Rigidity in mechanical biological networks

M. Lisa Manning, Department of Physics and BioInspired Institute, Syracuse University 13244

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Abstract: Multicellular organisms generate complex morphologies required for their function. Organisms control these morphologies by tuning active forces, and by altering the emergent "material properties" of a tissue, i.e. the rheology of the tissue. In many cases, organisms take advantage of dramatic changes in the rheology that occur when the material undergoes a rigidity transition from a fluid-like or floppy state to a solid-like or rigid state. This transition in turn depends on internal parameters at the scale of cells and molecules. This review highlights recent theoretical work identifying the *mechanisms* that drive such transitions, so that biologists can look for these mechanisms in *in vivo* or *in vitro* systems. We discuss two main types of transitions: a first-order rigidity transition that depends on the connectivity of small-scale structures, such as the number of contacts between cells or the number of branch points in a biopolymer network, and a second-order rigidity transition that depends on the geometry of small-scale structures, such as the shape of cells or the distance between crosslinks in a polymer network. We provide examples of each type of transition in model organisms and discuss methods for distinguishing between the mechanisms in future experiments.

Introduction

In multicellular organisms, developmental processes robustly generate specific morphologies of organs and body plans that are required for the organism to perform functions that ultimately enhance their fitness.[4] A key question is how organisms robustly construct this proper shape and organization of tissues – often at the scale of millimeters to meters -- given that the control mechanisms exist at the scale of molecules (e.g. DNA, epigenetics, biochemical signaling networks).

One potentially instructive non-biological example for how to frame this question is a hand-blown glass ornament. A skilled glass-blower controls the shape of the final ornament using two main mechanisms. The first is applied forces, controlled via a mandrel rod or air pressure. The second is applied heat to change the local temperature, which controls the material properties of the glass. Heating is effective because glass transforms from an elastic solid to a viscous fluid at a critical value of the temperature, which allows the glass to flow in local regions in response to applied forces.

Similarly, the ultimate shape and mechanical function of tissues and organisms is controlled by manipulation (perhaps internally) of applied forces and material properties. Unlike non-biological materials, biological systems are able to program their material properties to change in space and time, just like a glass-blower, to generate specific shapes.

In the physical sciences and engineering, the term "rheology" is used to describe how a material deforms in response to applied forces.[8] For example, in standard Newtonian fluids, an applied force generates a proportional change in the *rate* of deformation and vice versa, which is familiar to anyone who has tried to run though a body of water. In standard elastic solids, the applied force generates a proportional change in the *total amount* of deformation, e.g. stretching a rubber band. Many materials have a complex, viscoelastic rheology that simultaneously shares features of both liquids and solids, like toothpaste or ketchup. Some materials, like glass, can also undergo a rigidity transition where they change from fluid-like or floppy behavior to solid-like or rigid behavior as a function of an external parameter such as temperature or pressure.[9] Here, we use the term *solid-like* or *rigid* to describe materials that cost a finite energy to deform and where the constituent parts keep their same neighbors. In contrast, *floppy* materials can be slowly deformed with no energy cost and the constituent parts are able to move relative to one another. Some floppy materials are also *fluid-like*, so that in the presence of finite fluctuations the constituent parts completely rearrange and diffuse. A subtlety is that deforming floppy and fluid materials quickly (i.e. at finite rates) does cost energy to due to frictional and damping effects.

It is clear that evolution has been able to optimize the rheology-- and control floppy-to-rigid transitions or vice versa -- in biological "materials" at multiple scales. Examples of biological "materials" with specific rheologies that appear to be optimized for their function include intercellular fiber networks (stiffer actin-myosin cortex, softer but highly strain-stiffening intermediate filament networks), extracellular matrix (with different compositions and initial patterning that lead to different stiffnesses, porosities, alignment), as well as tissues that are cellularized, like early embryonic tissues, and acellularized, like cartilage.

As detailed below, sometimes a small change to the microscopic structure of a material can lead to big changes in its large-scale rheological behavior, and this is especially true close to a rigidity transition. In fact, it has been shown that some developmental and disease processes are aided or impeded by rigidity transitions.[3,10-13] In other words, "changing rigidity" could be a very useful mechanistic description of what is causing a disease or condition. Our hypothesis is that rigidity transitions are being actively controlled in many biological systems, but they have not yet been studied.

Other reviews have focused on describing the types of rigidity transitions that occur in biological systems, as well as the external parameters that can drive such transitions.[14,15] Therefore, this review instead seeks to distill recent theoretical work identifying the *mechanisms* that drive such transitions, so that biologists can look for these mechanisms in specific in vivo or in vitro systems. Ultimately, we hope to help researchers identify i) the mechanisms that biological systems can take advantage of to alter their rigidity, ii) the structural/mechanical signatures of such transitions, iii) as well as when and where such design principles might be important.

What is a Mechanical Biological Network?

