nature chemical biology

REVIEW

Force probing surfaces of living cells to molecular resolution

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Biological processes rely on molecular interactions that can be directly measured using force spectroscopy techniques. Here we review how atomic force microscopy can be applied to force probe surfaces of living cells to single-molecule resolution. Such probing of individual interactions can be used to map cell surface receptors, and to assay the receptors' functional states, binding kinetics and landscapes. This information provides unique insight into how cells structurally and functionally modulate the molecules of their surfaces to interact with the cellular environment.

Cells interact with their environments via their surfaces. Therefore, cell surfaces play essential roles in basic cellular processes such as signaling, communication, adhesion, sensing, transport, energy generation, embryonic and tissue development, tumor metastasis, and viral or bacterial infection[1–5]. The highly sophisticated functions of cell surfaces are mediated by the structurally complex and dynamic assembly of specific carbohydrates, proteins, lipids and other macromolecules. A crucial challenge in current cell biology is to understand how these cellular molecules structurally assemble, and how they modulate their functions to interact with the cellular environment[1,5,6].

Every process of the cell surface relies on molecular forces that are a complex interplay of chemical, biological and physical interactions[4,7,8]. Typical interactions occurring at biological surfaces include hydrophobic, hydrophilic, electrostatic, van der Waals and hydrogen bonding interactions, and the force measured for a specific biological interaction reflects the sum of all contributing interactions[7].

Elucidating when and where certain interactions determine cell biological processes is somewhat like deciphering a basic molecular language. Understanding this language includes describing how a cell surface receptor finds its ligand, how and where the ligand binds, and by which mechanism the ligand switches the functional state of its target. It also includes describing how cellular interactions modulate the assembly and functional state of cell surface receptors. Thus, a conceptual understanding of biological forces goes beyond simply describing the sensing, transduction and response of cells to mechanical stress (**Box 1**).

A range of force spectroscopy assays that allow measurement of inter- and intramolecular interactions of cell surfaces at the molecular scale have been established[9–12]. Notably, owing to its nanoscopic force sensor (~2–50 nm), atomic force microscopy (AFM)[13,14] is the only assay that allows investigators to sense and locate specific interactions of cell surfaces at high resolution (~10 nm). In addition, the sensitivity of AFM-based assays allows detection of the widest practical force range—from 5 pN to 100 nN (refs. 15–18). This makes AFM suited to detect forces ranging from the strength of single receptor-ligand bonds (~60–80 pN) to those covering the adhesion of entire cells (>>1 nN) (**Box 1** and **Table 1**).

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AFM of cell surfaces

Recent developments in AFM (**Box 2**) have extended its use from high-resolution imaging (**Fig. 1**) toward nanotechnological investigations that probe forces of biological, chemical and physical interactions to molecular resolution[14,16]. AFM works in solutions and at physiological temperatures, which is critical for biological applications[19]. Using extracted and immobilized membrane patches, the spatial resolution can approach ~1 nm[20], whereas the resolution of corrugated and dynamic surfaces of living cells is currently limited to ~50 nm for animal cells[21,22] and ~10 nm for microbes[23,24]. AFM imaging can be combined with modern light microscopy to correlate cellular structures down to the resolution limit of both microscopes[22,25].

Soon after its introduction, AFM was used to measure the force at which ligands (or receptors), attached to the stylus of the AFM cantilever, and receptors (or ligands), tethered to a support, unbind[26,27]. This technique, termed single-molecule force spectroscopy (SMFS), was used (i) to characterize the interaction of cell adhesion molecules (CAMs) including P-selectin[28], cadherin[29,30], oligosaccharides[31] and proteoglycans[32], (ii) to locate ligand or inhibitor binding to membrane proteins[33,34] and water-soluble proteins[17,35], and (iii) to characterize the anchoring forces of peptides in lipid membranes[36]. However, *in vitro* SMFS using purified biological molecules has an inherent flaw. The molecules studied are removed from their biological context, which controls their structural assembly and functional state[2,5,6,37]. It is of particular concern that CAMs such as cadherins and integrins have several substrate binding states[2,38,39]. In most cases, such transmembrane receptors are purified in truncated forms consisting only of their extracellular domains. Thus, in addition to being removed from their native environment, the receptors are characterized in absence of their regulatory domains. These problems can be circumvented using cells.

Box 1 Forces from the cellular to the molecular level

Cells generate, sense, resist and respond to mechanical forces. Cellular forces range (Table 1) from ~6 pN generated by single molecular motors of myosin II and extend to contraction forces over 2 μN generated by the elaborate assembly of such motors in a smooth muscle cell[89,90]. Only with well-coordinated complexes can cells generate and resist such forces. To understand how biological systems respond to mechanical stress, the forces at which biological assemblies come apart, unbind or unfold are relevant. Starting at the single-molecule level, single covalent C-C bonds rupture at ~4 nN (ref. 91). This is considerably higher than the force needed to unfold a protein domain—150–200 pN in the case of a titin immunoglobulin[92]. Slightly lower forces (100–150 pN) sequentially unfold and extract structural domains of transmembrane proteins from cellular membranes[33,93]. Of particular interest to this review are the binding strengths of receptor-ligand interactions, which fall between 20 and 200 pN (refs. 58,70). However, after a few minutes of attachment, integrin receptors cooperate to enhance cell attachment strength manyfold[54].

Table 1 Forces characterizing biological processes from the cellular to the molecular range

Process	Force required/ generated*	References
Cellular contraction	~2 μN	89
Rupture of covalent bond	~4 nN	91
Unfolding of water-soluble proteins	~100–200 pN	92
Unfolding and extracting a membrane protein from a lipid bilayer	~100–150 pN	36,93
Rupture of receptor-ligand interactions	~20–200 pN	58,70
Enzymatic activation	~10–50 pN	17,18
Cytoskeletal motor protein	~2–10 pN	90

*The rupture force of a bond depends on the time range in which the bond is forced to break.

The shape and functional state of eukaryotic cells is defined by cycles of mechanosensing, mechanotransduction and mechanoresponse[2,4]. Sensing of specific forces is transduced into biochemical signals[94] and the cell responses to complex properties such as substrate rigidity[3,95]. