening behaviour. However, these models neglect important components of biopolymer networks, such as the solvent that they are coupled to and the resulting poroelastic effects, as well as the hierarchical structure of the filaments. We therefore assess recent advances in understanding poroelastic effects and explore how the hierarchical nature of filaments gives rise to unexpected mechanical effects, such as their high extensibility. We further address how limitations to purely elastic models that neglect viscoelasticity can be overcome by considering time-dependent and plastic effects. There is a growing recognition of the importance of plastic effects in biopolymer networks, prompted by observations of permanent extracellular matrix remodelling in the context of tumour cell invasion[11]. In biopolymer networks, remodelling comes from not only external sources but also active processes made possible by ATP hydrolysis in the cytoskeleton, a feature we consider in a section on active material properties. Finally, we assess how nature combines all of the above mechanisms in composite networks, leading to, for example, the high extensibility of arteries and the ability of cells to deform while being mechanically stable. We conclude by pointing to the future research directions that are needed to bridge the gap between our understanding of in vitro model systems and real living systems.

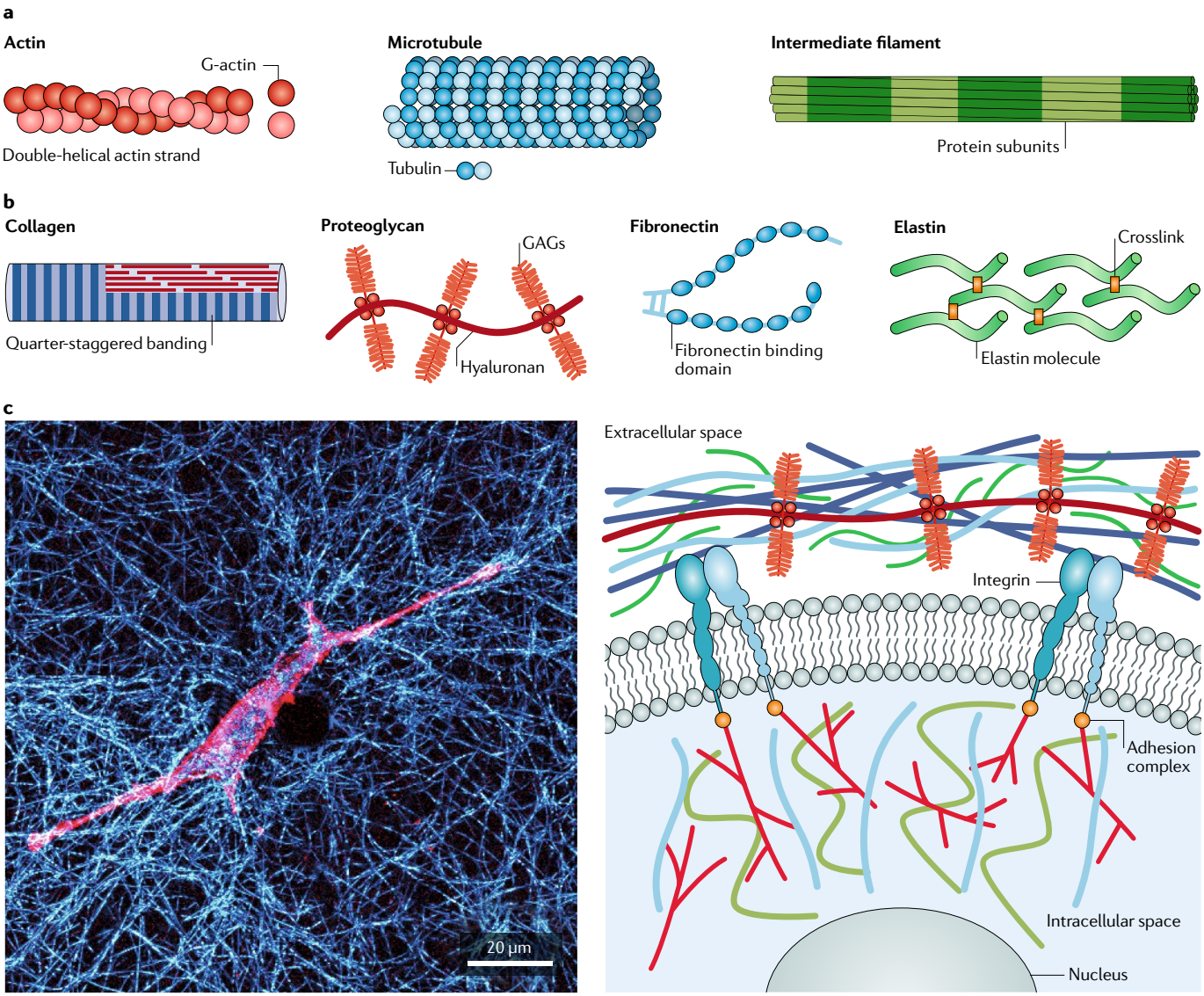

Fig. 1 | **Components of biopolymer networks.** Cells and tissues are mechanically supported by biopolymer networks known as the cytoskeleton and extracellular matrix, respectively. **a** | Illustration of the three filaments that constitute the cytoskeleton of a cell. **b** | Illustration of the most prevalent biopolymers in the extracellular matrix. **c** | The left part shows a confocal microscopy image of a cell (actin is shown in red) adhered to a collagen matrix (blue fibres). The right part shows a schematic of the cytoskeleton and extracellular matrix connected across the cell membrane by integrin adhesion proteins. Note that the extracellular matrix in vivo is 3D in some tissues, such as skin, but forms a 2D sheet in other tissues, such as epithelia. GAG, glycosaminoglycan. Panel **c** (left part) courtesy of C. Martinez-Torres and F. Burla, AMOLF, Netherlands.

Biopolymer network elasticity

Cytoskeletal and extracellular polymers are supramolecular filaments with a highly organized molecular structure dictated by specific interactions between the constituent proteins. Examples are the double-helical architecture of actin filaments and the quarter-staggered packing structure of collagen fibres (FIG. 1a,b). Cytoskeletal filament assembly is driven by reversible non-covalent interactions. This dynamic assembly is integral to the biological functions of the cytoskeleton, in which actin filaments and microtubules often need to assemble or disassemble rapidly in response to biochemical or mechanical signals. By contrast, extracellular matrix polymers such as collagen are more stable owing to covalent crosslinks created by enzymes[22].

Mechanical models of cytoskeletal and extracellular matrix polymers usually coarse-grain the filaments as smooth linear rods that resist bending with a modulus κ and stretching with a modulus μ. At finite temperatures, thermal fluctuations cause the filaments to bend as a function of their persistence length (l_p), which is defined as the decay length of angular correlations along the polymer contour. The persistence length is related to the bending modulus by $\kappa = k_B T l_p$, where k_B is Boltzmann's constant and T is the temperature. Biopolymers are categorized on the basis of the ratio between the persistence length and total (or contour) length (L) as flexible ($l_p \ll L$), semiflexible ($l_p \approx L$) or stiff ($l_p \gg L$). Collagen fibres and microtubules have persistence lengths in the range of a few millimetres and contour lengths on

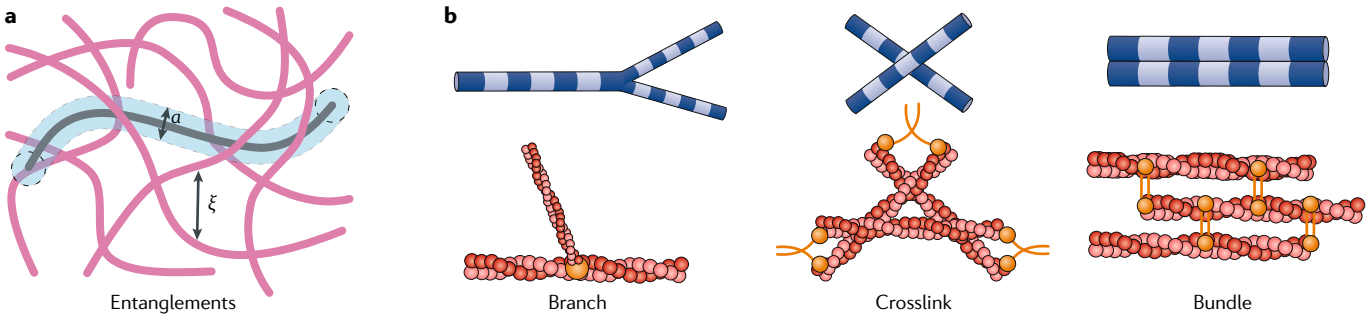

Fig. 2 | **Mechanisms of biopolymer network formation. a** | Biopolymers entangle when their density is sufficiently high such that they sterically hinder each other's transverse motion. The cylinder indicates the snake-like path along which each polymer is forced to reptate. The arrows indicate the tube width (*a*) and network mesh size (*ξ*). **b** | Branches, crosslinks and bundles are key components of filament networks and form through intermolecular interactions between the filaments, as in the case of collagen (top parts), or with the aid of accessory proteins, as in the case of actin (bottom parts). Panel **a** is adapted with permission from REF.[182], RSC.

the order of several micrometres, and thus collagen fibres are examples of stiff filaments. By contrast, actin filaments and intermediate filaments have persistence and contour lengths in the micrometre range and are therefore semiflexible[23–25]. An example of a flexible biopolymer is hyaluronan, a polysaccharide in the extracellular matrix with a persistence length of ~4–8 nm and contour length of several hundreds of nanometres[26].

Biopolymers are assembled into load-bearing networks by a variety of mechanisms. The simplest mechanism is by entanglements that naturally arise from steric interactions (FIG. 2a). At sufficiently high densities, polymers constrain each other's motions to snake-like paths along their contour, as conceptualized by the reptation model[27,28] (FIG. 2a). In this model, each filament is considered to be constrained in its motion to a narrow tube formed by contacts with the surrounding filaments[27]. As cytoskeletal filaments have lengths of hundreds of nanometres up to several micrometres, it has been possible to directly observe filament reptation by fluorescence microscopy[29]. Entangled biopolymer solutions can store elastic energy only on short timescales, because at longer timescales, the filaments escape the constraints imposed by entanglements[30]. Long-term mechanical stability is therefore possible only in the presence of long-lived filament interactions, which can occur by branching or crosslinking (FIG. 2b). In the cytoskeleton, actin filaments and microtubules are branched and crosslinked by a large set of specialized proteins[31,32], whereas intermediate filaments are crosslinked through a combination of accessory proteins and cation-mediated interactions[33]. The transient nature of these filament connections turns cytoskeletal networks into viscoelastic materials. By contrast, the extracellular matrix has a more elastic character owing to covalent crosslinking. For example, the collagen framework is covalently crosslinked by lysyl oxidase[22]. When polymerized on its own, purified collagen tends to form networks through a combination of branching and crosslinking[34,35], whereas in the body, collagen assembly and mechanics are tightly regulated in a tissue-specific manner by cells and accessory matrix molecules[36]. In order to recreate the complex regulation of collagen mechanics in vitro, artificial methods of collagen crosslinking have been used; for example, ribose[37] and transglutaminase[38] have been used as crosslinking agents.

Measurements on reconstituted biopolymer networks have revealed that these materials exhibit a general tendency to stress-stiffen in response to shear or uniaxial tensile loads and to stress-soften under compressive loads[39–42] (FIG. 3a). Theoretical modelling has shown that these nonlinear elastic properties are an intrinsic feature of filamentous networks. Compression-induced network softening involves a competition between softening due to polymer buckling and stiffening due to polymer densification upon solvent efflux[40–43]. Much more is known about the stiffening response upon tensile or shear loading. Interestingly, the mechanisms that govern stiffening are fundamentally different for semiflexible and rigid polymer networks. Semiflexible polymer networks stiffen because the conformational entropy of the polymers decreases as they are pulled taut along the direction of principal strain[44] (FIG. 3b). The elastic modulus can be calculated by averaging over the entropic force–extension response of the constituent filaments[39], provided that the network is densely crosslinked so that it deforms uniformly (that is, affinely) down to length scales on the order of the crosslink distance[45]. The elastic modulus is expected to increase with applied (shear) stress according to a power law with an exponent of 3/2, which is indeed observed for actin and intermediate filaments[46,47]. The onset strain at which stiffening sets in is governed by the amount of excess length stored in thermal fluctuations of polymer segments between adjacent crosslinks and is therefore a function of the persistence length and crosslink density. Networks of actin and intermediate filaments are highly strain-sensitive because stiffening sets in at strains of just a few per cent, and the stiffness can easily increase by a factor of 10–100 before rupture. This strain sensitivity is believed to mechanically protect cells by preventing large deformations. Moreover, the strain sensitivity enables cells to tune their stiffness by molecular motor activity, as discussed below. Given these advantages, there is a growing interest in mimicking strain sensitivity in synthetic polymer gels. Although synthetic polymers are typically flexible[39], several groups have successfully created synthetic polymers that are sufficiently stiff to exhibit strain sensitivity[48–50].

