Fracture in living tissues

Authors and affiliations:
Alessandra Bonfanti[1*], Julia Duque[2*], Alexandre Kabla[3], Guillaume Charras[2,4]

1. Department of Civil and Environmental Engineering, Politecnico di Milano, Milan, 20133, Italy
2. London Centre for Nanotechnology, University College London, London, WC1H 0AH, UK
3. Department of Engineering, University of Cambridge, Cambridge, UK
4. Department of Cell and Developmental Biology, University College London, London, WC1E 6BT, UK

* These authors contributed equally

Correspondence : Alexandre Kabla (ajk61@cam.ac.uk) or Guillaume Charras (g.charras@ucl.ac.uk).

Keywords:
Cytoskeleton, cadherin, desmosome, fracture

Highlights:
- Living tissue fracture is a multi-scale phenomenon.
- Fracture can occur as a result of externally applied stresses or active stresses generated by myosin motors.
- Microelectromechanical systems (MEMS) enable the characterisation of fracture at molecular, cellular, and tissue-scales.

Abstract:
During development and in adult physiology, living tissues are continuously subjected to mechanical stresses originating either from cellular processes intrinsic to the tissue or external forces. As a consequence, rupture is a constant risk and it can arise due to excessive stresses or because of tissue weakening by genetic abnormalities or pathologies. Tissue fracture is a multi-scale process involving the unzipping of intercellular adhesions at the molecular scale in response to stresses arising at the tissue-scale or cellular-scale transmitted to adhesion complexes through the cytoskeleton. In this review, we detail experimental characterisation and theoretical approaches for understanding the fracture of living tissues at the tissue-, cellular-, and molecular-scales.

Tissue fracture in normal physiology and disease

During development, tissues within the embryo are continuously subjected to mechanical stresses originating either from cellular processes intrinsic to the tissue or from morphogenetic movements occurring in adjacent tissues. For example, during *Drosophila* germ band extension, elongation of cells in the antero-posterior axis is the result of extrinsic stress generated by the endoderm invagination occurring in the posterior of the embryo [1]. During wing formation, contraction of cells within the hinge region exerts stretch on the cells in the wing blade [2]. Because of these mechanical stresses, perturbations to tissue mechanical properties associated with genetic abnormalities can result in fracture. For example, during gastrulation in the *Xenopus* embryo, convergence and extension in the blastopore leads to tissue tension in the ectoderm [3]. Depletion of myosin or E-cadherin both result in disruptions to the tissue leading to fracture [3, 4]. During adulthood, tissues must withstand mechanical stresses associated with normal physiological function, such as those produced during expansion and contraction of the lungs or the heart or stretching of the skin during limb movement [5]. A clear illustration of the mechanical role of tissues can be gained from diseases in which mutations or pathogens targeting the cytoskeleton or intercellular adhesion fragilise tissues leading to frequent rupture even though tissue stresses remain within physiological range [6, 7]. Such tissue failures have serious consequences. Local weakening of the blood vessel wall can lead to aneurysm and eventually ruptures, that often prove fatal. Rupture of tissues within the digestive system may lead to severe pain, internal bleeding and stomach perforation. Mutations to intermediate filaments and desmosomal proteins lead to a broad spectrum of pathologies associated with fragile tissues, such as epidermolysis bullosa [8] [9]. While tissue failure is usually undesired in adult organisms, the separation of cells is in some cases required for normal development, as during the formation of fluid-filled luminal cavities in the blastocoel [10, 11] or the gut [12] or during leg eversion in *Drosophila* [13]. Despite clear health implications and roles in development, we still know remarkably little about the physical limits of the stress that tissues can sustain and the associated fracture mechanisms.

Fracture as we experience in everyday life involves the permanent separation of continuous materials into two or more parts as a result of mechanical stress. In classical engineering materials, such as metals and ceramics, brittle failure is associated with small deformations of the bulk material (**Fig. 1a,b**). In this class of materials, fracture involves irreversible rupture at the atomic level in response to external stresses (**Fig. 1c**). By contrast, the most prominent feature of fracture in soft materials, like plastic and rubber, is the large deformation and crack blunting before the crack starts propagating (**Fig. 1a,d**). Hence, the strain field close to the crack tip is significantly different from the hypothesis of infinitesimal deformation that has been successfully applied in engineering to study fracture processes in metals or ceramics (**Fig. 1e**) [14, 15]. The effects of such large deformations during fracture are still poorly understood from a theoretical point of view and their characterisation forms an active area of research. Cellularised tissues also display large deformation during fracture, with yet further complexities. Tissue fracture involves permanent rupture of the molecular connections between cells that normally show a highly dynamic reversible binding-unbinding behaviour – a phenomenon not present in classical materials. Plasticity within tissues is often associated with active and dynamic processes, such as cytoskeletal remodelling at the molecular scale or rearrangement and division at the cellular-scale [16], all of which take place without loss of tissue integrity. In this review, we describe the subcellular structures involved during tissue rupture before summarising what we know about the mechanical conditions leading to fracture and the characterisation techniques enabling the study of fracture. Finally, we provide a broad overview of the current theoretical and numerical approaches for studying failure of living tissues and highlight open questions.

Tissue fracture is a complex multiscale process

The organisation of soft tissues is inherently multi-scale. Micron-sized cells form the basic building blocks of millimetre to metre-sized tissues and these cells are linked to one another by nanometre-sized intercellular adhesion molecules that dynamically turn over. The macroscopic defects that we identify as fractures are the final outcome of an accumulation of molecular- and then cellular-scale ruptures (**Fig 3a-c**). Indeed, within a tissue under stress, each cell bears a load, which is distributed across all of the protein complexes that link it to its neighbour. Rupture of one intercellular adhesion complex increases the load on the remaining ones, further increasing their probability of rupture. When a sufficient number of adhesion complexes have ruptured, the process becomes catastrophic and the cells detach from one another. At the tissue-scale, this cell-scale rupture increases the load on the remaining cells, increasing the probability that other cells will separate. Again, when a sufficient number of cells have detached from one another, the process becomes catastrophic and the tissue ruptures. Thus, soft tissue fracture arises from a multi-scale process.

