Soft matters in cell adhesion: rigidity sensing on soft elastic substrates†

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This contribution highlights recent advances in our understanding of the relation between soft matter and biological systems. We first discuss the physical scales of living cells which follow from simple scaling arguments developed in soft matter physics. Then we discuss the way cells sense and react to extracellular stiffness as revealed by recent experiments with soft elastic substrates. Theoretical modelling allows addressing of the physical basis of the underlying mechanotransduction processes and its consequences for the organization of single cells and cell communities in soft environments. In the future, these efforts will also lead to an improved understanding of physiological and artificial tissue.

1. Soft matter and biological systems

Living cells are made up of soft matter mainly because the intrinsically dynamic nature of soft matter allows the biological system to quickly react to changes in its environment. Soft matter at work in biological systems includes the biomembranes defining the various compartments of cells[1] and the polymer networks forming the cytoskeleton and the extracellular matrix.[2] Soft matter is held together by multiple weak interactions, each of the order of thermal energy $E = k_BT = 4.1$ pN nm. Here k_B is the Boltzmann constant and $T = 300$ K is the order of magnitude for room or body temperature. Moreover, soft matter is structured on molecular length scales l that are much larger than atomic length scales. Taking $l = 10$ nm for a large supramolecular aggregate, we get an elastic modulus of $Y = E/l^3 =$ kPa. Indeed this is a typical value for the rigidity of colloidal crystals or weakly crosslinked hydrogels, and several orders of magnitude below the typical rigidity of traditional condensed matter systems like atomic crystals. kPa is also a typical value for the stiffness of cells, which however are characterized by a large range of different energy and length scales. Similarly, the time scales relevant for cellular processes also span a large range. For example, the lifetime of biomolecular adhesion bonds ranges from milliseconds (*e.g.* L-selectin–PNAd) to hours (*e.g.* biotin–avidin). Viscosity in cellular systems strongly depends on the length scale of the probe used and ranges from a few times the viscosity $\eta = 10^{-3}$ Pa s of water for small probes to $\eta = 10^4$ Pa s on the scale of a whole cell.[3] This results in a typical time scale $\eta/Y = 10$ s for the viscoelastic flow of whole cells.

Soft matter is robust and fragile at the same time: due to the weak interaction energies, it can easily be perturbed by external fields, but also self-organizes again after the perturbation has ceased. The tendency of soft matter to self-organize is used over and over again in biological systems.[4] One prominent example is the self-assembly property of lipids in an aqueous environments, where they tend to form lipid bilayers over a large range of concentrations due to the hydrophobic effect. The most important physical property of lipid bilayers is their bending rigidity κ, which from elasticity theory follows as $\kappa = Yl^3$, where $l = 4$ nm is the thickness of the thin elastic sheet. Because $Y = E/l^3$ as explained above, it follows from theoretical grounds that lipid bilayers have bending rigidities of the order of thermal energy. For example, the bending rigidity in the case of the phospholipid DMPC has been found experimentally to be 15 k_BT. Therefore bending is easily excited by thermal fluctuations, which is put to good use by cells because the thermally excited flickering of the plasma membrane is one of the main mechanisms to avoid non-specific adhesion to external surfaces (the other main mechanism is the evolution of a polymer brush surrounding the cell, the so-called *glycocalix*). Despite these

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fluctuations, lipid bilayers are mechanically very stable: the nm-thick shell is able to hold together a whole cell, which is tens of microns large. Another important property of lipid bilayers is that they easily form suprastructures, like the bicontinuous cubic structures reminiscent of the tubular networks commonly observed in the endoplasmic reticulum, the Golgi apparatus and mitochondria.[5] Changes in membrane morphology are essential in many biological processes, including endo- and exocytosis, and therefore biological systems have developed many intricate ways of controlling the curvature of lipid bilayers, including demixing of different types of lipids and protein adsorption onto the membrane.[6]