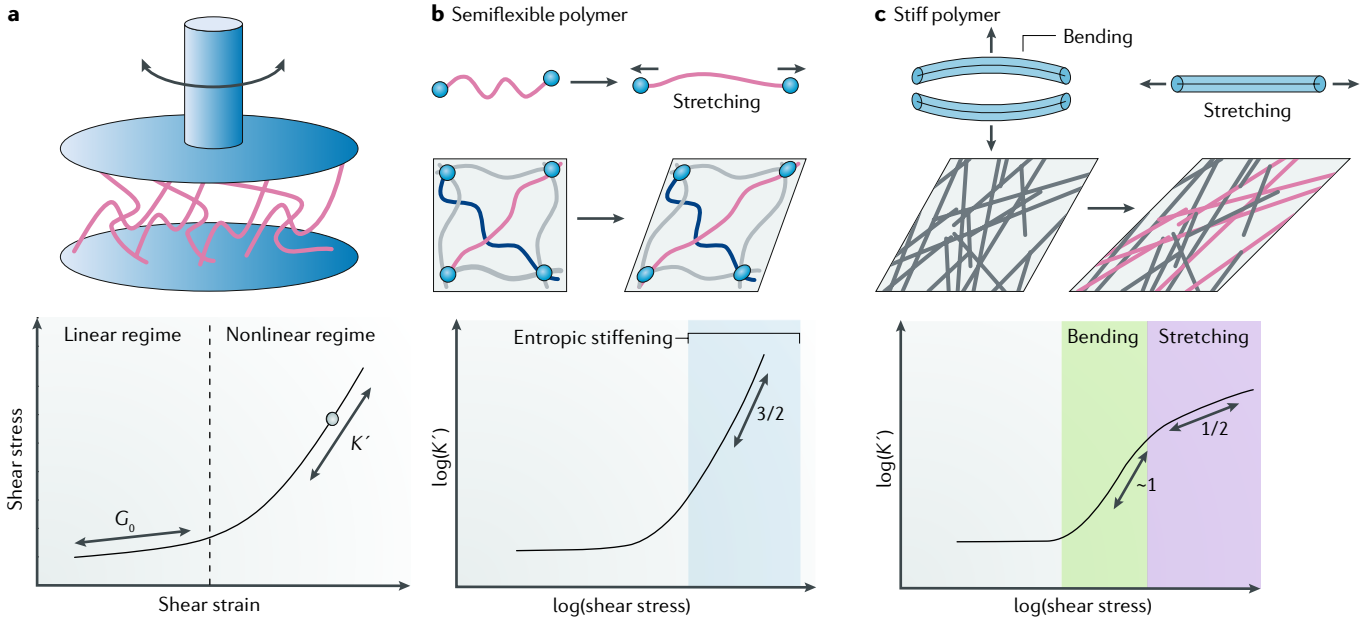

Fig. 3 | **Nonlinear elasticity in biopolymer networks. a** | The nonlinear elastic response of biopolymer networks to strain can be probed by subjecting networks polymerized between two plates to an oscillatory or steady shear deformation (shown in the top part). The stress–strain response (shown in the bottom part) is linear at low strain, at which the slope gives the linear modulus (G_0), but becomes nonlinear at high strain. In this nonlinear regime, the derivative of the stress–strain curve gives the differential modulus (K'). **b** | Semiflexible polymer networks strain-stiffen owing to the entropic resistance of the thermally undulating filaments against stretching (shown in the top part). Filaments under tension (pink, middle part) extend by stretching out thermal fluctuations, while filaments in the opposite direction (blue) are compressed. The pulling-out of thermal fluctuations gives rise to a characteristic 3/2 power-law stiffening. **c** | Stiff polymer networks strain-stiffen by undergoing a transition from a soft, bending-dominated state to a stiff, stretching-dominated state (shown in the top part), in which filaments are aligned along the direction of strain (pink, middle part). This gives rise to a power-law stiffening regime with an exponent of ~1 at moderate stress and a regime with an exponent of 1/2 at high stress.

Networks of stiff (that is, athermal) filaments such as collagen also strain-stiffen, but in this case, the nonlinearity is an emergent phenomenon that arises at the network level (FIG. 3c). This form of nonlinearity is related to the network connectivity. As biopolymers form networks through a combination of bundling, branching and crosslinking, the average coordination number (that is, the number of fibrils meeting at a junction) is in the range of three to four[34,35]. These networks are referred to as subisostatic because the coordination number is below the Maxwell criterion of six required for the mechanical stability of networks of springs[51]. However, unlike springs, fibres can form stable subisostatic networks because of their large bending rigidity[52]. Filamentous networks are soft at low strains because they deform in a non-affine manner dominated by fibre bending[53,54]. However, shear or tensile strains drive a transition to a rigid state dominated by fibre stretching, owing to the alignment of fibres along the principal direction of strain. This transition occurs at a critical strain determined by the network connectivity[35,54,55]. Collagen networks are highly strain-sensitive given that nonlinearity usually sets in at strains of only ~10% and the stiffness can increase by 100-fold before network rupture. Strain stiffening is thought to help prevent tissue rupture while also promoting long-range mechanical communication between cells[56].

The effects of poroelasticity

The network models described above neglect the fact that biopolymer networks are coupled to the solvent in which they are embedded. Biopolymer networks are biphasic systems that combine a solid porous phase composed of protein fibres with a fluid phase that occupies ~70% of the total volume in cells and >95% in reconstituted networks. Compressive or tensile deformations that change the volume of the system will necessarily induce fluid flow through the network owing to the incompressibility of water (FIG. 4a). This causes a time-dependent mechanical response that is referred to as poroelasticity[57]. When the deformation is fast, the system will respond like an incompressible material because the load is supported primarily by the incompressibility of the interstitial fluid[58]. By contrast, the system responds like a compressible material when the deformation is slow enough to allow for fluid outflow (in the case of compression) or inflow (in the case of extension). The typical timescale (τ) for a fluid of viscosity η to flow across a distance d through a polymer network with pore size ξ and shear modulus G can be estimated using a two-fluid model for a linearly elastic polymer network in a viscous background fluid[59,60], according to $\tau \approx \eta d^2/Gk$. Here, $k \approx \xi^2$ is the hydraulic permeability of the network.

Poroelastic effects are well known in the context of tissue biomechanics, for example, in cartilage, in which

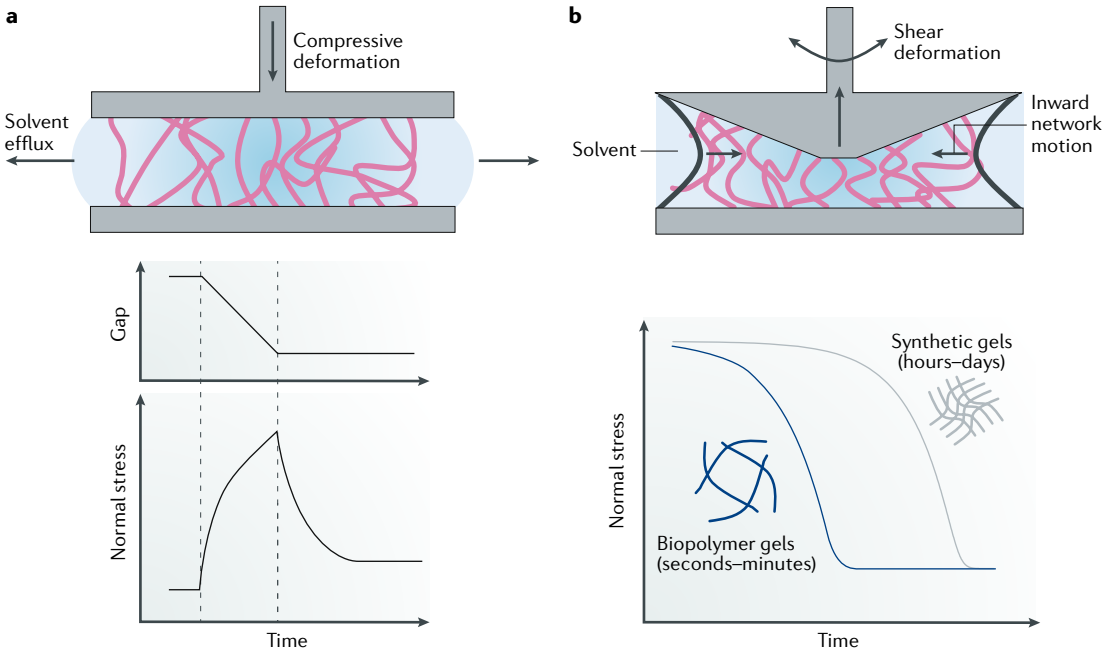

Fig. 4 | **Poroelasticity of biopolymer networks. a** | Upon compression, fluid is squeezed out of the network (shown in the top part), which generates a time-dependent normal force along the axial direction (shown in the bottom part). **b** | Upon shearing by rotation of the upper cone (shown in the top part), hydrostatic pressure is built up, which relaxes by an inward, radial contraction of the network relative to the solvent. The built-up normal stress decays exponentially (shown in the bottom part) as a function of time after the application of a constant shear stress at time = 0 with a time constant that is determined by the pore size. Therefore, the time constant tends to be much smaller for biopolymer gels than for synthetic gels.

flow through the porous extracellular matrix has an important role in load bearing and energy dissipation[61]. Poroelasticity was long thought to be unimportant inside cells because of their small size (5–20 μm). However, a seminal study on blebbing cells showed that poroelastic effects do affect cell mechanics on timescales relevant to cell motility[62]. When the cell membrane locally detaches from the cytoskeleton, spherical membrane protrusions called blebs are formed. Blebbing is driven by active contraction of a thin actin–myosin network that is directly located underneath the cell membrane (referred to as the actin cortex), which creates a compressive stress that initially only locally increases the hydrostatic pressure. Thereafter, fluid flow inflates the detached membrane. Pressure equilibration across the cell takes ~10 s owing to the small mesh size of the cytoskeleton (~10 nm) and the high viscosity of the cytoplasm[63]. Subsequent atomic force microscopy nanoindentation and microrheology measurements confirmed the importance of poroelasticity in determining cell mechanics[63,64]. Cells may exploit the slow equilibration of hydrostatic pressure to generate blebs or lamellipodial protrusions that drive locomotion[65–68]. Furthermore, cells can exploit poroelastic effects to modify their volume by water influx or efflux, which influences cell differentiation[69].

Poroelasticity also has a notable influence on the shear rheology of biopolymer networks even though shear deformations are volume conserving, unlike compressive and tensile deformations (FIG. 4b). Sheared polymer networks develop a normal force perpendicular to the direction of shear, which tends to be negative (that is, contractile) for semiflexible and rigid biopolymers and positive (that is, extensile) for flexible polymers[70]. This so-called Poynting effect[71] is of relevance in tissues such as the ventricular walls[72], which deform with a superposition of pure shear and extension and/or compression. In the presence of a fluid phase, the normal stress is always positive at short timescales because of the strong viscous coupling between the polymer network and the interstitial fluid. However, if the influence of the interstitial fluid is neglected, which is justified on timescales much longer than the timescale on which fluid flow occurs, the normal force from the Poynting effect is always calculated to be negative because network segments that develop tension outnumber nodes under compression for networks of springs[73], semiflexible polymers[70] and subisostatic networks of rigid fibres[54,55]. The normal stress switches in sign from positive to negative at timescales that correspond to the characteristic time for fluid flow (τ) introduced above[60]. In biopolymer systems, this timescale is comparable to experimentally observable and relevant in vivo timescales (seconds to minutes).