Within tissues, cells are interfaced by a variety of intercellular junctions that link their cytoskeletal networks or allow passage of solutes and small molecules (**Fig. 2**). Most research to date has focused on two types of intercellular junctions that play a central role in resisting fracture [17, 18]: adherens junctions (**Fig. 2b**), which link the actomyosin cytoskeletons of adjacent cells to one another, and desmosomes (**Fig. 2c**), which interface keratin intermediate filament networks. In both junctions, adhesion arises from multimolecular complexes with a similar organisation: intercellular adhesion is provided by interaction between the extracellular domains of transmembrane proteins of the cadherin family (*e.g.* E-Cadherin, N-Cadherin for adherens junctions; desmoglein, desmocollin for desmosomes) and their cytoplasmic tail is tethered to cytoskeletal filament networks via intracellular anchor proteins (α- and β-catenin for adherens junctions and desmoplakin, plakoglobin, and plakophilin for desmosomes). A third type of junction, tight junctions, whose primary function is to restrict the passage of ions and solutes across epithelia, may also bear load [19, 20], although their strength appears smaller than the previous two [21] and they may play more of a signalling role [20]. In addition to cadherins, cells express a number of other intercellular adhesion proteins, such as members of the immunoglobulin superfamily (JAMs, nectins, and nectin-like proteins), but their mechanical role is currently less well understood. Key differences with inert materials are that proteins within junctions continuously turn over (with time-scales of minutes to hours [22]), that cells can adapt the junctional complex structure and cytoskeletal organisation to load [23], and that broken bonds can readily heal. Downstream of adhesion complexes, force is transmitted to either actomyosin or the intermediate filament network, whose respective rheological behaviours will influence cellular and tissue stresses.

Due to their central role in tissue integrity, intercellular adhesion complexes have been the subject of much research and, as a result, our understanding of the complex interplay between molecular-scale interactions and load bearing properties is improving [24]. At the molecular scale, rupture in intercellular adhesion complexes can take place intracellularly between anchor proteins (for example between α- and β-catenin, **Fig. 3c-I**) or extracellularly between transmembrane adhesion proteins from adjacent cells [25] (**Fig. 3c-II**). As a consequence, researchers have sought to characterise the force-dissociation kinetics of the various proteins involved in intercellular adhesion complexes. This has revealed that both cadherins and desmogleins display catch-bond properties, signifying that their lifetime increases with applied force up to an optimum before decreasing with additional load [26, 27], and dissociate in response to forces on the order of 50 pN in vitro and in cells [26, 28-32]. Intracellular parts of these adhesion complexes display similar force-dependent properties. Cell-free experiments using purified proteins have revealed that the application of force can stabilise the binding of catenin complexes to F-actin [33]. Furthermore, the application of forces of ~10-15 pN to α-catenin leads to its unfolding, which reveals a cryptic binding domain that can recruit vinculin proteins to strengthen the adhesion complex [34]. Importantly, in cells, cadherin and desmosomal intercellular adhesion complexes arrange in clusters [35], which influences the stability of adhesion. Within cells, the forces borne by individual intercellular proteins during

normal function have been investigated using molecular strain gauges [36]. This has revealed that adhesion complexes are routinely subjected to forces ~10 pN [37]. However, a quantitative view of how large numbers and multiple types of intercellular adhesion complexes combine to set the cellular-scale and tissue-scale resistance to rupture remains missing.

In summary, the understanding of fracture in living tissues requires investigation of rupture at molecular- and cellular-scales as well as a comprehension of the interplay of mechanical forces with physiological molecular turnover processes and cellular-scale flow within tissues, making this a challenging conceptual and experimental undertaking.

Soft tissue fracture can be triggered by extrinsic or intrinsic factors
Conceptually, two distinct sources of stress can be distinguished in living tissues and each can give rise to fracture. Passive stress results from deformation of the material, as in classic engineering materials such as springs; whereas active stress is energy-consuming and arises from the local action of molecular motors on the cytoskeletal scaffold. In particular, the actomyosin machinery of cells generates pulling forces that are transmitted to other cells across intercellular adhesion complexes to give rise to a tissue-scale stress.

<u>Passive stresses.</u> At the tissue-scale, ruptures occurring in response to externally applied stress have mostly been studied in epithelia, sheet-like tissues that often consist of only one layer of cells. In some experimental systems, epithelia are interfaced at their extremities to test rods that can be prised apart until rupture (**Fig. 3d**). A common observation in these studies is the high deformation that epithelia can sustain. Indeed, adult epithelia and embryonic epidermis can withstand deformations larger than 50% before the appearance of the first tears which eventually result in complete failure for strains close to 100% [38, 39]. Perhaps unsurprisingly, the maximum strain that tissues can withstand is decreased by treatments weakening intercellular junctions [39]. Other tissues are less deformable, *Drosophila* amnioserosa tissues are only able to withstand a modest 6% strain before ruptures are observed at adherens junctions [40]. Similar observations have been reported for the rupture of a single junction between pairs of cells. Microelectromechanical systems (MEMS) consisting of two platforms that can be moved away from one another (**Fig. 3e**) have provided new insights into intercellular junction fracture. At high strain rates, stress accumulates at adherens junctions with ruptures occurring at strains of ~200%, similar to what is observed for complete tissue rupture [41]. When cells were depleted in cadherin, lower strains were needed for separation. Similarly, separation force between germ layers cells decreases with E-cadherin depletion in single-cell force spectroscopy experiments carried out with AFM [42]. Conversely, separation force increases with increasing E-Cadherin expression in micropipette cell separation experiments [43].