Although eventually we are interested in the material response of a biological material as a function of timescale or frequency, here we focus on the behavior in the limit of very long timescales. We also focus on idealized behavior in the limit that fluctuations, such as active

contractions due to cell cycle[16] or motor activity, vanish. Clearly, this zero-fluctuation limit rarely exists in biological systems, but past work in nonbiological systems and recent work in fiber networks[17,18] has demonstrated that the rheology in the presence of small fluctuations is controlled by the zero-fluctuation behavior. In the idealized case, a rigidity transition occurs when the system goes from being floppy, where components are able to move around freely, to rigid, where there is a mechanical energy cost to deforming the material.

We also focus here on a class of materials that can be described as "mechanical biological networks". These are materials that can be approximated mechanically as a network of edges and vertices, which is a surprisingly reasonable description for a wide range of structures. Examples include i) cytoskeleton composed of fibers such as actin, microtubules, and intermediate filaments that comprise the network edges, connected at vertices by crosslinkers and motor proteins,[19,20]; ii) extracellular matrices composed of stiff fibrous proteins such as collagen or fibronectin, sometimes also interspersed with softer networks composed of aggrecans and hyaluronic acid,[21] and iii) dense cellularized tissues, including epithelial layers with cell-cell interfaces as the edges of the network and tricellular junctions or rosettes as the vertices,[22] or very dense early embryonic tissues that are well-represented as a network of cell-cell contacts.[3]

All these networks have a relatively simple mathematical description: a physical configuration is given by a list of locations of all the vertices and then the edges that connect the vertices, (and possibly facets in 3D). In addition, there is a mechanical energy function that describes the interactions along the edges or facets, or between cell centers. For example, collagen networks are well described by an energy functional where the fibers between crosslinkers are modeled as an elastic spring.[23] This equation has a few parameters: the stiffness of the spring, the rest length of the spring, and sometimes the bending energy between two springs. Similarly, a dense packing of non-confluent cells can be represented by a spring network between touching cell centers.[3]

In what follows, it is useful to make a distinction between two types of variables: the vertices move around and are called the "degrees of freedom" in the mathematical model, while the edges (e.g. springs) can be thought of as constraints that restrict those degrees of freedom. We note that this is precisely the description used in the engineering literature for many other mechanical systems (bar-joint networks, tensegrity models, origami). As engineers have thought carefully about how to ensure that structures like bridges remain rigid, this similar mathematical structure enables us to use powerful results from those fields.

Two types of rigidity in mechanical networks

We will briefly review some of the key results from rigidity theory in bar-joint networks. First, there is an important mathematical object called the rigidity matrix that describes how changing a vertex (i.e. the degrees of freedom) changes the edges (i.e. the constraints). In many standard materials, the network becomes rigid when the number of degrees of freedom equals the number of constraints. This is because changing the vertices necessarily changes the edge lengths, which costs energy. This simple theory is referred to as "constraint counting".[1] It is also called "first-order rigidity" because the vertices directly impact the edge lengths.[24] In mathematics, this is called a "first-order perturbation in the vertex

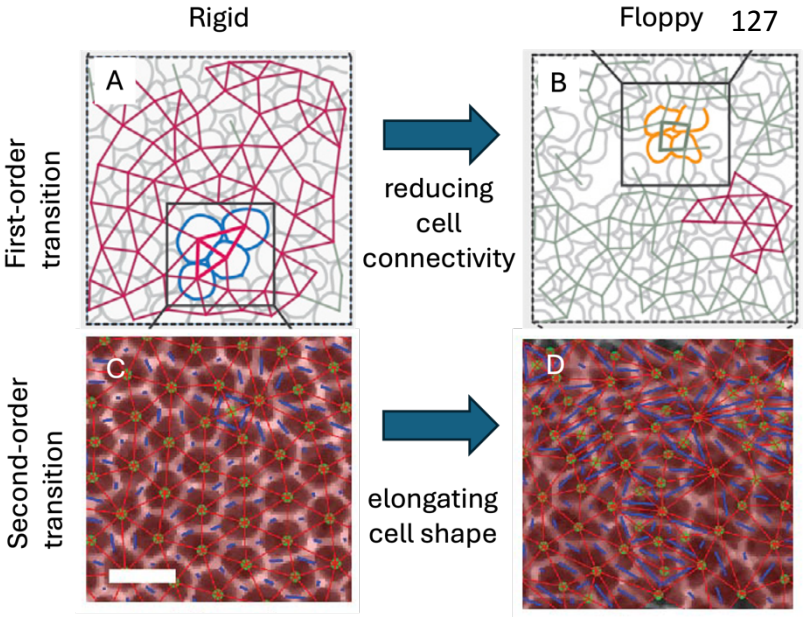

Fig 1. Examples of first-order (top row) and second order (bottom row) rigidity transitions in cellularized tissues. (A) is an illustration of the cell-cell contact network (red lines) in solid-like early zebrafish embryos just before the tissue undergoes large-scale flows. (B) shows that a short time later, a reduction in cell-cell adhesion reduces the number of cell-cell contacts, resulting in a non-percolating network of connections and a floppy network[3]. Because the transition depends on the number of contacts (constraints) compared to the degrees of freedom (cell centers), this is an example of a first-order rigidity transition. (C, D) show cell shapes in early *Drosophila* embryos during body axes elongation. Panel C has cell shapes that are more rounded, where D has cell-shapes that are more elongated, and this change in cell-scale geometry coincides with the onset of cellular rearrangement that allow large-scale tissue deformation, as predicted by second-order rigidity in vertex models. [6]

displacements". In what follows, we use the term "first-order rigidity" because it is standard in engineering and applied mathematics, but emphasize that it is distinct from the term "first-order phase transition" that often appears in physics. The latter term describes a phase transition, such as water to ice, where there is a discontinuous change in material properties at the transition. This contrasts with second-order phase transitions, such as the response of iron to a magnetic field, where the material becomes magnetic continuously below a certain temperature. The concepts of *rigidity* and *phase transitions* are somewhat independent; for example, jammed spheres are *first-order rigid*, but they show hallmarks of both first-order and second-order *phase transitions* when they become rigid.[25]