Due to its intrinsically dynamic nature, soft matter not only tends to self-assemble, it can also easily be coupled to biochemical control structures, allowing for robust and purposeful functioning of biological systems. One prominent example for this relation is the regulation of the actin cytoskeleton. The actin cytoskeleton is a network of protein filaments that gives structural strength to the cell, provides spatial organization to intracellular processes and reorganizes rapidly in response to extracellular signals. Its main regulators are the small GTPases from the Rho-family, small molecular switches which control the organization of the actin cytoskeleton into different architectures. In particular, lamellipodia, filopodia and stress fibers are regulated through Rac, Cdc42 and Rho, respectively.[7] This corresponds roughly to the physiological processes of motility, polarity and mature adhesion, respectively. The different GTPases are activated differently in space and time by different extracellular clues, including biochemistry, mechanics and topography of external surfaces. They also exhibit crosstalk to other signaling pathways and lead to changes in gene expression, thus contributing to the complexity of cellular behaviour.

2. Rigidity sensing in cell adhesion

Recent years have seen a renaissance for quantitative approaches to biology, mainly triggered by the human genome project. In the wake of the different omics-projects (most prominently genomics and proteomics), the regulative aspects implemented in signal transduction and gene expression networks have received a lot of attention from biology and neighboring disciplines. Less prominent, but also very important, however, is the steadily increasing transfer of concepts and methods from soft matter physics into the biosciences. One striking example for this development is cell-matrix adhesion. A large variety of new tools is now used to control cell adhesion to a much larger degree than formerly possible, including micro-contact printing of adhesive structures (*e.g.* polygonal islands[8] or extended dot-patterns[9]) and ligand-positioning on the nm-scale using block-copolymer lithography.[10] Moreover many biophysical tools have been developed to correlate the biochemical and physical states of adherent cells, including force microscopy of cells,[11,12] traction force microscopy,[13–16] cell stretchers[17,18] and activity maps of the cell contour.[19,20]

One of the most impressive advances triggered by this development is the realization that cell behaviour depends sensitively on the rigidity of the extracellular environment. Traditionally cell–matrix adhesion is studied by culturing cells on rigid glass or plastic dishes, where they typically develop micron-sized sites of cell–matrix adhesion (*focal contacts*) connected by contractile actin bundles (*stress fibers*). In 1997, it was found that these structures are gradually lost as cells are cultured on increasingly softer substrates which can be prepared by changing the crosslinker density for polyacrylamide gels.[21] Subsequent and more quantitative work with elastic substrates showed that for typical tissue cells the crossover occurs roughly at 10 kPa, that is on the scale of cellular rigidity.[22] Experimental studies with soft elastic substrates have shown that fibroblast-like cells spread to larger areas on stiffer substrates,[22] that they locomote for stiff or tensed regions in their environment,[23] and that cell growth and differentiation can be controlled by the stiffness of the environment.[24,25] The rigidity response seems to be coupled to growth inhibition on soft substrates, which distinguishes normal from cancer cells.[26,27] During recent years, the molecular basis of the mechanotransduction events underlying rigidity sensing have also been investigated in great detail.[28,29] It was found that larger stiffness of the environment correlates with a larger force at single focal adhesions,[30] which in turn correlates with larger protein aggregation.[14–16] Because both correlations have been measured to be linear (compare Fig. 1), cell traction seems to lead to