Natural processes taking place in some parts of a tissue generate enough force to deform other parts of the tissue and cause rupture. For example, lumenogenesis necessitates active transport of water towards the interior of a tissue. In mouse blastocyst, the blastocoel cavity is generated in a process that appears to involve multiple rupture events between cell-cell contacts of the inner cell mass and the trophectoderm [10]. Deformations and rupture can also result from migratory processes (**Fig. 3d**) as is the case for a sheet-like primitive marine animal called *Trichoplax adhaerens.* Some cells within the epithelium undergo contractions and the tissue between contractile foci becomes stretched, leading to localised intercellular detachment and eventually tissue rupture [44, 45]. Epithelia migrating collectively on thin micropatterned strips of extracellular matrix separated by non-adhesive regions form bridges suspended over the non-adherent areas. As migration progresses, the tension within these bridges increases, eventually giving rise to rupture where a cell division had taken place [46].

<u>Active stresses.</u> Local high concentrations in myosins have also been observed at the location of tissue rupture, suggesting that local build-up of active stresses can lead to tissue failure. For example, leg eversion in *Drosophila* necessitates rupture of a squamous epithelium called

the peripodial membrane. A localised rupture of this epithelium is initiated by Myosin-induced stress in some of the cells [13]. During *Drosophila* development, the actomyosin-mediated pulsatile behaviour of amnioserosa cells generates the main force driving dorsal closure [47, 48]. Inactivation of Myosin phosphatase in this tissue increases active stress and gives rise to severe fractures in the amnioserosa epithelium with loss of adherens junction integrity that prevents embryos from reaching the larval state [49]. Similar ruptures are also observed in mammalian epithelia in which myosin phosphatase is inhibited [23]. When examined in pairs of cells, active stresses appear to have a biphasic effect on the integrity of junctions. While some active stress promotes the lengthening of junctions, excess stress induced for example by myosin contractility downstream of vasoactive compounds leads to junction rupture [50]. Intriguingly, a key process in development and disease, Epithelial to Mesenchymal Transitions (EMT), may represent a type of rupture in response to an increase in active stress. During this process, a cohesive epithelium dissociates into individual cells by a combination of stresses generated by migration and contractility [51, 52]. Thus, tissues appear to intrinsically possess the capacity of self-tearing.

In summary, both categories of stress have been observed to trigger rupture in living tissues but a more in depth understanding of their respective role remains hindered by our current ability to quantitatively characterise the process of fracture.

Experimental techniques to study fracture from the molecular to tissue scale
The ability to characterise the mechanics of soft biological materials at different scales (i.e. tissue, cellular, molecular) is key for the study of soft tissue fracture. The multiscale nature of living tissue fracture signifies that the processes involved exhibit large variations in length-scales, from milli-metre sized tissues to nano-metre adhesion proteins, and the forces generated vary across orders of magnitude, from milli-Newton for tissues to pico-Newton for single bonds [53]. Accurate and sometimes creative solutions have been found for testing tissues on macroscopic scales [54]. Yet, testing small size specimens and measuring small ranges of forces have challenged traditional setups used in Material Science. In this context, the small-size and low forces within microfabricated setups are well-suited for characterising fracture and for mimicking the physiological environments or disease conditions in combination with pharmacological and genetic treatments. **Table 1** summarises some of the recently introduced systems, organized from molecular- to tissue-scale.

While experimental devices to characterise the force response of intermolecular bonds between proteins involved in intercellular adhesion are well established and commercially available (**Table 1A-B**), this is not the case for devices examining the cell and tissue scales. Key requirements for characterisation setups are: (i) the presence of regions in which the majority of stresses are borne by intercellular junctions to allow simple interpretation of the mechanical test, (ii) attachment of cells and tissues to the devices must require minimal manipulation, (iii) measurements must be quantitative with highly reproducible geometries to allow comparison across treatments and to theoretical predictions, (iv) live microscopy imaging must be possible during the mechanical test to assess the role of cellular structures. Many of the experimental setups rely on a similar principle with cells growing across a gap between two platforms whose distance can be varied over time (**Table 1C, E-H, J**). Here, MEMS (**Table 1E, J**) allow to manufacture devices with a high degree of control over the area for cell attachment as well as the dimensions of the gap region outlined in (i, iii), something challenging with more traditional systems that are often assembled by hand (**Table 1F-G**). Most experimental devices output a force-displacement relationship for which fine control of displacement and accurate measurement of force are essential (**Table 1E-J**). Again here, MEMS are well-suited because they allow force measurement with high accuracy and large dynamic range [41, 55, 56]. Furthermore, while traditional approaches only allow uniaxial tests, MEMS can perform shear testing (**Table 1J**, [57]), something that may prove invaluable given the reported sensitivity of intercellular adhesion to shear stresses [58]. Current challenges

include simplifying the manufacturing process and increasing experimental throughput to allow for quantitative testing of model predictions.

Current theoretical approaches for the study of soft tissue fracture
The study of tissue fracture aims at predicting if and how a tissue with given mechanical properties will break in response to a well-defined load or loading history. As highlighted earlier, tissue fracture is the result of separation of adjacent cells which in turn results from the unbinding of molecular adhesion complexes (**Fig. 3a-c**). Thus, tissue fracture couples processes with different time-scales and length-scales. Such hierarchy is also reflected in the theoretical approaches adopted to study fracture: at tissue-scale via continuum models, at cell-scale level via discrete models and at the molecular-scale level via stochastic models. The development of a predictive model for tissue fracture requires integration of these co-existent models at different length-scales, which is still an ongoing challenge. In the section below, we highlight the key mechanical processes that a fracture model should account for and we review the models currently available at different scales.

The first step to predicting fracture is understanding the spatiotemporal response of tissues to deformation. Given the large strains (>50%) observed during fracture around the crack tip and the relatively fast time-scales involved (seconds-minutes), taking into account material time-dependent behaviours and nonlinearity is essential to study tissue failure. Living materials dissipate stresses, resulting in a time-dependent response often referred to as viscoelasticity [22, 59]. At small deformation, this behaviour is commonly modelled by a linear theory via a network of elastic springs, viscous dashpots and springpots - a recently introduced rheological element based on fractional calculus that can capture the weak power-law regime typical of hierarchical materials [60, 61]. At larger strains, tissues show a highly nonlinear material behaviour, whereby they stiffen or fluidify under high mechanical stress or strain [59]. Tissues presenting apico-basal polarity may also exhibit curling behaviours that make it more difficult to model in-plane stress and crack geometries [62, 63].