Structural hierarchy of biopolymers

When protein biopolymer networks are subjected to large (>50%) strains, fracture is inevitable unless the constituent polymers are able to elongate. Several cytoskeletal and extracellular protein biopolymers have been shown to be extremely extensible. This extensibility is a result of their molecular packing structure, which can change under strain (FIG. 5). One mechanism for filament elongation is the sliding of protein subunits along one

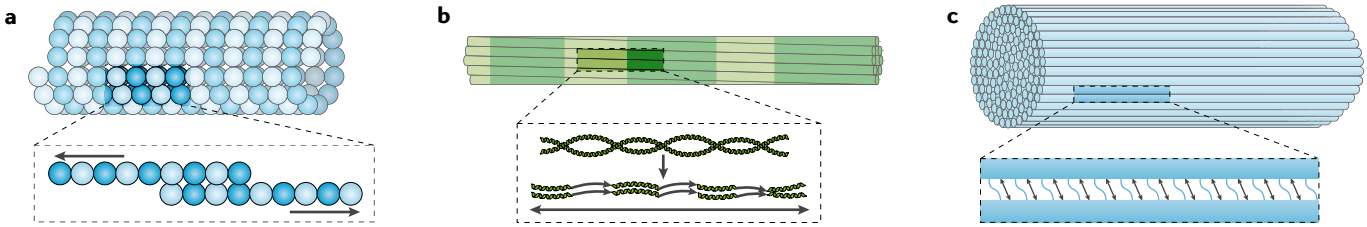

Fig. 5 | **Mechanisms of biopolymer stretching arising from the hierarchical structure.** The hierarchical assembly of biopolymers introduces several mechanisms for elongation of the constituent polymers. These mechanisms include sliding of the subunits, as observed with microtubules (panel **a**); forced unfolding of protein subunits, as observed with intermediate filaments (panel **b**); and stretching of disordered, flexible linkers that connect subunits, as observed with fibrin fibres (panel **c**).

another. Subunit sliding has been observed for microtubules and for collagen fibres, both of which are bundles of thin protofilaments associated by lateral interactions that are weaker than the longitudinal interactions[74,75]. Although the bending stiffness of both filament types is length dependent owing to protofilament sliding[74,75], the filaments are only moderately extensible and break at strains of 50–80%[23,76,77]. Bundling of actin filaments with crowding agents or crosslinker protein generates filamentous structures that can similarly lengthen by sliding, giving rise to rate-dependent force–extension behaviour[78,79].

An alternative mechanism for filament elongation is molecular unfolding of the protein subunits. This phenomenon is well documented for intermediate filaments, which can be stretched to more than three times their rest length using the tip of an atomic force microscope[25,80]. Spectroscopic measurements of the secondary structure content as well as X-ray scattering measurements of the molecular packing structure showed that stretching is mediated by a conformational transition of the protein subunits from α-helical to β-sheet[81,82], which sets in at tensile strains of ~10%. As a result of this conformational transition, the mechanical response of the filaments is strongly dependent on the loading rate[25]. A similar α-helix to β-sheet transition has been proposed to underlie the remarkable extensibility of the fibres formed by the blood clotting protein fibrin on the basis of X-ray scattering and spectroscopy measurements on fibrin networks[83–85] and single-molecule force spectroscopy[86,87]. However, this mechanism has not yet been definitively proved because the complex architecture of fibrin fibres also allows for alternative mechanisms for elongation. Fibrin fibres are thick bundles of ~100 protofibrils that are interconnected by long linker domains that are flexible because they are largely unstructured[88,89]. Several studies have suggested that linker stretching can account for the extreme extensibility of single fibrin fibres without the need to invoke unfolding of the structured domains[90,91]. It is possible that both mechanisms act in unison[92]. In a conceptually similar manner, the elastin filaments that confer resilience to skin, lung and vascular tissues combine long, disordered protein domains that are flexible and extensible with ordered domains that confer rigidity and tensile strength[93–95].

Owing to the numerous organizational levels of biopolymers, it remains a challenge to dissect the precise molecular mechanisms that orchestrate their elastomeric properties. Multi-technique approaches that correlate the mechanical response measured at the fibre or network level with molecular changes as measured through small-angle X-ray scattering[83,85] or vibrational spectroscopy[84] are needed, which should be coupled to multiscale modelling that connects molecular models to the coarse-grained network models described above through systematic coarse-graining[96]. The extensibility of intermediate filaments, fibrin and elastin enables cells and tissues to cope with large mechanical strains. Moreover, these filaments nonlinearly stiffen as they are stretched, which has been predicted to increase their flaw tolerance[97]. Both of these features would be highly desirable in synthetic tissues. Unfortunately, it is still difficult to realize the hierarchical structure that is characteristic of protein biopolymers in fully synthetic materials. Current efforts to make bioinspired resilient materials therefore mainly use either natural or designed recombinant proteins as building blocks[98–101]. DNA nanotechnology offers another promising route towards hierarchical materials[102].

The effects of dynamic crosslinking

Above, we have considered only the elastic properties of biopolymer networks. However, cells are viscoelastic materials with time-dependent mechanical properties owing to the transient binding of the linker proteins that mediate cytoskeletal crosslinking[103]. Dynamic crosslinking is crucial for cell functions such as migration, division and morphogenesis because it enables cells to dynamically remodel their interior and change shape[104,105].

The mechanical consequences of transient crosslinking have mainly been studied in the context of actin networks. At the single-molecule level, actin crosslinkers have typical bond lifetimes of several seconds[106,107], which, at the network level, translates into elastic behaviour at timescales shorter than the bond lifetime and viscoelastic flow on longer timescales[108]. This viscoelastic flow does not follow a simple Maxwell model with a single relaxation time but instead follows power-law behaviour characteristic of multiple relaxation times[109] (FIG. 6). In the linear elastic regime, both the elastic and viscous moduli show a frequency (ω) dependence of $\omega^{1/2}$. Although there is a single microscopic timescale for crosslinker unbinding, there is a broad range of macroscopic relaxation

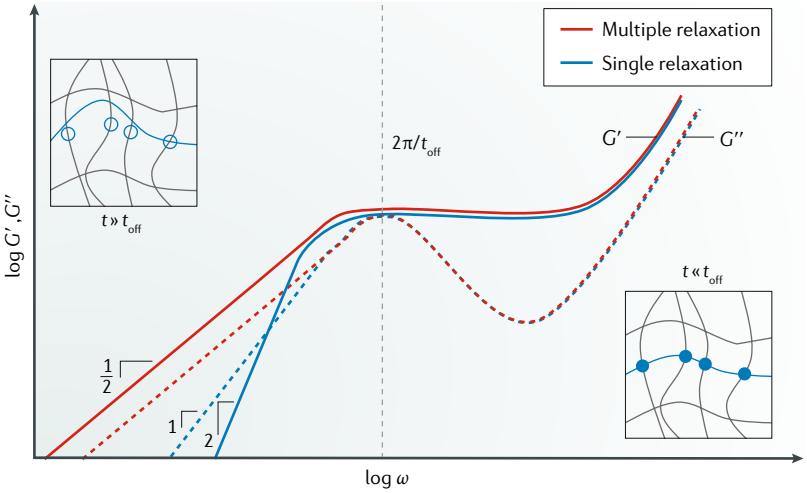

Fig. 6 | **Time-dependent rheology.** Polymer networks crosslinked by linkers that unbind at a rate of $1/t_{off}$ (where t_{off} is the unbinding time) show a time-dependent response to an oscillatory shear strain. Flexible polymer networks (blue lines) behave as Maxwell fluids that undergo a transition from elastic to fluid behaviour at a single characteristic frequency (ω), namely, ω_{off}. By contrast, semiflexible polymer networks (red lines) exhibit a broad distribution of relaxation times at frequencies below ω_{off} because stress relaxation requires many independent linker binding and unbinding events. This behaviour is shown in the insets, in which a single filament (blue) relaxes owing to multiple unbinding events (open circles) for time (t) $\gg t_{off}$, whereas it stays bound (filled circles) at $t \ll t_{off}$. The elastic and viscous shear moduli (G' and G'', respectively) increase at high frequencies owing to viscous drag, which hampers filament fluctuations. Adapted with permission from REF.[109], APS.

times, as each filament is crosslinked to the surrounding network by many crosslinker proteins. Stress relaxation therefore requires many independent binding and unbinding events[109,110]. In the nonlinear regime, the network response is dependent on time as well as stress because some crosslinker proteins exhibit slip-bond behaviour, which means that they dissociate faster upon application of force[106]. As a consequence of the slip-bond behaviour, actin networks soften at small loading rates owing to forced crosslinker unbinding, whereas they stiffen owing to nonlinear elasticity when the loading rate exceeds the crosslinker unbinding rate[111–113]. Intriguingly, several linkers, including α-actinin-4, filamin and vinculin, exhibit an opposite response to loading, known as catch-bond behaviour, whereby the bond lifetime initially increases with force because loading exposes a hidden binding site[114–116]. Indeed, catch bonds have been shown to delay the onset of relaxation and flow in actin networks[117]. A complication in studying reconstituted actin networks is that the structure is often determined by kinetics, owing to dynamic arrest during the polymerization process[118,119]. Kinetic trapping can cause the presence of long-lived internal stresses that take many hours to relax because of the slow dynamics of crosslinker-governed network relaxation[120,121]. It is unclear whether dynamic arrest is relevant in the context of cells, in which actin filaments are constantly disassembled and nucleated anew.

The extracellular matrix has a more elastic character than cells because the collagen framework is covalently crosslinked[22]. However, in reconstituted collagen networks, stress relaxation is significantly enhanced under strain owing to force-dependent unbinding of the bonds that hold the fibres together[122]. Furthermore, the interstitial space of collagen networks in tissues is filled with a soft hydrogel composed of hyaluronic acid and other transiently crosslinked components, which introduce additional mechanisms for stress relaxation[123]. It will be interesting to investigate the collective dynamics that result from the composite architecture of the extracellular matrix, especially as recent work has revealed that the viscous response of the matrix, in addition to rigidity, has a notable impact on the behaviour and function of cells[124,125].

Plasticity, fracturing and self-healing

Another important consequence of transient crosslinking is network plasticity, which is sometimes referred to as mechano-memory. Plasticity in cytoskeletal networks arises when mechanical loading causes dissociation of the crosslinkers, which subsequently diffuse and rebind elsewhere[103]. The redistributed crosslinks can freeze into a shear-induced fibre alignment, which causes network hardening[126,127]. In principle, structural changes in transiently crosslinked networks decay over time, but these effects are typically dynamically arrested owing to the slow, glassy stress relaxation[118,121]. When the shear stress is too high, actin networks completely lose mechanical percolation. Experimentally, the rupture strength is known to depend on the actin filament length and crosslink density[128] as well as on the microscopic properties of the crosslinkers, including their compliance[129,130]. The microscopic mechanism of rupture is still poorly understood. Through 1D modelling of bond arrays, it has recently been shown that the dynamic unbinding of crosslinks should make transient networks inherently prone to fracturing, as local fluctuations in crosslinker density propagate into large-scale cracks[131,132]. However, cytoskeletal networks are also inherently self-healing. Broken crosslinks are capable of re-forming[133], and the filaments themselves can even self-repair by the addition of new monomers[134,135]. In cells, the nucleation and growth of new filaments can further promote self-healing[136]. Nucleation and polymerization of actin[137] and extracellular filaments[138] is typically enhanced at sites undergoing mechanical stress, which further increases the self-healing capabilities of the network. The self-healing potential of transiently connected networks has already been adopted in materials science, as highlighted by several exciting examples of self-healing synthetic polymers[139,140].

Extracellular matrix networks, including collagen and fibrin, also exhibit plasticity upon cyclic loading, but in this case, the fibres themselves form new bonds in the deformed state[41,141]. Once the external stress is released, these new bonds are stretched, causing the build-up of internal contractile stress that nonlinearly stiffens the network. Owing to the complex molecular packing structure of the fibres, additional plasticity can arise at the level of the fibres themselves[142]. In the case of non-crosslinked networks of collagen or fibrin, cyclic shearing has been observed to cause fibre lengthening, presumably through subunit sliding, which delays the onset of strain stiffening[143]. There is a growing recognition that these mechano-memory effects are relevant for normal tissue

development as well as for pathological processes such as fibrosis and cancer progression. By exerting contractile forces, cells irreversibly remodel the extracellular matrix and generate rigid, aligned fibre tracts[142,144,145]. These rigid tracts in turn promote cellular force generation through positive mechanochemical feedback.