Interestingly, many biological mechanical networks are always "underconstrained", including most physiological collagen networks and vertex models for epithelial tissues. This means they have a lot more degrees of freedom than constraints. So how do they become rigid?

There is a simple example that illustrates the mechanism,[1] shown in Fig. 2, which is a chain of springs connected by vertices. Our argument works for any size chain, but for simplicity, imagine three springs, where each spring has the same rest length l_0 and spring constant k, and we have control over the distance between the two ends of the chain. We call this distance between the end points L. The energy for a harmonic spring is then $E = k\,(l - l_0)^2$, though it turns out that the exact form of the energy functional is not too important.

We can perform constraint counting on this chain: there are three constraints and four vertices, so the system is underconstrained – there are fewer constraints than degrees of freedom, and so we might expect the system to always be floppy. This is the case if $L < 3l_0$, as the chain wiggles around in space so that all the springs are "happy" -- they can each attain their rest length and the total energy of the system is zero, as shown in Fig 2A.

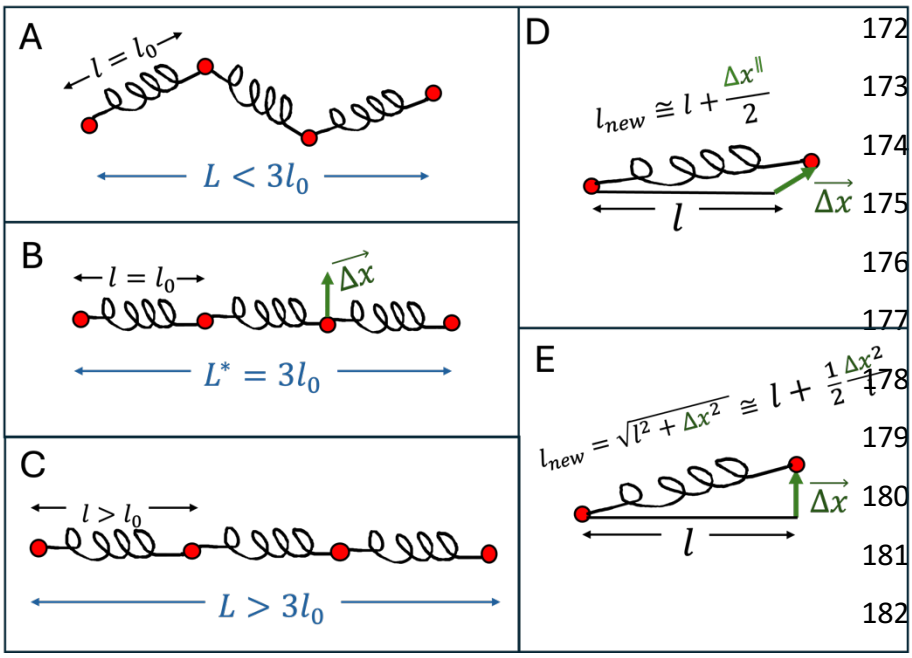

Fig 2. Mechanism for second order rigidity, described in Ref [1] (A-C) Geometry of a linear chain of three springs with rest length l_0 for different extension lengths L. A) When the distance between the endpoints $L < 3l_0$, the chain wiggles and the springs can all achieve their desired rest length, so that the system is floppy and at zero energy. B) At the critical value of the extension $L^* = 3l_0$, the chain becomes perfectly straight and each spring length l is precisely at its rest length l_0. C) If one keeps stretching, $L > 3l_0$, and each spring is stretched past its rest length so $l > l_0$, and the system is rigid and has a non-zero energy. (D,E) Illustration of a first-order and second-order response to displacing a vertex by Δx. (D) In a standard configuration, displacing a vertex by Δx causes the spring length to extend by Δx, i.e. to "linear order" in Δx. (E) In the special geometry at the critical point, the allowed displacements are perpendicular to the spring, and therefore a displacement by Δx extends the spring by a factor that is proportional to Δx^2, or to "second order" in Δx.

But, we all know that if we stretch the distance between the ends of the chain so that $L > 3l_0$ (Fig. 2C), then the system becomes rigid, and the system becomes stiffer and stiffer as L increases. This is a familiar concept to anyone who has tuned a string instrument, where the pitch is proportional to the stiffness. There is also a geometrical consequence: starting at the critical point $L^* = 3l_0$ (Fig. 2B) the chain forms a straight line. So why can't we understand this rigidity using constraint counting?