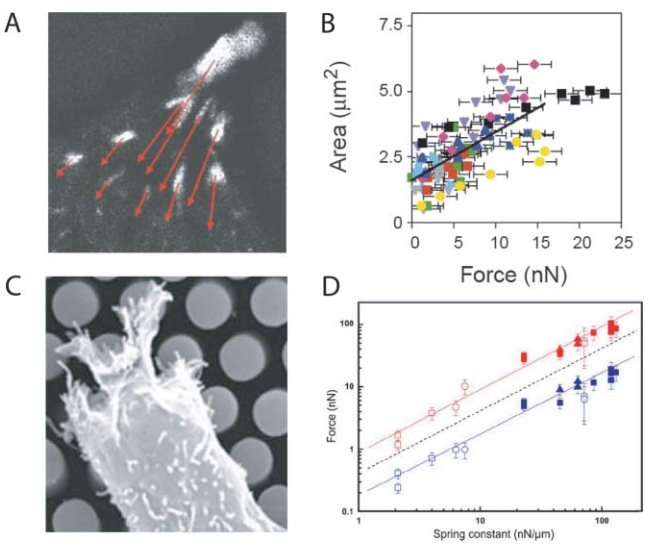

Fig. 1 (A) Forces measured at single focal adhesions of fibroblasts on micropatterned soft elastic substrates (reproduced, with permission, from an article published in *Nature Cell Biology*, see ref. 14). (B) Quantitative analysis revealed a linear correlation between force and lateral size. (C) MDCK epithelial cells on a micro-fabricated pillar array (reproduced, with permission, from an article published in *Biophysical Journal*, see ref. 30). (D) Quantitative analysis revealed a linear correlation between the local spring constant and the force at single focal adhesions (blue and red data points are average and maximum values).

constant deformation of the order of 130 nm[30] and constant stress of the order of 5.5 nN μm^{-2} at single focal adhesions.[14] Because single molecules are characterized by nm-dimensions and thermal energy k_BT = 4.1 pN nm, the force scale on the molecular level is pN, *e.g.* in regard to the rupture strength of a single adhesion bond. With a typical distance of 10 nm between the integrins in focal adhesions, this force scale is increased to F = 10 nN at micron-sized focal adhesions, as indeed measured experimentally. On a soft elastic substrate with Y = 10 kPa, this leads to a displacement of $l = (F/Y)^{1/2}$ = μm. On the cellular scale of tens of microns, this corresponds to a strain of up to 10 percent.

3. Models for rigidity sensing

Motivated by the experimental findings relating to rigidity sensing, the relation between extracellular rigidity and force generation at and growth of cell–matrix contacts has been subject to detailed modelling efforts.[31–37] Because the stiffness of the environment is a passive quantity, it has to be actively sensed by the cell by contracting it with actomyosin force and by measuring some kind of mechanical response, for example by monitoring the energy which has to be invested to reach a certain level of internal force.[38] A simple rationale for rigidity sensing at focal adhesions is provided by the two-spring model schematically depicted in Fig. 2.[39] We consider a cell which contracts its environment with one stress fiber anchored at two sites of adhesion. The spring constants K_e and K_i represent extracellular and intracellular stiffness, respectively. Because the situation has mirror symmetry, we only have to consider two springs. Starting at time t = 0, they are strained by a cytoskeletal molecular motor represented by a linearized force–velocity relation $v(F) = v_0\,(1 - F/F_s)$ with free velocity v_0 and stall force F_s. We also include a dynamic process, *e.g.* the rupture of a weak link in the structure, with rate k_0, for reasons which will become clear later. We first note that the effective spring constant follows from $1/K = 1/K_e + 1/K_i$, that is the softer spring determines the mechanical response. This implies that if the environment is much stiffer than the cell, it basically strains itself and cannot sense the exact value of the environmental stiffness. Indeed such a saturation behaviour has been found for the spreading area as a function of increasing substrate stiffness (in contrast, the cell behaviour has been found to be biphasic as a function of the density of adhesive ligand).[22] The dynamics of this system can be found analytically by equating the power vF invested by the motor with the power $\mathrm{d}/\mathrm{d}t(F^2/2K)$ stored in the springs. Integration leads to

$$F = F_s\,(1 - e^{-t/t_K}). \qquad (1)$$

Therefore the force first rises linearly and then saturates at the stall force F_s, irrespective of the stiffness K. If the cell wants to sense extracellular stiffness through some force-mediated process, it therefore has to resort to some dynamical process, because it is the timescale $t_K = F_s/v_0K$ for the build up of force which depends on rigidity K. For typical parameter values, this timescale rises from milliseconds to seconds as extracellular stiffness decreases from MPa to kPa. In the framework of a simple Poisson process with rate k_0, the average force which can be built up until the weak link ruptures is $F/_s(1 + k_0t_k)$, which rises with increasing stiffness K. Thus if the mechanosensor at the focal adhesions depends on a process which requires some threshold in force (*e.g.* the force-induced exposure of some cryptic binding site of one of the many proteins localized to focal adhesions), then stiff environments are more favorable because force is built up faster.