Active material properties

Above, we have considered how biopolymer networks respond to externally applied deformations. However, a unique feature of cells is the capability of the cytoskeleton to generate internal forces[146] that turn it into an active, out-of-equilibrium material. Actin filaments and microtubules provide tracks for the motion of motor proteins, as they have a structural polarity conferred by the head-to-tail assembly of the protein subunits. The two structurally distinct ends of actin filaments and microtubules are referred to as the plus and minus ends. Motor proteins recognize the polarity and step unidirectionally towards one end of each filament by coupling the energy released by ATP hydrolysis to a mechanical cycle.

The material properties of the actin cytoskeleton are mostly governed by a specific class of motors known as non-muscle myosin-II[13,147]. Individually, myosin-II motors cannot generate contractile stress because they have a low duty ratio, meaning that they are bound to actin for only a small fraction of the ATP hydrolysis cycle. Stress generation therefore requires myosin assembly into bipolar filaments of ~10–30 motors (FIG. 7a). As the motor domains are exposed at each end, bipolar myosin filaments can slide antiparallel actin filaments past one another. In the absence of crosslinks, this sliding activity can fluidize actin networks by relieving entanglement constraints[148], and this may contribute to myosin-driven softening of nonadherent (that is, suspended) cells[149]. By contrast, cells adhered to solid substrates through integrin-mediated adhesions are stiffened by myosin motor activity because the adhesions facilitate the build-up of contractile prestress. In vitro studies have shown that the build-up of contractile stress in actin networks requires a sufficiently high crosslink density such that the connectivity is above a percolation threshold[150,151]. In principle, extension should be equally likely as contraction. However, several mechanisms bias actin–myosin networks towards contraction[152]. An important contribution comes from the asymmetric response of crosslinked fibrous networks to compressive versus tensile strain[153,154]. Simulations have revealed that collective fibre buckling in the vicinity of a local contractile force centre will always rectify the stress towards strongly amplified isotropic contraction in disordered networks[154]. This same principle also applies to extracellular matrix networks containing contractile cells (FIG. 7b) and has been shown theoretically[154,155] and experimentally[156]. An alternative mechanism that biases actin–myosin networks towards contraction is polarity sorting[157]. In this case, myosin motors run towards the plus ends of actin filaments and form polar asters (an aster is a radial array of filaments with plus ends pointing inwards and motors accumulated in the centre) because they tend to end-dwell. These asters act as contractile nodes, which drive contraction in crosslinked networks. Irrespective of the mechanism of contraction, theoretical models predict that active stress will stiffen filamentous networks because of their nonlinear elastic response to stress[155,158,159] (FIG. 7c). Indeed, motor-driven stiffening has been experimentally confirmed for reconstituted actin–myosin networks[150,160] as well as for fibrin and collagen networks containing cells[56,156].

Microtubules combined with kinesin motors (FIG. 7a) exhibit a broader variety of behaviours than do actin–myosin networks depending on the presence of crosslinks and pre-existing filament alignment. Disordered crosslinked microtubule–kinesin networks contract because motors walk to the plus ends of microtubules and pause there, resulting in polarity sorting of microtubules into asters with the microtubule plus ends oriented inwards; motor activity on microtubules connecting adjacent asters leads to aster merging and ultimately macroscopic contraction[161]. By contrast, nematic solutions of microtubules exhibit extensile flow in the presence of kinesin motors because the motors move pairs of antiparallel microtubules in opposite directions[133,162]. Motor-driven expansion is, in principle, also possible in actin–myosin systems[163,164], but it is rarer,

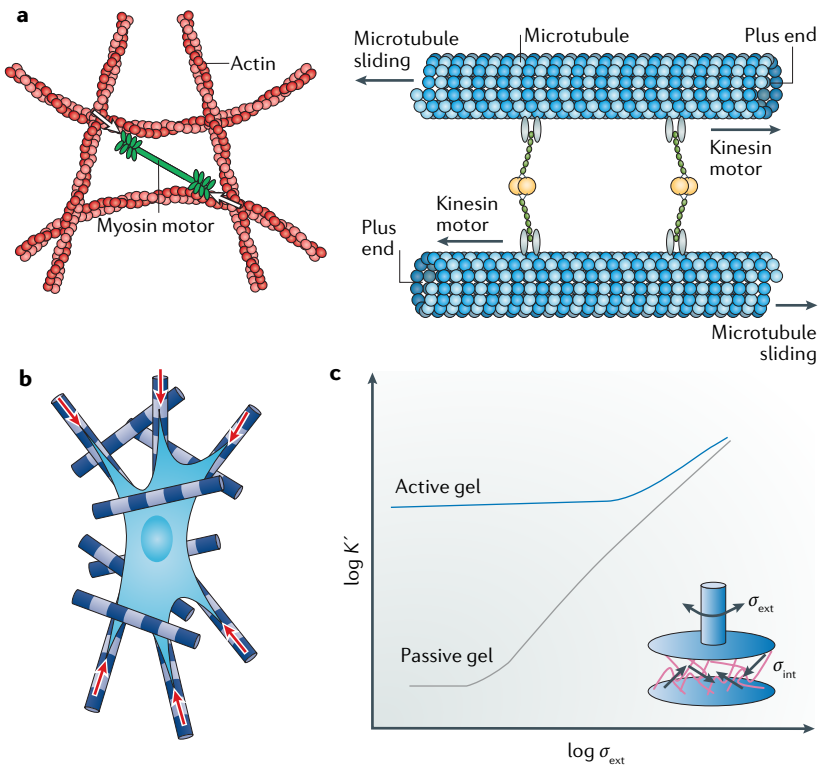

Fig. 7 | **Active control over biopolymer network mechanics by contractility.** **a** | Cytoskeletal contractility. Myosin motors form bipolar filaments that contract crosslinked actin networks by sliding antiparallel actin filaments past one another (shown on the left), and kinesin motors that crosslink two antiparallel microtubules slide them apart, reducing their overlap (shown on the right). **b** | Cells contract the extracellular matrix by transferring contractile forces generated by actin and myosin through focal adhesions. **c** | Active contraction makes cytoskeletal and extracellular matrix networks stiffer than their passive (equilibrium) counterparts because the elasticity of extracellular matrix networks responds nonlinearly to internal stress. In an active gel, the resulting differential shear modulus K'; is determined by a combination of external shear stress σ_{ext} and internal shear stress σ_{int} (shown in the inset).

probably owing to the smaller persistence length of actin filaments, which favours filament buckling.

Another important source of activity in the cytoskeleton is the constant turnover of actin filaments and microtubules, which is driven by nucleotide hydrolysis by the filaments themselves[165]. Although nucleotide hydrolysis also occurs in monomeric actin, monomers rapidly exchange nucleotides with the environment, whereas nucleotides are fixed in filamentous actin. Hydrolysis of ATP enables actin filaments to polymerize at one end while depolymerizing at the other end (a process known as treadmilling)[165], whereas hydrolysis of GTP enables microtubules to undergo stochastic switching between growth and shrinking (which is known as dynamic instability)[166]. The classical picture of actin treadmilling has been disputed, with the suggestion that ATP hydrolysis favours depolymerization of all old actin filaments[167]. Regardless of whether actin turnover occurs by treadmilling or depolymerization of old filaments, it is expected to dissipate motor-driven stress because depolymerization removes tensed filaments while new filaments are produced in a stress-free state[168–170]. Indeed, an experimental study confirmed that filament treadmilling speeds up stress relaxation in actin networks[171]. Experiments on cell extracts showed that the combination of motor activity and actin turnover leads to multiple dynamic steady states, including long-range flow patterns[172]. Given the complexity of cell extracts, which contain many thousands of distinct proteins[173], it will be interesting to test these findings in reconstituted networks.

The active material properties of cytoskeletal networks have already inspired several exciting synthetic active materials, such as synthetic polymer networks activated by fuel-dependent polymer treadmilling[174] or light-driven molecular rotors[175], and DNA-based networks driven by fuel-dependent processive enzymes[176].

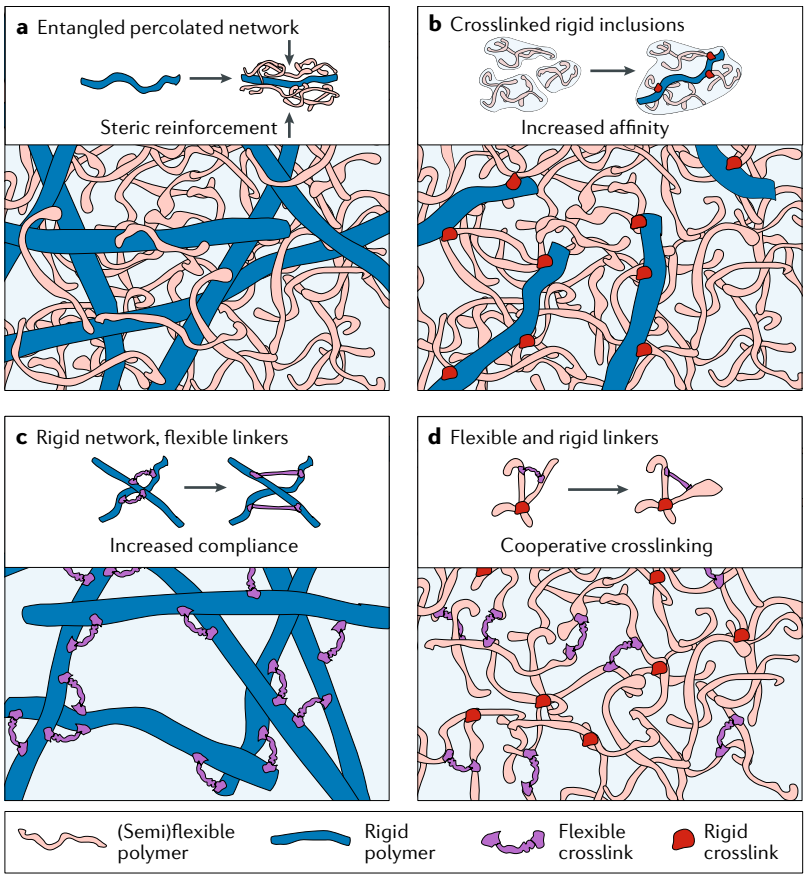

Fig. 8 | **Mechanical synergy in multicomponent biopolymer networks.** Composite networks offer additional degrees of freedom in mechanical function. Even simple combinations of rigid and flexible polymers and crosslinks produce diverse mechanical behaviours. **a** | In the absence of crosslinks, a dense background of flexible polymers can increase the rigidity of a second component by steric reinforcement. **b** | In composite networks, synergistic effects can occur even when one of the networks is not fully percolated. Here, rigid inclusions act as crosslinkers, which can make deformations of a second component more affine (uniform). **c** | Flexible crosslinkers can act as shock absorbers by increasing the compliance of a rigid network. **d** | Multiple crosslinkers with different rigidities can cooperate to fine-tune the mechanical response of a network. Panel **c** is adapted with permission from REF.[197], APS. Panel **d** is adapted from REF.[198], CC-BY-4.0.

Synergy in composite networks

In nature, the various processes described above often occur simultaneously, as both the cytoskeleton and the extracellular matrix are composite mixtures of biopolymers with different mechanical and dynamic properties. Despite using the same building blocks, the synergy between individual polymers with diverse properties enables access to a wide range of mechanical properties to meet the requirements of different cell and tissue types. Cartilage, for example, needs to simultaneously resist tensile and compressive loads and achieves this through the interplay between a fibrous collagen network and a proteoglycan meshwork[177]. Migrating cells need to combine resilience with directional motion through fluidization and therefore rely on coupling between actin, intermediate filaments and microtubules[178]. Composite biopolymer networks have only recently begun to be investigated by quantitative rheological measurements and theoretical modelling. The focus thus far has been on two-component systems, but even these simplified systems have a large parameter space in which the network mechanics can be tuned by variations in the persistence lengths of the two polymers, their relative and absolute densities and the interconnectivity between the two components (FIG. 8).