It is because the straight line corresponds to a very special geometry. The vertices in the middle of the chain are still constrained by the springs, but the geometry makes those constraints less effective. Normally, if a vertex gets displaced by a vector $\overrightarrow{\Delta x}$ with a magnitude that is small compared to the typical spring length, $\Delta x \ll l$, then the length of the spring attached to that vertex is also stretched by approximately $\frac{\Delta x^{\parallel}}{2}$, where $\Delta x^{\parallel}$ is the component of $\overrightarrow{\Delta x}$ that is parallel to the spring (Fig 2D). In math terms, the spring changes to "first order" in Δx. But in the special geometry, the allowed displacements are in the vertical direction, and one can draw a triangle

and use a so-called Taylor expansion to show that a vertical displacement of the vertex by a magnitude Δx only stretches the springs by a factor proportional to $\Delta x^2/l \ll \Delta x$, or in math terms, to "second order" in Δx (Fig 2E). In other words, changing the position of the middle vertices in Fig 2B doesn't change the edge lengths as much as in the first-order case. This is called second-order rigidity because mathematicians would say that changes to vertices only impact the edge lengths to second order.

Lastly, since we are interested in biological systems that are actively evolving their small-scale properties, it is useful to think about what might happen if the springs in the network could tune their own rest lengths. Just by looking at the geometry of Figure 2 (A-C), it is clear that keeping the total length L fixed and shrinking the rest lengths is exactly equivalent to keeping the rest lengths fixed and increasing L. In other words, if biological networks can tune the relevant internal geometric parameter -- such as rest length or a target cell shape -- they can directly tune across this rigidity transition without changing the "size of the box". For example, a cell could tune the balance between E-cadherin-based adhesion and actin/myosin-based cortical tension to decrease the surface area in contact with other cells in a confluent monolayer. This would reduce the target cell shape and drive the tissue towards the rigid phase.

In one dimension, there is an obvious link between the geometry and the transition. However, it is not immediately clear that the same mechanism occurs in disordered networks in 2D and 3D, like epithelial tissues, such as those in Fig 1C,D, and fiber networks, such as those in Fig 3A-D. Recent work has established that this is in fact the case for mathematical models for tissues and fiber networks,[26] and it is also true for structures like origami[27] and other non-biological networks with exotic mechanical properties.[28] So, we now understand that there are two distinct mechanisms for rigidity: first-order rigidity that depends on the number of connections (the connectivity) of the network, and second-order rigidity that depends on internal small-scale geometric parameters.

Examples of rigidity in biological mechanical networks

An immediate question is which mechanism causes rigidity in known biological mechanical networks? In the past five years several examples have emerged. First-order rigidity has been implicated in embryonic development, including in the zebrafish tailbud,[11] and in epiboly at the very early stages of zebrafish development.[3] In the former case, labeling of the interstitial fluid demonstrated that the rigidity was dependent on the packing fraction of the cells (the space taken up by the cells divided the total space),[11] which is a hallmark of first-order jamming transitions in soft spheres. In the latter, researchers actually counted the total number of cell-cell contacts and demonstrated that the tissue fluidized precisely when the number of contacts dropped below the constraint counting prediction.[3]

Another set of examples than interpolates between first-order and second-order rigidity are collagen networks. In reconstituted *in vitro* systems, one can control the effective connectivity of the collagen network,[7] as highlighted in the inset to Fig 3E. The main panel of Fig 3E illustrates that in collagen networks where the reconstituted network connectivity is above the critical constraint counting value, the networks are rigid and quite stiff, but when the connectivity is below the critical threshold, the networks are orders of magnitude less stiff (i.e. floppy), as predicted by first-order rigidity. In direct analogy to the distance L for the 1D chain example, when researchers change the size or shape of the "box" for the collagen network, applying a dilation or shear strain to the network, there is a critical strain (the analogue of L^* above) at which the material stiffens by orders of magnitude [7,29]. One can show mathematically that this strain-stiffening of physiological fiber networks is a second-order rigidity transition.[26] Similarly, there is significant evidence that epithelial layers can tune their geometric individual cell shapes to cross a second-order fluid-to-solid transition, in an *in vitro* cultured system for asthmatic and non-asthmatic human bronchial epithelial cells,[12] during body axis elongation of developing Drosophila embryos,[6] and in a cell line model for breast cancer.[30] In many of these examples, it is also the case that mutants or disease states change the internal geometric parameters and lead to rigidity transitions that are enhanced or delayed in comparison to non-diseased states.[13,30,31] Ongoing work is focused on understanding whether these corrupted transitions are responsible for downstream breakdown in function associated with the disease.

How to identify rigidity transitions and their mechanisms in biological materials

As this is an emerging idea, it may be the case that similar rigidity transitions are being tuned by organisms at a multitude of scales. Here we discuss some features that researchers might look for in experimental systems – to determine if a rigidity transition is occurring in biological materials, and if so to identify the mechanism that is causing rigidification.