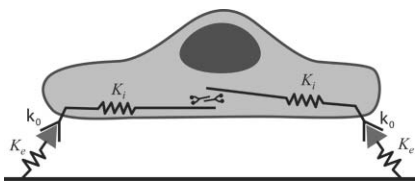

Fig. 2 Two-spring model for rigidity sensing through cell–matrix contacts:[39] one stress fiber connects two focal adhesions. Force is generated by myosin II molecular motors sliding actin filaments relative to each other. The time scale for the build-up of force is determined both by internal rigidity K_i and external rigidity K_e. The larger K_e, the faster force is built up, as long as $K_e < K_i$. This dynamic competes with other internal dynamics of the cell, *e.g.* rupture of weak links with rate k_0.

In the future, simple models like the two-spring model have to be extended to include three-dimensional elasticity as well as other important features of cell mechanics like cytoskeletal prestress and strain stiffening. Although the two-spring model does provide interesting insights into the physical aspects of cell adhesion, it is important to note that at the present state of affairs, the experimental data are far from allowing definite conclusions to be drawn. In particular, cellular activation on stiff substrates can also be explained by arguments based on displacement rather than force.[29,40] For example, it has been argued that the small deformations resulting on stiff substrates ensure spatial proximity of the kinase Fyn and its substrate Cas, which are essential for the cellular response to rigidity, acting downstream of integrin-based cell–matrix contacts and upstream of regulators of the actin cytoskeleton like Rac or Vav.[27] Given the complexity of biological systems, it should not be surprising if in practise different mechanisms act in parallel (or even in concert) at cell–matrix contacts.

4. Towards a science of tissues

Cell–matrix adhesion is also the functional basis for the large scale organization of tissue, whose mechanical aspects have been investigated for a long time.[41] In general, tissue is a composite material comprising cells and the extracellular matrix. For example, the connective tissue beneath our skin is a mixture of collagen, which resists tensile strain, proteoglycans like hyaluronan, which resist compressive strain, and living cells, mainly fibroblasts, which degrade old matrix, secrete new matrix and mechanically reorganize existing matrix in response to external stimuli, especially in the case of severe damage (*wound healing*).[42] It has long been clear that an intricate interplay exists between the mechanical properties of the matrix and the activity of tissue cells, leading to the maintenance of a functional mechanical state of healthy tissue (*tensional homeostasis*).[26,43] Active mechanosensing at focal adhesions has been argued theoretically to lead to non-trivial effects in regard to the way cells position and orient themselves in soft environments, in good agreement with experimental

observations.[38] Moreover, recent experimental studies using similar tools as mentioned above for single cells have shown that substrate mechanics determine the fate of growing tissue,[44,45] possibly through a negative feedback mechanism mediated by mechanical strain.[46] Because tissues are based on the collective behaviour of a large number of cells, one of the big challenges in this field is to develop a statistical mechanics approach to ensembles of active particles interacting through the extracellular matrix. In particular, it has to be seen if a Gibbs ensemble is appropriate to describe the disorder present in cell ensembles. The first advances in this direction have indeed been made by using the concept of an effective temperature, which for cellular systems turns out to correspond to an energy scale of 4×10^{-15} J.[47,48] This is six order of magnitude larger than thermal energy k_BT and corresponds nicely to the fact that in order to reorganize, the cell has to detach of the order of 10^5 adhesion bonds, each with an energy around 10 k_BT.

In summary, *soft matters in cell adhesion* not only because cells are made from soft material, but also because cells actively sense and react to the rigidity of their environment. The cellular response is built on generic aspects of soft matter systems, in particular self-assembly of the polymer networks of the cytoskeleton and coupling to biochemical control structures like the small GTPases from the Rho-family regulating the actin cytoskeleton. By designing biomimetic models for the extracellular matrix and by quantitatively analyzing their effect on cell–matrix adhesion, we might hope to eventually unravel the basic principles at work at the interface between living cells and their environment. One particularly rewarding line of research is how the cellular response to local rigidity relates to the large-scale organization of tissue, because in the long run, such an understanding might pave the way for rational design of scaffolds for tissue engineering and for new strategies in regenerative medicine and cancer therapy.

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