A simplified approach to fracture prediction is by modelling the tissue as a homogeneous continuum material. By neglecting the complex multiscale tissue structure, this approach allows the qualitative description of fracture processes with a limited number of parameters. With continuous models, the effects of the processes occurring at cellular and subcellular levels are phenomenologically captured by a constitutive equation that relates stress $\sigma(t)$ to strain $\varepsilon(t)$, which involves model/material parameters. Therefore, such an approach does not directly relate tissue scale fracture processes to underlying rupture at cellular and molecular scales. Due to the complexity of fracture in soft living tissues, efforts have mostly focused on characterizing failure in gels, which are comparatively simpler soft materials. Knauss [64] and Schapery [65] pioneered the Linear Viscoelastic Fracture (LVF) theory, where nonlinearities due to large deformation are neglected. However, LVF theory predicts an unrealistically small dissipative zone around the crack [66]. This well-known paradox was attributed to material nonlinearities appearing around the crack tip because of the extremely large strain [67].

Accounting for the nonlinear viscoelastic behaviour necessitates the introduction of a new length scale l that represents the distance from the crack tip below which linear viscoelasticity breaks down and large dissipation of energy takes place ("nonlinear zone", **Fig. 4a**). Recently, it has been shown that material softening due to large deformations around the tip shields the crack from high stresses, thus hindering crack propagation [68]. While this softening at the crack tip is exhibited by gels and polymers at large deformations, evidence that cellularised tissues manifest a similar behaviour is still emerging [62]. A more detailed review on continuum models for fracture of highly deformable materials can be found in [69]. Currently, models accounting for both material and geometrical nonlinearities are limited to one-dimension because of the challenge in coupling these two effects. As a result, the crack path cannot currently be predicted.

A good understanding of stress patterns at the crack site and the effects of cell-level processes on tissue-level rupture requires a discrete modelling approach. This can be implemented at the cellular-scale *via computational models* that account for individual cell behaviours and cell separation and allow prediction of the crack path. Two main approaches have been applied (**Fig. 4b**): (i) cell-based models where the cells are reduced to particles connected via deformable links; (ii) models that account for the cell geometry, of which vertex models are a well-established class [70, 71]. The cell-based models tend to be simple, yet successful in capturing mechanical behaviours that are not influenced by the exact geometry of cells or the area of cell-cell contact. A first cell-based model accounting for link breakage has yielded insight into the connection between the macroscopic visco-plastic behaviour of tissues and that of single cells [72]. More recently, an extension of such a cell-based model was used to study *Trichoplax adhaerens* [45] and revealed that if a tissue is subjected to a loading rate faster than the characteristic maturation time of cell-cell connections it will rapidly fracture, in contrast to the ductile behaviour otherwise shown. Yet, some fracture processes are highly dependent on cell shape and cell-cell contact area; therefore, accounting for cell geometry in these contexts is key. Recently, a tissue vertex model has revealed the role of tension at cell-cell junctions and cell adhesion in controlling the transition from a solid to a fluid state in tissues, which can lead to tissue fracture [73]. By accounting for extracellular spaces between cells, complex cell shapes and cortical tension dynamics, the model shows that an increase in relative cell adhesion results in a transition from many small gaps between cells to large defects, which are a manifestation of tissue fracture. Although these models faithfully replicate cell morphologies and tissue structure at the onset of fracture, capturing the creation and progression of a crack is challenging. The integration of cortical tension and adhesion into a surface energy at the cell membranes is primarily an equilibrium consideration. Fracture is an out-of-equilibrium process that requires to carefully consider the kinetics of the adhesive processes at cell junctions.

To capture the maximum tension that a junction can bear, the dynamics at the molecular scale must be modelled. Connections between cells are mediated by proteins that are characterised by clusters of short-lived bonds. Thus, each individual receptor-ligand bond has a lifetime that depends on competition between dissociation and rebinding rate (**Fig. 4c**). A number of theoretical models were developed to capture the behaviour of a single receptor-ligand bond subjected to mechanical forces. The seminal work of Bell [74, 75] describes the bond dynamics as a competition between dissociation due to electrostatic forces and attraction due to ligand-receptor interaction, which has been represented via the classical theory of chemical reactions. This original work uncovered an exponential relationship between the bond dissociation rate k_{off} and the applied force F, $k_{off} \propto e^{F}$. Yet, the understanding of cell connections requires the study of the collective dynamics of a large number of bonds. A common assumption is that of equally spaced bonds within clusters, all subjected to the same level of force from the tissue. An early study of cluster sites revealed the existence of a critical size below which clusters behave like a single bond and above which the cluster lifetime increases due to the collective effect of bonds. In the small force regime, cluster lifetime t_{bound} is highly dependent on rebinding strength: for weak rebinding, the lifetime grows logarithmically with bond number N, $t_{bound} \propto log(N)$; while for strong rebinding, the lifetime grows exponentially with bond number, $t_{bound} \propto e^{N}$ [76, 77]. Therefore, larger clusters have a longer lifetime and are more stable than smaller clusters, which can easily switch to an unbound state. Subsequent studies have examined binding between adhesive clusters linked to soft matrices subjected to stress [78, 79]. They revealed that, when bonds are tethered to soft matrices, the unbinding probability increases due to stress concentration at the edge of the cluster [79] and that the elastic recoil of the matrix after unbinding inhibits bond rebinding by increasing the distance between ligand and receptor, thus reducing adhesion stability [78]. A more recent model incorporating the viscoelastic behaviours observed in cells indicates that viscosity increases the lifetime and stability of bonds [80]. Indeed, the non-instantaneous relaxation behaviour of viscoelastic materials signifies that bond separation necessitates more

time than when tethered to elastic matrices, thus increasing the probability of rebinding (**Fig 4c**). Further, due to viscoelasticity, stress concentrations at the adhesion sites are dissipated. From experiments, we know that cells are characterized by high cortical tension. A recent study has demonstrated that such surface tension strongly increases cluster lifetime and their sensitivity to flaws by bringing receptor and ligand closer to one another and favouring rebinding at crack initiation [81].