To understand whether a system is becoming rigid, researchers can study the *dynamics* of their systems. If researchers have access to microscopic imaging, they may be able to track the motion of cells or fibers using particle-tracking algorithms, and compute a quantity such as the mean-squared displacement.[32] Fluid-like systems have components that exhibit mean-squared displacements that scale linearly with time on long timescales, while systems that are in the process of rigidifying exhibit subdiffusive behavior (sub linear scaling, or even a flat plateau) on intermediate timescales.[33] If the resolution is not good enough to allow for particle tracking, particle image velocimetry or image cross-correlation approaches can also be used to estimate structural flows.[34] Another possibility is to explicitly identify rearrangements to network structure, which is often a signature of a fluid-like state. One example is T1 transitions in epithelial monolayers, where two cells that do not initially have a contacting interface switch neighbors and come in contact.[35] Systems that do not have network rearrangements are more solid-like.[36] Finally, in some cases it may be possible to directly measure the viscosity of a structure using a mechanical measurement such as micropipette aspiration.[3,37] Solid-like states have a diverging viscosity, while fluid-like states do not.

To understand the *mechanisms* that are driving rigidification, researchers should study the microscopic *structure* of their systems. First, it is useful to characterize the network connectivity – e.g. the number of branches per crosslink in fiber networks (Fig 3E) or the number of touching neighbors in cell packing.[3,7] Then, one can study whether an increase in fluidity occurs at times or in spatial locations where the connectivity drops below the critical constraint counting value, which would indicate first-order rigidity is controlling the transition. This, in turn, would suggest

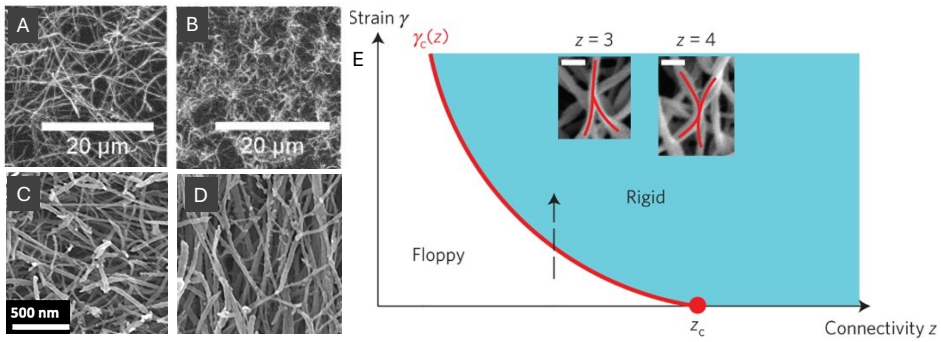

Fig 3. Relationship between small-scale structure (geometry, connectivity) and large-scale material properties in collagen networks. (A,B) Confocal images illustrating architecture of *in vitro* reconstituted collagen networks prepared at two different temperatures, 25°C (A) and 37°C (B).[2] (C,D) SEM images of bovine knee cartilage at two different tissue depths: (C) closer to the surface and (D) deeper in the tissue, highlighting the collagen network structure. The collagen in the deeper network is more aligned.[5] (E) Schematic diagram illustrating observed rigidity transition in reconstituted collagen networks as a function of the connectivity/branching of the polymer network, z, and the applied external strain, which corresponds to a change in the "shape of the box" that contains the network.[7]

that mutations or environmental conditions that perturb connectivity could alter global tissue mechanics and morphology.

For second-order rigidity, the observables are a little less direct.

Recently, several researchers have suggested using a "straightness" parameter to estimate the tension along edges (cell-cell interfaces, fibers between crosslinks) in biomechanical networks.[38,39] This method is based on the hypothesis that active processes at the subcellular scale generate fluctuating forces that act as an effective temperature forcing the edge. In that case, one expects the magnitude of the observed fluctuations in the shape of the edge to be inversely proportional to the tension along the edge. This matches our intuition that a less wiggly string is under higher tension. In second-order rigid systems, the tension along edges is proportional to how rigid the system is. Therefore, one could study whether an increase in fluidity is associated with less tension along edges, which would indicate second-order rigidity is controlling the transition.

In addition, some researchers have seen that alignment of edges, as seen in Fig 3D, increases significantly in strain-stiffening systems, and this even forms the basis for some engineering models of collagen networks.[40] Although it is not clear that edge alignment occurs in all second-order systems, it does seem to be associated with large shear strains in both tissue [6] and fiber [41] systems. It is also not typically seen in first-order rigid systems, and so it could potentially be used to distinguish between the two mechanisms.