In theoretical studies, this complex phase space has been mainly explored in the limit of permanently crosslinked networks that combine rigid and flexible or semiflexible polymers (FIG. 8a). When both polymers form percolating networks, the linear elastic modulus of the composite can become substantially larger than the sum of the moduli of the separate networks[179]. In such systems, the biopolymer with a lower rigidity forms a denser elastic background owing to its smaller mesh size, which in turn increases the effective bending rigidity of the more rigid biopolymer[179,180]. Such a synergistic increase in the linear elastic modulus has been experimentally observed in composites of actin and the intermediate filament protein vimentin, which differ in persistence length by a factor of 10 (with $l_p = 10\,\mu m$ and $1\,\mu m$, respectively)[181]. Although this synergistic increase was not confirmed in a more recent study, this inconsistency could be a result of subtle differences in the filament

interactions[182]. Networks of flexible or semiflexible polymers have also been predicted to reinforce rigid polymers against compressive loads[180], an effect that has indeed been observed in actin–microtubule composites[183] and is thought to be important for cells crawling through soft matrices[184]. Recently, active superelasticity (that is, the ability to undergo large and reversible deformation by accommodating strain in an inhomogeneous manner) was observed in epithelial cells and attributed to the synergistic behaviour of actin and intermediate filament networks[185]. In the context of the extracellular matrix, collagen–hyaluronan composites were also reported to exhibit an enhanced resistance to compressive loading compared with collagen alone[186]. However, in this case, the mechanism was not elastic but viscous in origin: hyaluronan increases the viscosity of the fluid in the interstices of the collagen matrix and thus increases the hydraulic resistance to fluid outflow. As glycosaminoglycans tend to swell in hypotonic solutions, they can also induce prestress when interpenetrated with a collagen network[187]; this prestress can change the nonlinear elastic response of collagen owing to its stress sensitivity[188].

Mechanical enhancement can also be achieved for composites in which only one of the two polymers forms a percolating network (FIG. 8b). In this case, the percolated component determines the linear elastic modulus, whereas the non-percolated one influences the nonlinear elastic response[189]. Rigid polymer inclusions are expected to lower the threshold shear strain required to induce strain stiffening of semiflexible polymers by making the strain field more affine[189–192]. This effect has been confirmed experimentally for composite networks of actin and microtubules[193,194]. Furthermore, rigid polymer inclusions are predicted to induce compressibility in an otherwise almost incompressible matrix because they constrain the displacement field[195], a phenomenon observed in co-entangled actin and microtubule composites[196].

In the cytoskeleton, the crosslinks that connect the filaments are proteins that, in some cases, directly influence the network mechanics by contributing their own compliance (FIG. 8c). An extreme example is filamin, a V-shaped protein that has two actin binding domains that are connected by long and flexible linker domains. Filamin drastically changes the nonlinear elastic response of actin networks, from the 3/2 power-law stiffening observed with rigid crosslinks, such as α-actinin, to an approximately linear stiffening response[130]. This effect has been explained by modelling actin–filamin networks as composites of rigid filaments and worm-like chain crosslinkers[197]. Compliant crosslinks or combinations of crosslinkers with different rigidities thus provide additional parameters to tune the nonlinear mechanics of cytoskeletal networks[129,198,199] (FIG. 8d).

A challenge in experimental studies of composite networks is that the constituent polymers can influence each other's organization through steric constraints, direct interactions or depletion effects. Structural changes caused by such mutual interactions have been reported in, for example, composites of actin and intermediate filaments[200,201] and composites of collagen and glycosaminoglycan[202]. It will be important in future studies to gain better control over the network structure of composites through the assembly kinetics and the use of bifunctional crosslinking agents, such as plectins and spectraplakins[178]. An alternative approach is to create hybrids of biopolymers and synthetic polymers or fully synthetic hybrid networks; these systems enable better control over the interaction between the constituents and assembly conditions of the polymers[100,203]. Furthermore, the theoretical predictions of the relationship between the stress and strain fields in composite networks have yet to be examined experimentally by, for example, confocal rheometry[45].

Conclusions and outlook

Living matter is able to combine two contradictory mechanical functionalities: the capacity to resist substantial loads and the ability to actively change its shape, architecture and mechanics. Experiments on reconstituted biopolymers coupled with theoretical modelling have successfully unveiled some of the design principles that underlie these functionalities, but many open-ended questions remain. Arguably the most challenging of these is how living systems maintain mechanical strength while actively deforming. This ability is especially difficult to understand in the context of cells, because cell deformability requires transient crosslinking, but transient bond dynamics makes materials vulnerable to rupture. We speculate that catch-bond crosslinkers may help cells to circumvent this problem, as they tend to accumulate in stressed regions[204]. A further factor is the complementarity of the three cytoskeletal systems, which have traditionally been regarded as independent with separate cellular tasks. However, there is mounting evidence that these cytoskeletal systems function in a coupled manner through interactions mediated by crosslinker and motor proteins and shared signalling pathways[178]. Microtubules and actin stress fibres, for instance, align and polarize intermediate filaments, while aligned intermediate filament structures in turn serve as a long-lived template that guides microtubule growth[205]. Intermediate filaments also integrate the contractile forces generated by actin across the cell[206]. We anticipate that studies of reconstituted composite cytoskeletal networks will provide a powerful strategy to elucidate the collective active and passive material properties that emerge from cytoskeletal cooperation. The development of engineered selective crosslinkers will also help to elucidate the role of the interaction between different cytoskeletal components[207]. Future experimental progress will be aided by advanced techniques developed for measuring mechanics in situ, such as optical microrheology and molecular force sensors[7], whereas progress in modelling will benefit from advances in coarse-grained approaches and statistical frameworks to describe active matter[96,146,208]. Importantly, the emergence of optogenetic tools now makes it possible to bridge the gap between in vivo and in vitro experiments by selectively controlling signalling pathways with high spatiotemporal resolution[209]. Through this technique, it was, for example, revealed that viscoelastic timescales probed in vivo can exceed those of the internal components[210], which indicates that a wide variety of interactions exist in the same cell.

Although connective tissues are often regarded as more static structures than cells, everyone who has recovered from a broken bone or has performed body building knows that bones and muscles adapt to mechanical loading. In fact, the architecture of our bones is precisely optimized for the local loading conditions in the body. The dynamics that mediate this adaptivity are driven by cells, which constantly synthesize collagen and other extracellular matrix constituents and degrade the matrix by secreting proteolytic enzymes[211]. There is intriguing evidence that collagen displays a 'use it or lose it' functionality: collagen fibrils under high strain are protected from enzymatic degradation, whereas fibrils under low strain are enzymatically destroyed[212]. As a result, collagenous materials dynamically adapt to physiological loads, selectively strengthening and pruning themselves to retain a structure in the principal loading direction. Determining the mechanisms that lead to this counter-intuitive behaviour would be helpful in understanding pathologies such as fibrosis and would guide the design of materials for tissue regeneration.

Understanding the mechanical design principles of living matter is fundamental to elucidating the mechanistic basis of diseases associated with genetic defects in cytoskeletal and matrix proteins, such as skin fragility and heart muscle failure[213,214]. Furthermore, living matter has come to be regarded as a paradigmatic example of a growing class of soft condensed matter known as active matter[215]. Studies of reconstituted systems are providing an instructive road map for the creation of synthetic materials with life-like features. It remains a challenge to realize the active driving and hierarchical structuring that is unique to living matter. However, hybrid materials that combine synthetic and biological building blocks (proteins or even cells) provide a promising avenue.