In contrast to the main assumption of homogenous force distribution across bonds within a cluster, imaging of tissues under stress has revealed an inhomogeneous force distribution across connections [82]. A recent theoretical study has demonstrated that inhomogeneous distribution of forces across bonds influences the critical size for crack initiation, giving rise to a critical cluster size above which the lifetime of the cluster decreases [83]. Despite tremendous progress in modelling cell-cell adhesion, we know little about the effects of unequally spaced bonds subjected to inhomogeneous and non-constant forces. Mechanotransductory signalling within cells may recruit proteins to sites exposed to larger forces, thus resulting in inhomogeneously spaced bonds (e.g. [41, 84]). It has been speculated that such clustering density has been optimised in relation to the critical length for crack initiation [83]. Further, as mentioned above, two types of junctions simultaneously play a crucial role in maintaining tissue integrity, each of which possess different dynamics, thus resulting in a complex stochastic behaviour still not investigated theoretically. Therefore, future work modelling adhesion lifetime should focus in these directions.

Concluding remarks:
Rupture is a constant risk for tissues that can arise in both physiological conditions and disease due to either stresses intrinsic to the tissue or externally applied. Tissue fracture is a multi-scale process involving the unzipping of intercellular adhesions at the molecular scale in response to stresses arising at the tissue-scale or cellular-scale transmitted to adhesion complexes through the cytoskeleton. As a result, predicting fracture necessitates understanding not only the unbinding of bonds under force but also the complex rheological behaviour arising in soft tissues in response to large deformations, the active stresses generated by myosin contractility as well as the contribution of molecular turnover and adaptation in cytoskeletal and adhesive structures. While great progress has been made in understanding the complex rheology of cells and tissues, characterising rupture at the molecular-, cellular-, and tissue-scales remains challenging (see Outstanding Questions). MEMS are particularly well-suited to studying these questions because they can replicate the length- and force-scales present in physiological conditions and offer a great degree of control and reproducibility. Although receptor-ligand bond fracture under force is well characterised, this understanding cannot currently be leveraged for prediction of tissue rupture because we do not know how stresses are distributed across the various cytoskeletal networks, the density of intercellular adhesion complexes within cell junctions, or the magnitude of force each adhesion complex is subjected to during normal function. Thus, the understanding of tissue rupture in physiological and pathological conditions remains a vast experimental and theoretical challenge for years to come.

Figure 1: Mechanical behaviour of classical engineering materials and soft non-living materials. (**a**) Schematic plot of the true stress as a function of the true strain during a tensile test. The asterisk identifies the formation of the first crack and the cross denotes complete failure of the specimen. (**b-e**) Schematics showing the stress free condition, the appearance of the first crack, and complete rupture of brittle and soft materials. (**b**) In classic engineering materials, fracture occurs at small deformation at the macroscale. (**c**) Crack propagation is characterised by permanent rupture of bonds within the rigid atomic lattice and deformations in the microscale architecture remain small. (**d**) In soft materials, fracture occurs for large deformations at the macroscopic scale. (**e**) The high deformations at the macroscale are due to the presence of large deformations in the microscale architecture. The large deformation that the microscale can accommodate slow crack propagation.

Figure 2: Diagram of cellular adhesions present in epithelia. (**a**) General representation of the main types of cell-cell adhesion complexes (tight junctions, adherens junctions, desmosomes) and cell-matrix adhesion complexes (focal adhesions, hemi-desmosomes). (**b**) Molecular organisation of the adherens junction complex. (**c**) Molecular organisation of desmosomes.

Figure 3: **Fracture is a multi-scale process**. (**a-c**) Tissue-level fractures result from detachment of cells and those, in turn, arise from molecular-scale ruptures. At the molecular scale, rupture can occur intracellularly at connections between adhesion complexes and the cytoskeleton or extracellularly as the result of separation of the ectodomain of intercellular adhesion proteins. (**d**) Left - Natural movements of *Trichoplax adhaerens* lead to formation of holes in both the dorsal and ventral sides of the animal [45]. Right - Laser-ablation of suspended epithelial monolayers gives rise to large size holes within minutes [62]. (**e**) A novel Microelectromechanical system (MEMS) allows to directly apply strain on cell pairs forming an intercellular junction while recording the applied stress [41]. (**f**) Single-molecule force clamp experiment where individual E-cadherin pairs are pulled apart to determine their rupture force [26]. (**g**) Rupture force for control and cadherin compromised monolayers [39] and (**h**) cells [41]. Red crosses depict the occurrence of complete fracture. (**i**) Rupture between cadherins ectodomains [30]. Images within this figure are from references [26, 30, 41, 45, 62].

Figure 4: Modelling approaches to study soft tissue fracture at different length-scales. Tissue fracture is a hierarchical process that involves separation of adjacent cells, which is itself due to failure of single intercellular bonds. This characteristic is also reflected in the current modelling approaches. (**a**) Schematic of a continuum model where the tissue is homogenised as a continuum material without representation of the cellular scale. (**b**) Diagrams of discrete cell scale models. Top: a cell-based model where the tissue is represented via particles connected through deformable links. Bottom: a vertex model that accounts for cell shape and cell-cell contact. (**c**) Schematic representation of cell junction bond dynamics when the cells are assumed to be an elastic material. N represents the number of bonds present at the cell-cell interface.