Summary and Future Directions

As with any interesting science problem, there are still a lot of open questions about rigidity in biomechanical networks. One class of questions focuses on systems that might interpolate between first-order and second-order rigidity. For example, fiber networks that have a lot of embedded cells (or even beads) possess a rheology that is quite different from bare fiber networks.[42] Bare fiber networks are second-order rigid, while packing of beads and rounded cells are first-order rigid, so understanding what happens to the composite is an active area of research.[43] Another type of interpolation is deformable particles[44] or active foams,[45] where the system can continuously transform between first-order soft spheres to second-order vertex models, though there may be interesting feedbacks that arise when the cells are highly deformable[46] A third type of interpolation might be related to the rheology behavior of composite networks, like cartilage, composed of a stiffer fiber backbone and a softer hydrogel like hyaluronic acid. Recent work has suggested that the softer network might act as an additional set of constraints on the motion of the stiff backbone, effectively increasing the connectivity of the network and reducing the amount of strain needed to reach the second-order rigidity strain-stiffening regime.[47]

Another open question is how the rheology of biomechanical networks change in the presence of finite fluctuations in forces or tensions. Is there an important difference between fluids and networks which are technically always "rigid" but have very small barriers to rearrangement?[48] Or fluids that behave as solids if you strain them too far? [49] Also, what happens to the rheological properties if cross-linkers are dynamic, [50] or if the fibers themselves are being assembled and dissembled (e.g. actin polymerization and depolymerization)?[51]

Some of the more interesting open questions concerns how biological tissues *control* changes to their cell-or fiber-scale properties. It seems likely that biological systems are tuning cell-scale properties in order to drive the system towards or away from a rigidity transition. Recent work suggests that cell shapes in fruit flies are carefully controlled in order to maintain rigidity in the

amniocerosa.[38] Even more speculatively, it may be that some biological systems have simple mechanical feedback control loops that help the organism achieve a particular rheology. For example, there are enzymes that preferentially sever low-tension edges in extra-cellular matrix networks,[52] and theoretical work suggests that a rule of this type could allow a network to maintain rigidity despite rapid turnover.[53] More broadly, many tissues contain mechanosensitive components[54] that could drive new and interesting feedbacks between small-scale features like cell connectivity and large-scale material properties.

Although this review focuses on understanding biological process through the lens of materials science, it seems likely that biological insights could facilitate the design of smart materials that change their morphology. Can we design new types of materials that behave like biological networks?[23,55]

In summary, biological materials can tune their emergent behavior at the scale of tissues and organs by exerting spatio-temporal control over the material rheology. Dramatic changes in the rheology occur when the material undergoes a transition from fluid-like/floppy to solid-like/rigid mechanics, which in turn depends on internal parameters at the scale of cells and molecules. There are two main types of transitions: first-order rigidity that depends on the connectivity of small-scale structures, and second-order rigidity that depends on the geometry (shape or length) of small-scale structures, which can be distinguished in biology experiments.

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References Cited

1. Lubensky, T., Kane, C., Mao, X., Souslov, A., and Sun, K. (2015). Phonons and elasticity in critically coordinated lattices. Reports on Progress in Physics *78*, 073901.
2. Licup, A.J., Münster, S., Sharma, A., Sheinman, M., Jawerth, L.M., Fabry, B., Weitz, D.A., and MacKintosh, F.C. (2015). Stress controls the mechanics of collagen networks. Proceedings of the National Academy of Sciences *112*, 9573-9578.

3. Petridou, N.I., Corominas-Murtra, B., Heisenberg, C.-P., and Hannezo, E. (2021). Rigidity percolation uncovers a structural basis for embryonic tissue phase transitions. Cell *184*, 1914-1928. e1919.
4. Thomson, J.A. (1917). On growth and form. Nature Publishing Group UK London.
5. Brown, E.T.T., Damen, A.H.A., and Thambyah, A. (2020). The mechanical significance of the zonally differentiated collagen network of articular cartilage in relation to tissue swelling. Clinical Biomechanics *79*, 104926. https://doi.org/10.1016/j.clinbiomech.2019.12.008.
6. Wang, X., Merkel, M., Sutter, L.B., Erdemci-Tandogan, G., Manning, M.L., and Kasza, K.E. (2020). Anisotropy links cell shapes to tissue flow during convergent extension. Proceedings of the National Academy of Sciences *117*, 13541-13551. 10.1073/pnas.1916418117.
7. Sharma, A., Licup, A., Jansen, K., Rens, R., Sheinman, M., Koenderink, G., and MacKintosh, F. (2016). Strain-controlled criticality governs the nonlinear mechanics of fibre networks. Nature Physics *12*, 584.
8. Wilson, D.I. (2018). What is rheology? Eye *32*, 179-183. 10.1038/eye.2017.267.
9. Debenedetti, P., and Stillinger, F. (2001). Supercooled liquids and the glass transition. Nature.
10. Angelini, T.E., Hannezo, E., Trepat, X., Marquez, M., Fredberg, J.J., and Weitz, D.A. (2011). Glass-like dynamics of collective cell migration. Proceedings of the National Academy of Sciences *108*, 4714-4719.
11. Mongera, A., Rowghanian, P., Gustafson, H.J., Shelton, E., Kealhofer, D.A., Carn, E.K., Serwane, F., Lucio, A.A., Giammona, J., and Campàs, O. (2018). A fluid-to-solid jamming transition underlies vertebrate body axis elongation. Nature, 1.
12. Park, J.A., Kim, J.H., Bi, D., Mitchel, J.A., Qazvini, N.T., Tantisira, K., Park, C.Y., McGill, M., Kim, S.H., Gweon, B., et al. (2015). Unjamming and cell shape in the asthmatic airway epithelium. Nat Mater *14*, 1040-1048. 10.1038/nmat4357.
13. Oswald, L., Grosser, S., Smith, D.M., and Käs, J.A. (2017). Jamming transitions in cancer. Journal of Physics D: Applied Physics *50*, 483001. 10.1088/1361-6463/aa8e83.
14. Sadati, M., Taheri Qazvini, N., Krishnan, R., Park, C.Y., and Fredberg, J.J. (2013). Collective migration and cell jamming. Differentiation; research in biological diversity *86*, 121-125. 10.1016/j.diff.2013.02.005.
15. Lawson-Keister, E., and Manning, M.L. (2021). Jamming and arrest of cell motion in biological tissues. Current Opinion in Cell Biology *72*, 146-155. 10.1016/j.ceb.2021.07.011.
16. Vianay, B., Senger, F., Alamos, S., Anjur-Dietrich, M., Bearce, E., Cheeseman, B., Lee, L., and Théry, M. (2018). Variation in traction forces during cell cycle progression. Biol Cell *110*, 91-96. 10.1111/boc.201800006.
17. Arzash, S., Gannavarapu, A., and MacKintosh, F.C. (2023). Mechanical criticality of fiber networks at a finite temperature. Physical Review E *108*, 054403. 10.1103/PhysRevE.108.054403.
18. Lee, C.-T., and Merkel, M. (2023). Generic elasticity of thermal, under-constrained systems. arXiv preprint arXiv:2304.07266.