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1. Freedman, B. R. et al. The (dys)functional extracellular matrix. *Biochim. Biophys. Acta* **1853**, 3153–3164 (2015).
2. Fletcher, D. A. & Mullins, R. D. Cell mechanics and the cytoskeleton. *Nature* **463**, 485–492 (2010).
3. Mouw, J. K., Ou, G. & Weaver, V. M. Extracellular matrix assembly: a multiscale deconstruction. *Nat. Rev. Mol. Cell Biol.* **15**, 771–785 (2014).
4. Horton, E. R. et al. Definition of a consensus integrin adhesome and its dynamics during adhesion complex assembly and disassembly. *Nat. Cell Biol.* **17**, 1577–1587 (2015).
5. Barriga, E. H., Franze, K., Charras, G. & Mayor, R. Tissue stiffening coordinates morphogenesis by triggering collective cell migration in vivo. *Nature* **554**, 523–527 (2018).
6. Discher, D. E. et al. Matrix mechanosensing: from scaling concepts in 'omics data to mechanisms in the nucleus, regeneration, and cancer. *Annu. Rev. Biophys.* **46**, 295–315 (2017).
7. Roca-Cusachs, P., Conte, V. & Trepat, X. Quantifying forces in cell biology. *Nat. Cell Biol.* **19**, 742–751 (2017).
8. Muncie, J. M. & Weaver, V. M. The physical and biochemical properties of the extracellular matrix regulate cell fate. *Curr. Top. Dev. Biol.* **130**, 1–37 (2018).
9. Vogel, V. Unraveling the mechanobiology of extracellular matrix. *Annu. Rev. Physiol.* **80**, 353–387 (2018).
10. Kirby, T. J. & Lammerding, J. Emerging views of the nucleus as a cellular mechanosensor. *Nat. Cell Biol.* **20**, 373–381 (2018).
11. van Helvert, S., Storm, C. & Friedl, P. Mechanoreciprocity in cell migration. *Nat. Cell Biol.* **20**, 8–20 (2018).
12. Fabry, B. et al. Scaling the microrheology of living cells. *Phys. Rev. Lett.* **87**, 148102 (2001).
13. Fernandez, P., Pullarkat, P. A. & Ott, A. A master relation defines the nonlinear viscoelasticity of single fibroblasts. *Biophys. J.* **90**, 3796–3805 (2006).
14. Trepat, X. et al. Universal physical responses to stretch in the living cell. *Nature* **447**, 592–595 (2007).
15. Perepelyuk, M. et al. Normal and fibrotic rat livers demonstrate shear strain softening and compression stiffening: a model for soft tissue mechanics. *PloS One* **11**, e0146588 (2016).
16. Ingber, D. E., Wang, N. & Stamenovic, D. Tensegrity, cellular biophysics, and the mechanics of living systems. *Rep. Prog. Phys.* **77**, 046603 (2014).
17. Grinnell, F. Fibroblast biology in three-dimensional collagen matrices. *Trends Cell Biol.* **13**, 264–269 (2003).
18. Lanir, Y. Mechanisms of residual stress in soft tissues. *J. Biomech. Eng.* **131**, 044506 (2009).
19. Kreis, T. & Vale, R. (eds) *Guidebook to the Cytoskeletal and Motor Proteins* (Oxford Univ. Press, 1993).
20. Broedersz, C. P. & MacKintosh, F. C. Modeling semiflexible polymer networks. *Rev. Mod. Phys.* **86**, 995 (2014).
21. Pritchard, R. H., Huang, Y. Y. & Terentjev, E. M. Mechanics of biological networks: from the cell cytoskeleton to connective tissue. *Soft Matter* **10**, 1864–1884 (2014).
22. Kagan, H. M. & Li, W. Lysyl oxidase: properties, specificity, and biological roles inside and outside of the cell. *J. Cell. Biochem.* **88**, 660–672 (2003).
23. van Mameren, J., Vermeulen, K. C., Gittes, F. & Schmidt, C. F. Leveraging single protein polymers to measure flexural rigidity. *J. Phys. Chem. B* **113**, 3837–3844 (2009).
24. Bartsch, T. F., Kochanczyk, M. D., Lissek, E. N., Lange, J. R. & Florin, E. L. Nanoscopic imaging of thick heterogeneous soft-matter structures in aqueous solution. *Nat. Commun.* **7**, 12729 (2016).
25. Block, J. et al. Nonlinear loading-rate-dependent force response of individual vimentin intermediate filaments to applied strain. *Phys. Rev. Lett.* **118**, 048101 (2017).
A pioneering experimental study of the stress–strain response of single intermediate filaments, demonstrating that monomer unfolding explains their remarkable extensibility.
26. Berezney, J. P. & Saleh, O. A. Electrostatic effects on the conformation and elasticity of hyaluronic acid, a moderately flexible polyelectrolyte. *Macromolecules* **50**, 1085–1089 (2017).
27. De Gennes, P. G. Reptation of a polymer chain in the presence of fixed obstacles. *J. Chem. Phys.* **55**, 572–579 (1971).
28. Doi, M. & Edwards, S. F. Dynamics of concentrated polymer systems. Part 1. — Brownian motion in the equilibrium state. *J. Chem. Soc. Faraday Trans. 2* **74**, 1789–1801 (1978).
29. Kas, J., Strey, H. & Sackmann, E. Direct imaging of reptation for semiflexible actin filaments. *Nature* **368**, 226–229 (1994).
30. Lang, P. & Frey, E. Disentangling entanglements in biopolymer solutions. *Nat. Commun.* **9**, 494 (2018).
31. Blanchoin, L., Boujemaa-Paterski, R., Sykes, C. & Plastino, J. Actin dynamics, architecture, and mechanics in cell motility. *Physiol. Rev.* **94**, 235–263 (2014).
32. Basnet, N. et al. Direct induction of microtubule branching by microtubule nucleation factor SSNA1. *Nat. Cell Biol.* **20**, 1172–1180 (2018).
33. Lin, Y. C. et al. Divalent cations crosslink vimentin intermediate filament tail domains to regulate network mechanics. *J. Mol. Biol.* **399**, 637–644 (2010).
34. Licup, A. J. et al. Stress controls the mechanics of collagen networks. *Proc. Natl Acad. Sci. USA* **112**, 9573–9578 (2015).
35. Sharma, A. et al. Strain-controlled criticality governs the nonlinear mechanics of fibre networks. *Nat. Phys.* **12**, 584–587 (2016).
A combined experimental and theoretical study showing that the nonlinear elasticity of fibrous networks such as collagen corresponds to a strain-controlled continuous phase transition.
36. Holmes, D. F., Lu, Y., Starborg, T. & Kadler, K. E. Collagen fibril assembly and function. *Curr. Top. Dev. Biol.* **130**, 107–142 (2018).
37. Gostynska, N. et al. 3D porous collagen scaffolds reinforced by glycation with ribose for tissue engineering application. *Biomed. Mater.* **12**, 055002 (2017).
38. Valero, C., Amaveda, H., Mora, M. & Garcia-Aznar, J. M. Combined experimental and computational characterization of crosslinked collagen-based hydrogels. *PloS One* **13**, e0195820 (2018).
39. Storm, C., Pastore, J. J., MacKintosh, F. C., Lubensky, T. C. & Janmey, P. A. Nonlinear elasticity in biological gels. *Nature* **435**, 191–194 (2005).
40. Chaudhuri, O., Parekh, S. H. & Fletcher, D. A. Reversible stress softening of actin networks. *Nature* **445**, 295–298 (2007).
41. Vos, B. E. et al. Programming the mechanics of cohesive fiber networks by compression. *Soft Matter* **13**, 8886–8893 (2017).
42. van Oosten, A. S. et al. Uncoupling shear and uniaxial elastic moduli of semiflexible biopolymer networks: compression-softening and stretch-stiffening. *Sci. Rep.* **6**, 19270 (2016).
43. Xu, X. & Safran, S. A. Compressive elasticity of polydisperse biopolymer gels. *Phys. Rev. E* **95**, 052415 (2017).
44. MacKintosh, F. C., Kas, J. & Janmey, P. A. Elasticity of semiflexible biopolymer networks. *Phys. Rev. Lett.* **75**, 4425–4428 (1995).
45. Liu, J., Koenderink, G. H., Kasza, K. E., Mackintosh, F. C. & Weitz, D. A. Visualizing the strain field in semiflexible polymer networks: strain fluctuations and nonlinear rheology of F-actin gels. *Phys. Rev. Lett.* **98**, 198304 (2007).
46. Gardel, M. L. et al. Elastic behavior of cross-linked and bundled actin networks. *Science* **304**, 1301–1305 (2004).
47. Lin, Y. C. et al. Origins of elasticity in intermediate filament networks. *Phys. Rev. Lett.* **104**, 058101 (2010).
48. Kouwer, P. H. et al. Responsive biomimetic networks from polyisocyanopeptide hydrogels. *Nature* **493**, 651–655 (2013).
49. Fernandez-Castano Romera, M. et al. Strain stiffening hydrogels through self-assembly and covalent fixation of semi-flexible fibers. *Angew. Chem. Int. Ed.* **56**, 8771–8775 (2017).
50. Yan, B. et al. Duplicating dynamic strain-stiffening behavior and nanomechanics of biological tissues in a synthetic self-healing flexible network hydrogel. *ACS Nano* **11**, 11074–11081 (2017).
51. Maxwell, J. C. L. On the calculation of the equilibrium and stiffness of frames. *Lond. Edinb. Dublin Philos. Mag. J. Sci.* **27**, 294–299 (1864).
52. Broedersz, C. P., Xiaoming, M., Lubensky, T. C. & MacKintosh, F. C. Criticality and isostaticity in fibre networks. *Nat. Phys.* **7**, 983–988 (2011).
53. Onck, P. R., Koeman, T., van Dillen, T. & van der Giessen, E. Alternative explanation of stiffening in cross-linked semiflexible networks. *Phys. Rev. Lett.* **95**, 178102 (2005).