Table 1: Experimental devices to study soft tissue fracture at different length-scales.
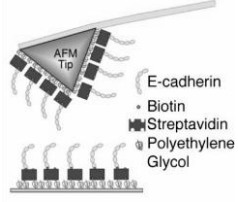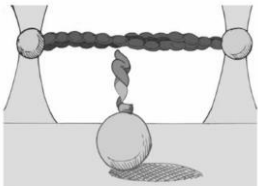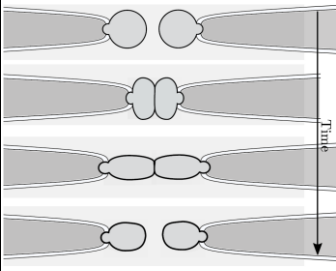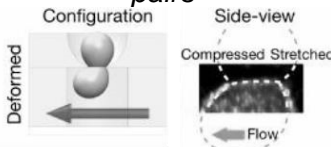
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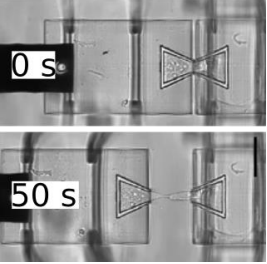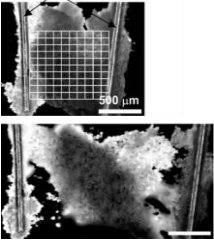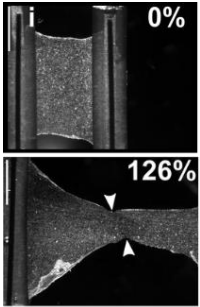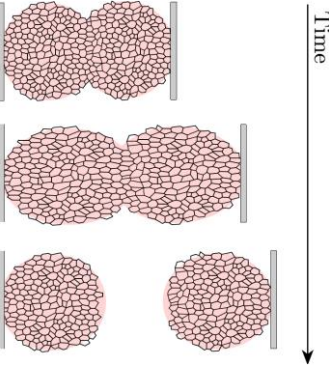
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Table 1:

	Testing Set-up	Description
	(a) *Single-molecule force spectroscopy with AFM* Image from [26]	**Single molecule force spectroscopy** implemented **with atomic force microscopy** allows measurement of the strength of intermolecular bonds. The AFM tip and the substrate are both functionalised with ligands (for example the ectodomain of E-cadherin) and brought into contact for different durations--thus resulting in different bond maturations. When the AFM tip is rapidly withdrawn from the substrate, deflection of the cantilever is recorded and sudden jumps in the deflection allow to characterise the rupture force of bonds [26]. Similar experiments are also performed by bringing two cells into contact (a technique called **Single cell force spectroscopy**) [26, 30, 85].
	(b) *Single-molecule force spectroscopy with optical tweezers* Image from [33]	**Single molecule force spectroscopy** can also be implemented **with optical tweezers.** Optical tweezers use a focused beam of light to trap microscopic particles in all three dimensions. Several configurations for the measurement of force and displacement have been introduced [86, 87]. For example, a recent study has demonstrated that the link between the cadherin-catenin complex and F-actin is force-sensitive [33]. Thus, this complex transitions to a strongly bound state at high forces.
	(c) *Micropipette cell separation*	**Micropipette cell separation** is an assay to test cell junction strength. A cell is held stationary by a micropipette while another one is brought into contact by a motorised micromanipulator. Once the cell-cell connections have been established, the micropipettes are moved apart, resulting in junction failure [43]. The two cells may be of different types [88]. A similar test can also be implemented with AFM (**Single cell force spectroscopy**).
	(d) *Shear test on cell pairs* Image from [55]	**Shear testing MEMS** apply shear stresses via hydrodynamic drag forces to two suspended cells placed in an hourglass-like aperture. Protein recruitment at the junctions in response to shear can be quantified via confocal imaging [55]. Different forces and loading rates can be applied by controlling the flow rate.
	(e) *Micro-tensile test on cell pairs*	**Tensile testing MEMS** enable displacement-controlled tensile test on cell pairs with mature adhesions until junction failure [41]. The cells are seeded within a bow-tie structure located on top of two platforms mounted on pillars. Displacement is applied to one of the platforms via an AFM while the force applied to the cells is measured by considering the pillars as cantilevered beams.

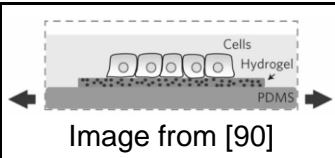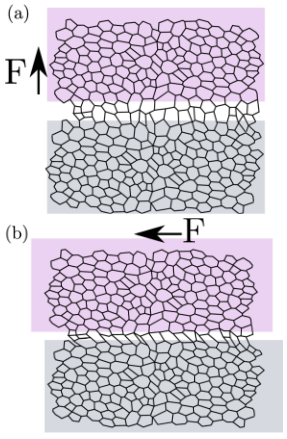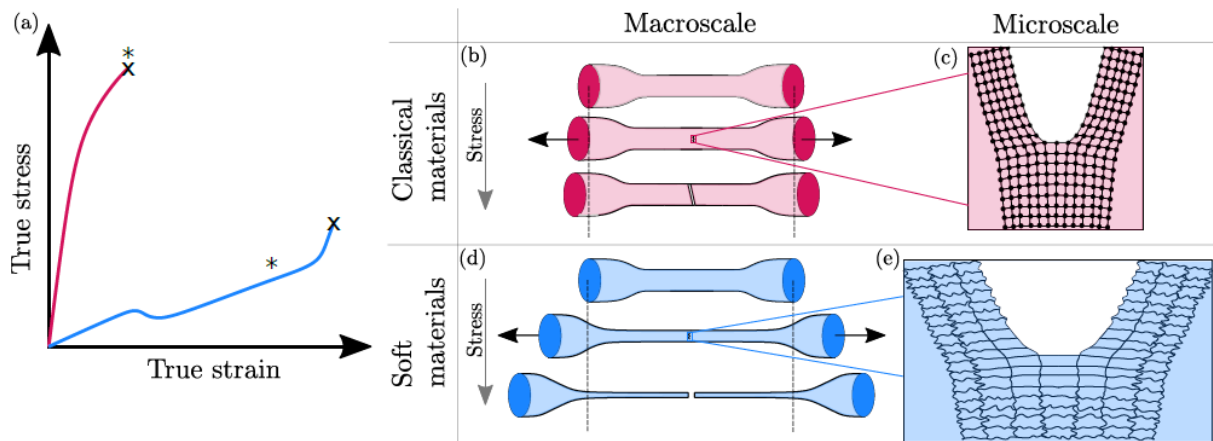|		
	 Image from [41]	Protein recruitment can be quantified via simultaneous confocal microscopy imaging.
Tissue-scale fracture	**(f)** *Tensile test of embryonic epithelia* Image from [38]	**Tensile testing platform** for the measurement of embryonic epithelial properties [38]. Live embryonic tissues are glued to the test rods. One rod is moved under computer control and force is calculated from the deflection of the second wire. This first investigation of its kind revealed that tearing in embryonic epithelia occurs at strains ~50%. (scale bars 500 μm).
	(g) *Tensile test of suspended monolayers* Image from [39]	A variation of the above has been introduced to study the mechanical response of suspended epithelial monolayers. Epithelia are cultured on a collagen substrate that is polymerised between two test rods. When the epithelia cover the whole collagen and part of the rods, the collagen substrate is enzymatically removed, leaving a monolayer suspended between the two rods. Deformation of the monolayer is applied via a motorised micromanipulator while force is recorded by a digital transducer. The device allows the study of the complex tissue rheology up to complete failure [39]. Imaging can be carried out simultaneously by confocal microscopy. (scale bar $1000\mu m$)
	(h) *Tensile test of cell aggregates* 	**Microplate devices** measure the strength of cell junctions between cell aggregates [89]. The right plate is moved at a predefined velocity while the deflection of the leftt plate allows measurement of the force (scale bar 100 μm).
	(i) *Stretching of monolayers on PDMS membrane*	**Substrate stretchers** allow to apply deformation to epithelial tissues cultured on a soft hydrogel substrate attached to a polydimethylsiloxane (PDMS) membrane. Deformation of the PDMS membrane stretches the hydrogel and the epithelial layer [90]. Tissue stress can be inferred by traction force microscopy.