19. Head, D., Levine, A., and MacKintosh, F. (2003). Distinct regimes of elastic response and deformation modes of cross-linked cytoskeletal and semiflexible polymer networks. Physical Review E *68*, 061907.
20. Zhu, J., and Mogilner, A. (2012). Mesoscopic model of actin-based propulsion. PLoS computational biology *8*, e1002764.
21. Frantz, C., Stewart, K.M., and Weaver, V.M. (2010). The extracellular matrix at a glance. Journal of Cell Science *123*, 4195-4200. 10.1242/jcs.023820.
22. Farhadifar, R., Röper, J.-C., Aigouy, B., Eaton, S., and Jülicher, F. (2007). The Influence of Cell Mechanics, Cell-Cell Interactions, and Proliferation on Epithelial Packing. Current Biology *17*, 2095-2104.
23. Arzash, S., Tah, I., Liu, A.J., and Manning, M.L. (2023). Tuning for fluidity using fluctuations in biological tissue models. arXiv preprint arXiv:2312.11683.
24. Connelly, R. (1980). The rigidity of certain cabled frameworks and the second-order rigidity of arbitrarily triangulated convex surfaces. Advances in Mathematics *37*, 272-299. https://doi.org/10.1016/0001-8708(80)90037-7.
25. Liu, A.J., and Nagel, S.R. (2010). The jamming transition and the marginally jammed solid. Annual Review of Condensed Matter Physics.
26. Damavandi, O.K., Hagh, V.F., Santangelo, C.D., and Manning, M.L. (2022). Energetic rigidity. I. A unifying theory of mechanical stability. Physical Review E *105*, 025003. 10.1103/PhysRevE.105.025003.
27. Chen, B.G.-g., and Santangelo, C.D. (2018). Branches of triangulated origami near the unfolded state. Physical Review X *8*, 011034.
28. Berry, M., Limberg, D., Lee-Trimble, M.E., Hayward, R., and Santangelo, C.D. (2022). Controlling the configuration space topology of mechanical structures. Physical Review E *106*, 055002. 10.1103/PhysRevE.106.055002.
29. Kang, H., Wen, Q., Janmey, P.A., Tang, J.X., Conti, E., and MacKintosh, F.C. (2009). Nonlinear elasticity of stiff filament networks: strain stiffening, negative normal stress, and filament alignment in fibrin gels. The Journal of Physical Chemistry B *113*, 3799-3805.
30. Grosser, S., Lippoldt, J., Oswald, L., Merkel, M., Sussman, D.M., Renner, F., Gottheil, P., Morawetz, E.W., Fuhs, T., Xie, X., et al. (2021). Cell and Nucleus Shape as an Indicator of Tissue Fluidity in Carcinoma. Physical Review X *11*, 011033. 10.1103/PhysRevX.11.011033.
31. Ilina, O., Gritsenko, P.G., Syga, S., Lippoldt, J., La Porta, C.A., Chepizhko, O., Grosser, S., Vullings, M., Bakker, G.-J., and Starruß, J. (2020). Cell–cell adhesion and 3D matrix confinement determine jamming transitions in breast cancer invasion. Nat Cell Biol *22*, 1103-1115.
32. Schoetz, E.M., Lanio, M., Talbot, J.A., and Manning, M.L. (2013). Glassy dynamics in three dimensional embryonic tissues. submitted, http://arxiv.org/abs/1307.4454
33. Mao, Y., Nielsen, P., and Ali, J. (2022). Passive and active microrheology for biomedical systems. Frontiers in bioengineering and biotechnology *10*, 916354.
34. Ahmadzadegan, A., Mitra, H., Vlachos, P.P., and Ardekani, A.M. (2023). Particle Image micro-Rheology (PIR) using displacement probability density function. Journal of Rheology *67*, 823-823.