54. Licup, A. J., Sharma, A. & MacKintosh, F. C. Elastic regimes of subisostatic athermal fiber networks. *Phys. Rev. E* **93**, 012407 (2016).
55. Jansen, K. A. et al. The role of network architecture in collagen mechanics. *Biophys. J.* **114**, 2665–2678 (2018).
56. Han, Y. L. et al. Cell contraction induces long-ranged stress stiffening in the extracellular matrix. *Proc. Natl Acad. Sci. USA* **115**, 4075–4080 (2018).
57. Biot, M. A. General theory of three-dimensional consolidation. *J. Appl. Phys.* **12**, 155–164 (1941).
58. Knapp, D. M. et al. Rheology of reconstituted type I collagen gel in confined compression. *J. Rheol.* **41**, 971–993 (1997).
59. De Gennes, P. G. Dynamics of entangled polymer solutions (I&II). *Macromolecules* **9**, 587–598 (1976).
60. de Cagny, H. C. et al. Porosity governs normal stresses in polymer gels. *Phys. Rev. Lett.* **117**, 217802 (2016).
61. Nia, H. T., Han, L., Li, Y., Ortiz, C. & Grodzinsky, A. Poroelasticity of cartilage at the nanoscale. *Biophys. J.* **101**, 2304–2313 (2011).
62. Charras, G. T., Yarrow, J. C., Horton, M. A., Mahadevan, L. & Mitchison, T. J. Non-equilibration of hydrostatic pressure in blebbing cells. *Nature* **435**, 365–369 (2005).
63. Moeendarbary, E. et al. The cytoplasm of living cells behaves as a poroelastic material. *Nat. Mater.* **12**, 253–261 (2013).
A study providing direct evidence of the importance of poroelasticity in cell rheology on the basis of microindentation and microrheology experiments.
64. Hu, J. et al. Size- and speed-dependent mechanical behavior in living mammalian cytoplasm. *Proc. Natl Acad. Sci. USA* **114**, 9529–9534 (2017).
65. Tao, J., Li, Y., Vig, D. K. & Sun, S. X. Cell mechanics: a dialogue. *Rep. Prog. Phys.* **80**, 036601 (2017).
66. Maiuri, P. et al. Actin flows mediate a universal coupling between cell speed and cell persistence. *Cell* **161**, 374–386 (2015).
67. Ruprecht, V. et al. Cortical contractility triggers a stochastic switch to fast amoeboid cell motility. *Cell* **160**, 673–685 (2015).
68. Petrie, R. J., Koo, H. & Yamada, K. M. Generation of compartmentalized pressure by a nuclear piston governs cell motility in a 3D matrix. *Science* **345**, 1062–1065 (2014).
69. Guo, M. et al. Cell volume change through water efflux impacts cell stiffness and stem cell fate. *Proc. Natl Acad. Sci. USA* **114**, E8618–E8627 (2017).
70. Janmey, P. A. et al. Negative normal stress in semiflexible biopolymer gels. *Nat. Mater.* **6**, 48–51 (2007).
71. Poynting, J. H. On pressure perpendicular to the shear planes in finite pure shears, and on the lengthening of loaded wires when twisted. *Proc. R. Soc. A* **82**, 546–559 (1909).
72. Taber, L. A., Yang, M. & Podszus, W. W. Mechanics of ventricular torsion. *J. Biomech.* **29**, 745–752 (1996).
73. Baumgarten, K. & Tighe, B. P. Normal stresses, contraction, and stiffening in sheared elastic networks. *Phys. Rev. Lett.* **120**, 148004 (2018).
74. Taute, K. M., Pampaloni, F., Frey, E. & Florin, E. L. Microtubule dynamics depart from the wormlike chain model. *Phys. Rev. Lett.* **100**, 028102 (2008).
75. Yang, L. et al. Mechanical properties of native and cross-linked type I collagen fibrils. *Biophys. J.* **94**, 2204–2211 (2008).
76. Shen, Z. L., Dodge, M. R., Kahn, H., Ballarini, R. & Eppell, S. J. In vitro fracture testing of submicron diameter collagen fibril specimens. *Biophys. J.* **99**, 1986–1995 (2010).
77. Wu, Y. T. & Adnan, A. Damage and failure of axonal microtubule under extreme high strain rate: an in-silico molecular dynamics study. *Sci. Rep.* **8**, 12260 (2018).
78. Schnauss, J. et al. Transition from a linear to a harmonic potential in collective dynamics of a multifilament actin bundle. *Phys. Rev. Lett.* **116**, 108102 (2016).
79. Ward, A. et al. Solid friction between soft filaments. *Nat. Mater.* **14**, 583–588 (2015).
80. Kreplak, L., Herrmann, H. & Aebi, U. Tensile properties of single desmin intermediate filaments. *Biophys. J.* **94**, 2790–2799 (2008).
81. Fudge, D. S., Gardner, K. H., Forsyth, V. T., Riekel, C. & Gosline, J. M. The mechanical properties of hydrated intermediate filaments: insights from hagfish slime threads. *Biophys. J.* **85**, 2015–2027 (2003).
82. Kreplak, L., Doucet, J., Dumas, P. & Briki, F. New aspects of the alpha-helix to beta-sheet transition in stretched hard alpha-keratin fibers. *Biophys. J.* **87**, 640–647 (2004).
83. Brown, A. E., Litvinov, R. I., Discher, D. E., Purohit, P. K. & Weisel, J. W. Multiscale mechanics of fibrin polymer: gel stretching with protein unfolding and loss of water. *Science* **325**, 741–744 (2009).
An impressive multiscale experimental study of the role of structural hierarchy in increasing the mechanical strength of fibrin.
84. Fleissner, F., Bonn, M. & Parekh, S. H. Microscale spatial heterogeneity of protein structural transitions in fibrin matrices. *Sci. Adv.* **2**, e1501778 (2016).
85. Portale, G. & Torbet, J. Complex strain induced structural changes observed in fibrin assembled in human plasma. *Nanoscale* **10**, 10063–10072 (2018).
86. Brown, A. E., Litvinov, R. I., Discher, D. E. & Weisel, J. W. Forced unfolding of coiled-coils in fibrinogen by single-molecule AFM. *Biophys. J.* **92**, L39–41 (2007).
87. Zhmurov, A. et al. Mechanical transition from alpha-helical coiled coils to beta-sheets in fibrin(ogen). *J. Am. Chem. Soc.* **134**, 20396–20402 (2012).
88. Protopopova, A. D. et al. Visualization of fibrinogen alphaC regions and their arrangement during fibrin network formation by high-resolution AFM. *J. Thromb. Haemos.* **13**, 570–579 (2015).
89. Piechocka, I. K., Bacabac, R. G., Potters, M., Mackintosh, F. C. & Koenderink, G. H. Structural hierarchy governs fibrin gel mechanics. *Biophys. J.* **98**, 2281–2289 (2010).
90. Houser, J. R. et al. Evidence that alphaC region is origin of low modulus, high extensibility, and strain stiffening in fibrin fibers. *Biophys. J.* **99**, 3038–3047 (2010).
91. Hudson, N. E. et al. Submillisecond elastic recoil reveals molecular origins of fibrin fiber mechanics. *Biophys. J.* **104**, 2671–2680 (2013).
92. Averett, R. D. et al. A modular fibrinogen model that captures the stress-strain behavior of fibrin fibers. *Biophys. J.* **103**, 1537–1544 (2012).
93. Elvin, C. M. et al. Synthesis and properties of crosslinked recombinant pro-resilin. *Nature* **437**, 999–1002 (2005).
94. Baldock, C. et al. Shape of tropoelastin, the highly extensible protein that controls human tissue elasticity. *Proc. Natl Acad. Sci. USA* **108**, 4322–4327 (2011).
95. Reichheld, S. E., Muiznieks, L. D., Keeley, F. W. & Sharpe, S. Direct observation of structure and dynamics during phase separation of an elastomeric protein. *Proc. Natl Acad. Sci. USA* **114**, E4408–E4415 (2017).
96. Amuasi, H. E. & Storm, C. Off-lattice Monte Carlo simulation of supramolecular polymer architectures. *Phys. Rev. Lett.* **105**, 248105 (2010).
97. Giesa, T., Pugno, N. M. & Buehler, M. J. Natural stiffening increases flaw tolerance of biological fibers. *Phys. Rev. E* **86**, 041902 (2012).
98. Aghaei-Ghareh-Bolagh, B., Mithieux, S. M. & Weiss, A. S. Elastic proteins and elastomeric protein alloys. *Curr. Opin. Biotechnol.* **39**, 56–60 (2016).
99. Pinto, N. et al. Self-assembly enhances the strength of fibers made from vimentin intermediate filament proteins. *Biomacromolecules* **15**, 574–581 (2014).
100. Rombouts, W. H., Giesbers, M., van Lent, J., de Wolf, F. A. & van der Gucht, J. Synergistic stiffening in double-fiber networks. *Biomacromolecules* **15**, 1233–1239 (2014).
101. Wu, J. et al. Rationally designed synthetic protein hydrogels with predictable mechanical properties. *Nat. Commun.* **9**, 620 (2018).
102. Schuldt, C. et al. Tuning synthetic semiflexible networks by bending stiffness. *Phys. Rev. Lett.* **117**, 197801 (2016).
103. Bonakdar, N. et al. Mechanical plasticity of cells. *Nat. Mater.* **15**, 1090–1094 (2016).
A single-cell rheology study demonstrating that cells exhibit a reversible viscoelastic deformation response and additive plastic deformation that originates from bond ruptures within the cytoskeleton.
104. Fischer-Friedrich, E. et al. Rheology of the active cell cortex in mitosis. *Biophys. J.* **111**, 589–600 (2016).
105. Clement, R., Dehapiot, B., Collinet, C., Lecuit, T. & Lenne, P. F. Viscoelastic dissipation stabilizes cell shape changes during tissue morphogenesis. *Curr. Biol.* **27**, 3132–3142.e4 (2017).
106. Ferrer, J. M. et al. Measuring molecular rupture forces between single actin filaments and actin-binding proteins. *Proc. Natl Acad. Sci. USA* **105**, 9221–9226 (2008).
107. Courson, D. S. & Rock, R. S. Actin cross-link assembly and disassembly mechanics for alpha-actinin and fascin. *J. Biol. Chem.* **285**, 26350–26357 (2010).
108. Lieleg, O., Schmoller, K. M., Claessens, M. M. & Bausch, A. R. Cytoskeletal polymer networks: viscoelastic properties are determined by the microscopic interaction potential of cross-links. *Biophys. J.* **96**, 4725–4732 (2009).
109. Broedersz, C. P. et al. Cross-link-governed dynamics of biopolymer networks. *Phys. Rev. Lett.* **105**, 238101 (2010).
110. Muller, K. W. et al. Rheology of semiflexible bundle networks with transient linkers. *Phys. Rev. Lett.* **112**, 238102 (2014).
111. Wolff, L., Fernandez, P. & Kroy, K. Resolving the stiffening-softening paradox in cell mechanics. *PloS One* **7**, e40063 (2012).
112. Maier, M. et al. A single charge in the actin binding domain of fascin can independently tune the linear and non-linear response of an actin bundle network. *Eur. Phys. J. E* **38**, 50 (2015).
113. Gralka, M. & Kroy, K. Inelastic mechanics: a unifying principle in biomechanics. *Biochim. Biophys. Acta* **1853**, 3025–3037 (2015).
114. Ehrlicher, A. J., Nakamura, F., Hartwig, J. H., Weitz, D. A. & Stossel, T. P. Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. *Nature* **478**, 260–263 (2011).
115. Huang, D. L., Bax, N. A., Buckley, C. D., Weis, W. I. & Dunn, A. R. Vinculin forms a directionally asymmetric catch bond with F-actin. *Science* **357**, 703–706 (2017).
116. Weins, A. et al. Disease-associated mutant alpha-actinin-4 reveals a mechanism for regulating its F-actin-binding affinity. *Proc. Natl Acad. Sci. USA* **104**, 16080–16085 (2007).
117. Yao, N. Y. et al. Stress-enhanced gelation: a dynamic nonlinearity of elasticity. *Phys. Rev. Lett.* **110**, 018103 (2013).
118. Falzone, T. T., Lenz, M., Kovar, D. R. & Gardel, M. L. Assembly kinetics determine the architecture of alpha-actinin crosslinked F-actin networks. *Nat. Commun.* **3**, 861 (2012).
119. Foffano, G., Levernier, N. & Lenz, M. The dynamics of filament assembly define cytoskeletal network morphology. *Nat. Commun.* **7**, 13827 (2016).
120. Schmoller, K. M., Lieleg, O. & Bausch, A. R. Internal stress in kinetically trapped actin bundle networks. *Soft Matter* **4**, 2365–2367 (2008).
121. Lieleg, O., Kayser, J., Brambilla, G., Cipelletti, L. & Bausch, A. R. Slow dynamics and internal stress relaxation in bundled cytoskeletal networks. *Nat. Mater.* **10**, 236–242 (2011).
122. Nam, S., Hu, K. H., Butte, M. J. & Chaudhuri, O. Strain-enhanced stress relaxation impacts nonlinear elasticity in collagen gels. *Proc. Natl Acad. Sci. USA* **113**, 5492–5497 (2016).
123. Lou, J., Stowers, R., Nam, S., Xia, Y. & Chaudhuri, O. Stress relaxing hyaluronic acid-collagen hydrogels promote cell spreading, fiber remodeling, and focal adhesion formation in 3D cell culture. *Biomaterials* **154**, 213–222 (2018).
124. Gong, Z. et al. Matching material and cellular timescales maximizes cell spreading on viscoelastic substrates. *Proc. Natl Acad. Sci. USA* **115**, E2686–E2695 (2018).
125. Bennett, M. et al. Molecular clutch drives cell response to surface viscosity. *Proc. Natl Acad. Sci. USA* **115**, 1192–1197 (2018).
126. Schmoller, K. M., Fernandez, P., Arevalo, R. C., Blair, D. L. & Bausch, A. R. Cyclic hardening in bundled actin networks. *Nat. Commun.* **1**, 134 (2010).
127. Majumdar, S., Foucard, L. C., Levine, A. J. & Gardel, M. L. Mechanical hysteresis in actin networks. *Soft Matter* **14**, 2052–2058 (2018).
128. Kasza, K. E. et al. Actin filament length tunes elasticity of flexibly cross-linked actin networks. *Biophys. J.* **99**, 1091–1100 (2010).
129. Wagner, B., Tharmann, R., Haase, I., Fischer, M. & Bausch, A. R. Cytoskeletal polymer networks: the molecular structure of cross-linkers determines macroscopic properties. *Proc. Natl Acad. Sci. USA* **103**, 13974–13978 (2006).
130. Gardel, M. L. et al. Prestressed F-actin networks cross-linked by hinged filamins replicate mechanical properties of cells. *Proc. Natl Acad. Sci. USA* **103**, 1762–1767 (2006).
131. Mulla, Y., Oliveri, G., Overvelde, J. T. B. & Koenderink, G. H. Crack initiation in viscoelastic materials. *Phys. Rev. Lett.* **120**, 268002 (2018).
Theoretical modelling reveals that transient networks are susceptible to spontaneous crack initiation, with a critical crack length that can be predicted from the nonlinear local bond dynamics.