Image from [90]

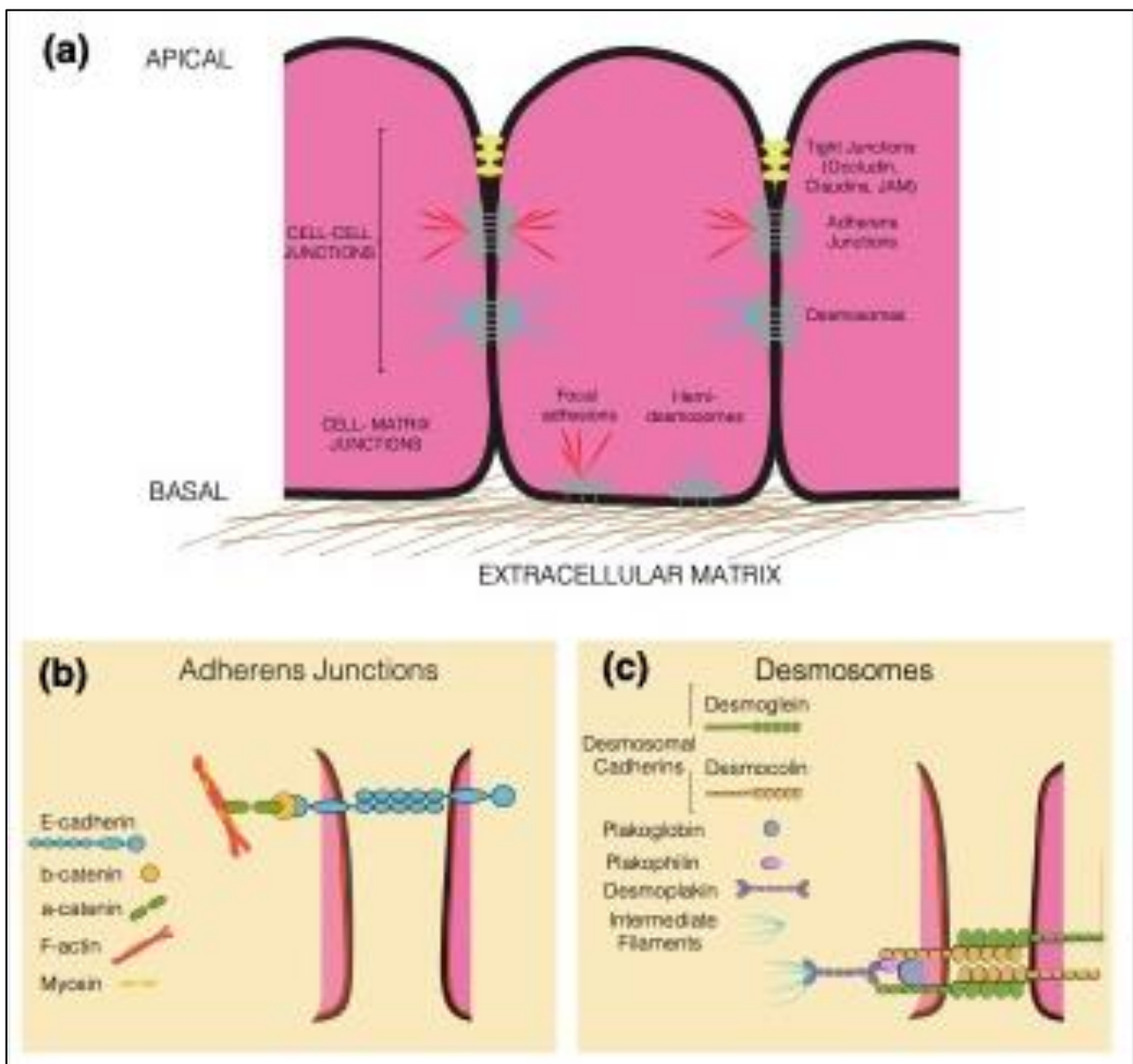**(j)** *Tensile and shear tests of adherent monolayers*