35. Weaire, D., and Hutzler, S. (2001). The Physics of Foams (Oxford University Press).
36. Bi, D.P., Lopez, J.H., Schwarz, J.M., and Manning, M.L. (2015). A density-independent rigidity transition in biological tissues. Nature Physics *11*, 1074-+. 10.1038/Nphys3471.
37. Evans, E., and Yeung, A. (1989). Apparent viscosity and cortical tension of blood granulocytes determined by micropipet aspiration. Biophys J *56*, 151-160.
38. Tah, I., Haertter, D., Crawford, J.M., Kiehart, D.P., Schmidt, C.F., and Liu, A.J. (2023). Minimal vertex model explains how the amnioserosa avoids fluidization during Drosophila dorsal closure. arXiv preprint arXiv:2312.12926.
39. Weng, S., Devitt, C.C., Nyaoga, B.M., Havnen, A.E., Alvarado, J., and Wallingford, J.B. (2023). New tools reveal PCP-dependent polarized mechanics in the cortex and cytoplasm of single cells during convergent extension. bioRxiv, 2023.2011.2007.566066. 10.1101/2023.11.07.566066.
40. Wang, H., Abhilash, A., Chen, C.S., Wells, R.G., and Shenoy, V.B. (2014). Long-range force transmission in fibrous matrices enabled by tension-driven alignment of fibers. Biophys J *107*, 2592-2603.
41. Hall, M.S., Alisafaei, F., Ban, E., Feng, X., Hui, C.-Y., Shenoy, V.B., and Wu, M. (2016). Fibrous nonlinear elasticity enables positive mechanical feedback between cells and ECMs. Proceedings of the National Academy of Sciences *113*, 14043-14048. 10.1073/pnas.1613058113.
42. van Oosten, A.S., Chen, X., Chin, L., Cruz, K., Patteson, A.E., Pogoda, K., Shenoy, V.B., and Janmey, P.A. (2019). Emergence of tissue-like mechanics from fibrous networks confined by close-packed cells. Nature *573*, 96-101.
43. Gandikota, M.C., Pogoda, K., Van Oosten, A., Engstrom, T.A., Patteson, A.E., Janmey, P., and Schwarz, J. (2020). Loops versus lines and the compression stiffening of cells. Soft matter *16*, 4389-4406.
44. Boromand, A., Signoriello, A., Ye, F., O'Hern, C.S., and Shattuck, M.D. (2018). Jamming of Deformable Polygons. Phys Rev Lett *121*, 248003. 10.1103/PhysRevLett.121.248003.
45. Kim, S., Pochitaloff, M., Stooke-Vaughan, G.A., and Campàs, O. (2021). Embryonic tissues as active foams. Nature Physics *17*, 859-866. 10.1038/s41567-021-01215-1.
46. Latorre, E., Kale, S., Casares, L., Gómez-González, M., Uroz, M., Valon, L., Nair, R.V., Garreta, E., Montserrat, N., del Campo, A., et al. (2018). Active superelasticity in three-dimensional epithelia of controlled shape. Nature *563*, 203-208. 10.1038/s41586-018-0671-4.
47. Wyse Jackson, T., Michel, J., Lwin, P., Fortier, L.A., Das, M., Bonassar, L.J., and Cohen, I. (2022). Structural origins of cartilage shear mechanics. Science Advances *8*, eabk2805.
48. Claussen, N.H., Brauns, F., and Shraiman, B.I. (2023). A Geometric Tension Dynamics Model of Epithelial Convergent Extension. arXiv preprint arXiv:2311.16384.
49. Huang, J., Cochran, J.O., Fielding, S.M., Marchetti, M.C., and Bi, D. (2022). Shear-driven solidification and nonlinear elasticity in epithelial tissues. Phys Rev Lett *128*, 178001.
50. Maxian, O., Peláez, R.P., Mogilner, A., and Donev, A. (2021). Simulations of dynamically cross-linked actin networks: morphology, rheology, and hydrodynamic interactions. PLoS computational biology *17*, e1009240.

51. Pfaendtner, J., De La Cruz, E.M., and Voth, G.A. (2010). Actin filament remodeling by actin depolymerization factor/cofilin. Proceedings of the National Academy of Sciences *107*, 7299-7304.
52. Saini, K., Cho, S., Dooling, L.J., and Discher, D.E. (2020). Tension in fibrils suppresses their enzymatic degradation–A molecular mechanism for 'use it or lose it'. Matrix Biology *85*, 34-46.
53. Cunha, M.A.G., Crocker, J.C., and Liu, A.J. (2023). Building rigid networks with prestress and selective pruning. arXiv preprint arXiv:2312.06119.
54. Orr, A.W., Helmke, B.P., Blackman, B.R., and Schwartz, M.A. (2006). Mechanisms of mechanotransduction. Developmental cell *10*, 11-20.
55. Hagh, V.F., Nagel, S.R., Liu, A.J., Manning, M.L., and Corwin, E.I. (2022). Transient learning degrees of freedom for introducing function in materials. Proceedings of the National Academy of Sciences *119*, e2117622119. doi:10.1073/pnas.2117622119.