132. Mulla, Y. & Koenderink, G.H. Crosslinker mobility weakens transient polymer networks. *Phys. Rev. E.* **6**, 062503 (2018).
133. Sanchez, T., Chen, D. T., DeCamp, S. J., Heymann, M. & Dogic, Z. Spontaneous motion in hierarchically assembled active matter. *Nature* **491**, 431–434 (2012).
134. Schaedel, L. et al. Microtubules self-repair in response to mechanical stress. *Nat. Mater.* **14**, 1156–1163 (2015).
135. Noding, B., Herrmann, H. & Koster, S. Direct observation of subunit exchange along mature vimentin intermediate filaments. *Biophys. J.* **107**, 2923–2931 (2014).
136. Sano, K. et al. Self-repairing filamentous actin hydrogel with hierarchical structure. *Biomacromolecules* **12**, 4173–4177 (2011).
137. Jegou, A., Carlier, M. F. & Romet-Lemonne, G. Formin mDia1 senses and generates mechanical forces on actin filaments. *Nat. Commun.* **4**, 1883 (2013).
138. Humphrey, J. D., Dufresne, E. R. & Schwartz, M. A. Mechanotransduction and extracellular matrix homeostasis. *Nat. Rev. Mol. Cell Biol.* **15**, 802–812 (2014).
139. Yang, Y. & Urban, M. W. Self-healing polymeric materials. *Chem. Soc. Rev.* **42**, 7446–7467 (2013).
140. Yanagisawa, Y., Nan, Y., Okuro, K. & Aida, T. Mechanically robust, readily repairable polymers via tailored noncovalent cross-linking. *Science* **359**, 72–76 (2018).
141. Kurniawan, N. A. et al. Fibrin networks support recurring mechanical loads by adapting their structure across multiple scales. *Biophys. J.* **111**, 1026–1034 (2016).
142. Ban, E. et al. Mechanisms of plastic deformation in collagen networks induced by cellular forces. *Biophys. J.* **114**, 450–461 (2018).
143. Munster, S. et al. Strain history dependence of the nonlinear stress response of fibrin and collagen networks. *Proc. Natl Acad. Sci. USA* **110**, 12197–12202 (2013).
Confocal imaging directly reveals that fibrin and collagen networks exhibit plasticity owing to a combination of network-level and fibre-level remodelling in response to cyclic shear.
144. Kim, J. et al. Stress-induced plasticity of dynamic collagen networks. *Nat. Commun.* **8**, 842 (2017).
145. Hall, M. S. et al. Fibrous nonlinear elasticity enables positive mechanical feedback between cells and ECMs. *Proc. Natl Acad. Sci. USA* **113**, 14043–14048 (2016).
A study highlighting the importance of nonlinear elasticity in cellular mechanosensing.
146. Gnesotto, F. S., Mura, F., Gladrow, J. & Broedersz, C. P. Broken detailed balance and non-equilibrium dynamics in living systems: a review. *Rep. Prog. Phys.* **81**, 066601 (2018).
147. Kollmannsberger, P., Mierke, C. T. & Fabry, B. Nonlinear viscoelasticity of adherent cells is controlled by cytoskeletal tension. *Soft Matter* **7**, 3127–3132 (2011).
148. Humphrey, D., Duggan, C., Saha, D., Smith, D. & Kas, J. Active fluidization of polymer networks through molecular motors. *Nature* **416**, 413–416 (2002).
149. Chan, C. J. et al. Myosin II activity softens cells in suspension. *Biophys. J.* **108**, 1856–1869 (2015).
150. Koenderink, G. H. et al. An active biopolymer network controlled by molecular motors. *Proc. Natl Acad. Sci. USA* **106**, 15192–15197 (2009).
151. Alvarado, J., Sheinman, M., Abhinav, S., MacKintosh, F. C. & Koenderink, G. H. Molecular motors robustly drive active gels to a critically connected state. *Nat. Phys.* **9**, 591–597 (2013).
152. Koenderink, G. H. & Paluch, E. K. Architecture shapes contractility in actomyosin networks. *Curr. Opin. Cell Biol.* **50**, 79–85 (2018).
153. Murrell, M. P. & Gardel, M. L. F-actin buckling coordinates contractility and severing in a biomimetic actomyosin cortex. *Proc. Natl Acad. Sci. USA* **109**, 20820–20825 (2012).
154. Ronceray, P., Broedersz, C. P. & Lenz, M. Fiber networks amplify active stress. *Proc. Natl Acad. Sci. USA* **113**, 2827–2832 (2016).
155. Zemel, A., Bischofs, I. B. & Safran, S. A. Active elasticity of gels with contractile cells. *Phys. Rev. Lett.* **97**, 128103 (2006).
156. Jansen, K. A., Bacabac, R. G., Piechocka, I. K. & Koenderink, G. H. Cells actively stiffen fibrin networks by generating contractile stress. *Biophys. J.* **105**, 2240–2251 (2013).
157. Wollrab, V. et al. Polarity sorting drives remodeling of actin-myosin networks. *J. Cell Sci.* **132**, 219717 (2018).
158. MacKintosh, F. C. & Levine, A. J. Nonequilibrium mechanics and dynamics of motor-activated gels. *Phys. Rev. Lett.* **100**, 018104 (2008).
159. Sheinman, M., Broedersz, C. P. & MacKintosh, F. C. Actively stressed marginal networks. *Phys. Rev. Lett.* **109**, 238101 (2012).
160. Mizuno, D., Tardin, C., Schmidt, C. F. & Mackintosh, F. C. Nonequilibrium mechanics of active cytoskeletal networks. *Science* **315**, 370–373 (2007).
A seminal study unveiling the effects of stresses generated by molecular motor activity on cytoskeletal mechanics.
161. Foster, P. J., Furthauer, S., Shelley, M. J. & Needleman, D. J. Active contraction of microtubule networks. *eLife* **4**, e10837 (2015).
162. Gao, T., Blackwell, R., Glaser, M. A., Betterton, M. D. & Shelley, M. J. Multiscale polar theory of microtubule and motor-protein assemblies. *Phys. Rev. Lett.* **114**, 048101 (2015).
163. Stam, S. et al. Filament rigidity and connectivity tune the deformation modes of active biopolymer networks. *Proc. Natl Acad. Sci. USA* **114**, E10037–E10045 (2017).
164. Zenker, J. et al. Expanding actin rings zipper the mouse embryo for blastocyst formation. *Cell* **173**, 776–791.e17 (2018).
165. Carlier, M. F. & Shekhar, S. Global treadmilling coordinates actin turnover and controls the size of actin networks. *Nat. Rev. Mol. Cell Biol.* **18**, 389–401 (2017).
166. Brouhard, G. J. & Rice, L. M. Microtubule dynamics: an interplay of biochemistry and mechanics. *Nat. Rev. Mol. Cell Biol.* **19**, 451–463 (2018).
167. Wioland, H. et al. ADF/cofilin accelerates actin dynamics by severing filaments and promoting their depolymerization at both ends. *Curr. Biol.* **27**, 1956–1967 (2017).
168. Hiraiwa, T. & Salbreux, G. Role of turnover in active stress generation in a filament network. *Phys. Rev. Lett.* **116**, 188101 (2016).
169. Mak, M., Zaman, M. H., Kamm, R. D. & Kim, T. Interplay of active processes modulates tension and drives phase transition in self-renewing, motor-driven cytoskeletal networks. *Nat. Commun.* **7**, 10323 (2016).
170. McFadden, W. M., McCall, P. M., Gardel, M. L. & Munro, E. M. Filament turnover tunes both force generation and dissipation to control long-range flows in a model actomyosin cortex. *PLoS Comput. Biol.* **13**, e1005811 (2017).
171. McCall, P. M., MacKintosh, F. C., Kovar, D. R. & Gardel, M. L. Cofilin drives rapid turnover and fluidization of entangled F-actin. Preprint at *bioRxiv* https://www.biorxiv.org/content/10.1101/156224v1 (2017)..
172. Tan, T. H. et al. Self-organized stress patterns drive state transitions in actin cortices. *Sci. Adv.* **4**, eaar2847 (2018).
173. Wuhr, M. et al. Deep proteomics of the *Xenopus laevis* egg using an mRNA-derived reference database. *Curr. Biol.* **24**, 1467–1475 (2014).
174. Boekhoven, J., Hendriksen, W. E., Koper, G. J., Eelkema, R. & van Esch, J. H. Transient assembly of active materials fueled by a chemical reaction. *Science* **349**, 1075–1079 (2015).
175. Li, Q. et al. Macroscopic contraction of a gel induced by the integrated motion of light-driven molecular motors. *Nat. Nanotechnol.* **10**, 161–165 (2015).
176. Bertrand, O. J., Fygenson, D. K. & Saleh, O. A. Active, motor-driven mechanics in a DNA gel. *Proc. Natl Acad. Sci. USA* **109**, 17342–17347 (2012).
177. Han, E. H., Chen, S. S., Klisch, S. M. & Sah, R. L. Contribution of proteoglycan osmotic swelling pressure to the compressive properties of articular cartilage. *Biophys. J.* **101**, 916–924 (2011).
178. Huber, F., Boire, A., Lopez, M. P. & Koenderink, G. H. Cytoskeletal crosstalk: when three different personalities team up. *Curr. Opin. Cell Biol.* **32**, 39–47 (2015).
179. van Doorn, J. M., Lageschaar, L., Sprakel, J. & van der Gucht, J. Criticality and mechanical enhancement in composite fiber networks. *Phys. Rev. E* **95**, 042503 (2017).
180. Das, M. & Mackintosh, F. C. Poisson's ratio in composite elastic media with rigid rods. *Phys. Rev. Lett.* **105**, 138102 (2010).
181. Esue, O., Carson, A. A., Tseng, Y. & Wirtz, D. A direct interaction between actin and vimentin filaments mediated by the tail domain of vimentin. *J. Biol. Chem.* **281**, 30393–30399 (2006).
182. Golde, T. et al. Glassy dynamics in composite biopolymer networks. *Soft Matter* **14**, 7970–7978 (2018).
183. Brangwynne, C. P. et al. Microtubules can bear enhanced compressive loads in living cells because of lateral reinforcement. *J. Cell Biol.* **173**, 733–741 (2006).
A combined in vivo and in vitro study showing the importance of the composite architecture of the cytoskeleton for cell mechanics.
184. Bouchet, B. P. et al. Mesenchymal cell invasion requires cooperative regulation of persistent microtubule growth by SLAIN2 and CLASP1. *Dev. Cell* **39**, 708–723 (2016).
185. Latorre, E. et al. Active superelasticity in three-dimensional epithelia of controlled shape. *Nature* **563**, 203–208 (2018).
186. Kreger, S. T. & Voytik-Harbin, S. L. Hyaluronan concentration within a 3D collagen matrix modulates matrix viscoelasticity, but not fibroblast response. *Matrix Biol.* **28**, 336–346 (2009).
187. Lai, V. K. et al. Swelling of collagen-hyaluronic acid co-gels: an in vitro residual stress model. *Ann. Biomed. Eng.* **44**, 2984–2993 (2016).
188. Burla, F., Tauber, J., Dussi, S., van der Gucht, J. & Koenderink, G.H. Stress management in composite biopolymer networks. *Nat. Phys.* https://doi.org/10.1038/s41567-019-0443-6 (2019).
189. Huisman, E. M., Heussinger, C., Storm, C. & Barkema, G. T. Semiflexible filamentous composites. *Phys. Rev. Lett.* **105**, 118101 (2010).
190. Wada, H. & Tanaka, Y. Mechanics and size-dependent elasticity of composite networks. *Europhys. Lett.* **87**, 58001 (2009).
191. Bai, M., Missel, A. R., Klug, W. S. & Levine, A. J. The mechanics and affine-nonaffine transition in polydisperse semiflexible networks. *Soft Matter* **7**, 907–914 (2011).
192. Shahsavari, A. S. & Picu, R. C. Exceptional stiffening in composite fiber networks. *Phys. Rev. E* **92**, 012401 (2015).
193. Lin, Y.-C., Koenderink, G. H., MacKintosh, F. C. & Weitz, D. A. Control of non-linear elasticity in F-actin networks with microtubules. *Soft Matter* **7**, 902–906 (2011).
194. Ricketts, S. N., Ross, J. L. & Robertson-Anderson, R. M. Co-entangled actin-microtubule composites exhibit tunable stiffness and power-law stress relaxation. *Biophys. J.* **115**, 1055–1067 (2018).
195. Das, M. & MacKintosh, F. C. Mechanics of soft composites of rods in elastic gels. *Phys. Rev. E* **84**, 061906 (2011).
196. Pelletier, V., Gal, N., Fournier, P. & Kilfoil, M. L. Microrheology of microtubule solutions and actin-microtubule composite networks. *Phys. Rev. Lett.* **102**, 188303 (2009).
197. Broedersz, C. P., Storm, C. & MacKintosh, F. C. Nonlinear elasticity of composite networks of stiff biopolymers with flexible linkers. *Phys. Rev. Lett.* **101**, 118103 (2008).
198. Das, M., Quint, D. A. & Schwarz, J. M. Redundancy and cooperativity in the mechanics of compositely crosslinked filamentous networks. *PloS One* **7**, e35939 (2012).
199. Schmoller, K. M., Lieleg, O. & Bausch, A. R. Cross-linking molecules modify composite actin networks independently. *Phys. Rev. Lett.* **101**, 118102 (2008).
200. Jensen, M. H., Morris, E. J., Goldman, R. D. & Weitz, D. A. Emergent properties of composite semiflexible biopolymer networks. *Bioarchitecture* **4**, 138–143 (2014).
201. Deek, J., Maan, R., Loiseau, E. & Bausch, A. R. Reconstitution of composite actin and keratin networks in vesicles. *Soft Matter* **14**, 1897–1902 (2018).
202. Yang, Y. L. et al. Influence of chondroitin sulfate and hyaluronic acid on structure, mechanical properties, and glioma invasion of collagen I gels. *Biomaterials* **32**, 7932–7940 (2011).
203. Jaspers, M. et al. Nonlinear mechanics of hybrid polymer networks that mimic the complex mechanical environment of cells. *Nat. Commun.* **8**, 15478 (2017).
204. Schiffhauer, E. S. et al. Mechanoaccumulative elements of the mammalian actin cytoskeleton. *Curr. Biol.* **26**, 1473–1479 (2016).
205. Gan, Z. et al. Vimentin intermediate filaments template microtubule networks to enhance persistence in cell polarity and directed migration. *Cell Syst.* **3**, 500–501 (2016).

206. Costigliola, N. et al. Vimentin fibers orient traction stress. *Proc. Natl Acad. Sci. USA* **114**, 5195–5200 (2017).
207. Preciado Lopez, M. et al. Actin–microtubule coordination at growing microtubule ends. *Nat. Commun.* **5**, 4778 (2014).
208. Gautieri, A., Vesentini, S., Redaelli, A. & Buehler, M. Hierarchical structure and nanomechanics of collagen microfibrils from the atomistic scale up. *Nano Lett.* **11**, 757–766 (2011).
209. Valon, L., Marin-Llaurado, A., Wyatt, T., Charras, G. & Trepat, X. Optogenetic control of cellular forces and mechanotransduction. *Nat. Commun.* **8**, 14396 (2017).
210. Oakes, P. W. et al. Optogenetic control of RhoA reveals zyxin-mediated elasticity of stress fibres. *Nat. Commun.* **8**, 15817 (2017).
211. Bonnans, C., Chou, J. & Werb, Z. Remodelling the extracellular matrix in development and disease. *Nat. Rev. Cell Biol.* **15**, 786–801 (2014).
212. Chang, S. W., Flynn, B. P., Ruberti, J. W. & Buehler, M. J. Molecular mechanism of force induced stabilization of collagen against enzymatic breakdown. *Biomaterials* **33**, 3852–3859 (2012).
213. Bouameur, J. E. & Magin, T. M. Lessons from animal models of cytoplasmic intermediate filament proteins. *Subcell. Biochem.* **82**, 171–230 (2017).
214. Arseni, L., Lombardi, A. & Orioli, D. From structure to phenotype: impact of collagen alterations on human health. *Int. J. Mol. Sci.* **19**, 1407 (2018).
215. Needleman, D. & Dogic, Z. Active matter at the interface between materials science and cell biology. *Nat. Rev. Mater.* **2**, 17048 (2017).

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