MEMS tissue testers apply tensile (a) or shear (b) forces [56, 57] to an epithelial monolayer. Cells are cultured on two platforms (pink and grey) separated by a small gap. Platforms can be moved to deform cells spanning the gap. Integration with a fluorescence microscope enables imaging at single cell level. The setup is compatible with pharmacological treatments and applicable to different cell types. (a) shear test, (b) tensile test. (scale bar 500 μm)
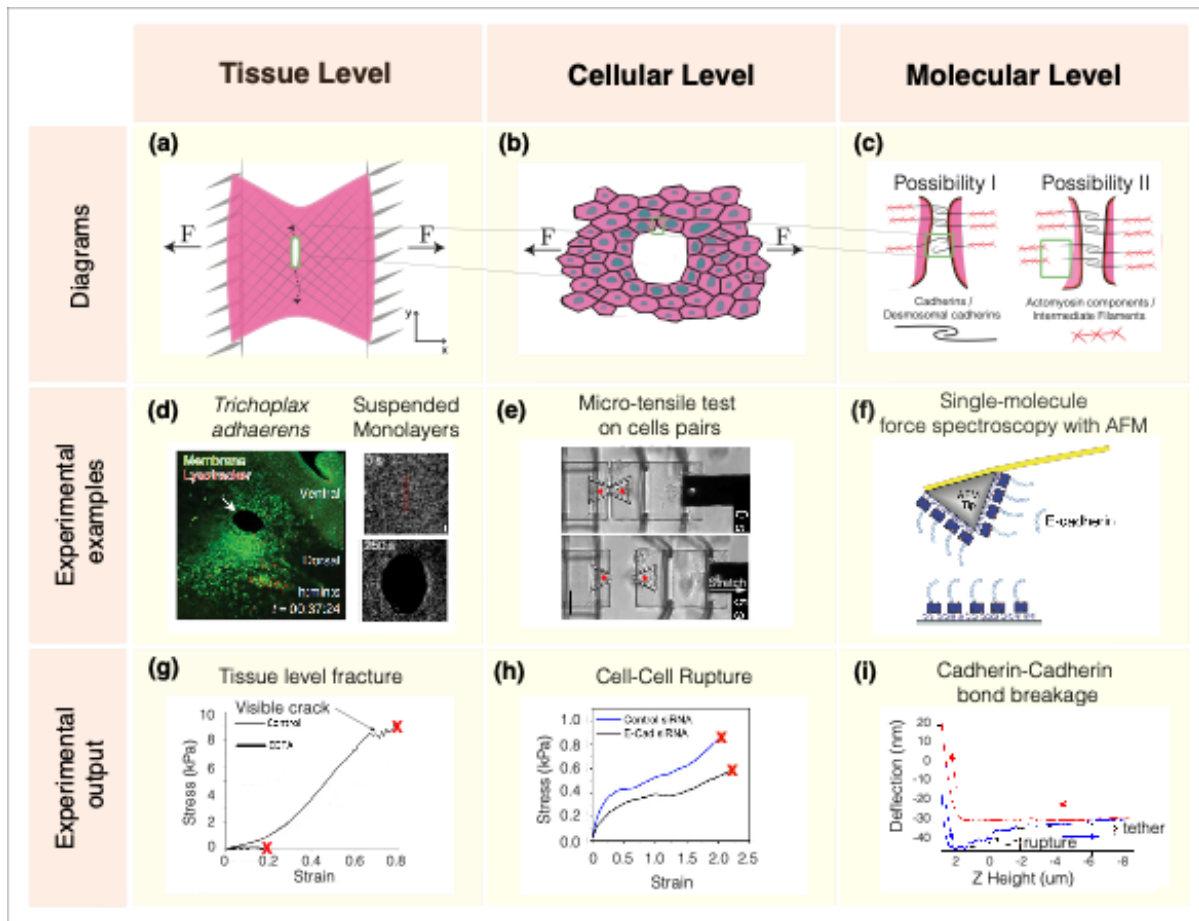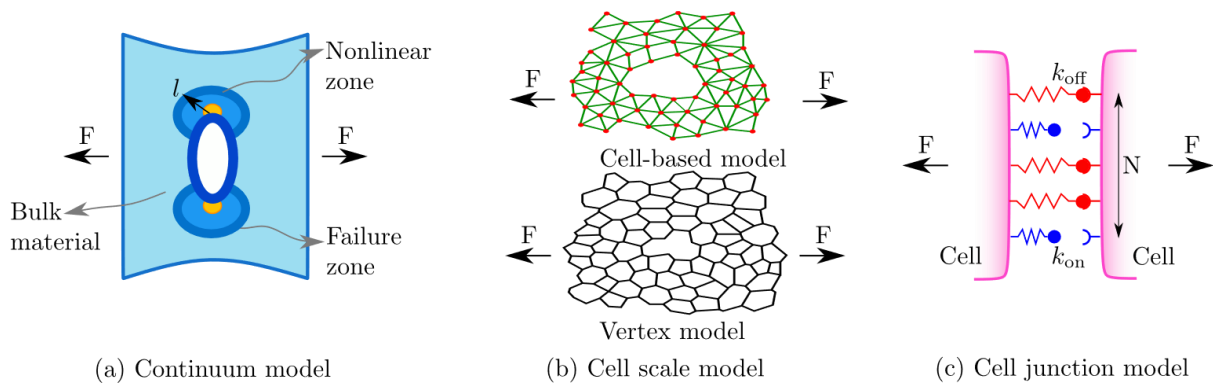
Figures
Figure 1:

Figure 2:

Figure 3:

Figure 4:

(a) Continuum model

(b) Cell scale model

(c) Cell junction model

Outstanding questions box:
- How are stresses shared across the cell's different cytoskeletal networks and intercellular adhesion complexes? What is the density and spacing of intermolecular complexes at junctions? What forces are intercellular adhesion complexes subjected to in vivo?

- How does macroscopic tissue fracture result from molecular-scale phenomena (e.g. turnover of adhesion complexes and the rheology of cytoskeletal networks) and cellular-scale behaviours (such as rearrangements and oriented divisions)?

- What is the best theoretical approach to predict tissue fracture from molecular- and cellular-scale characterization?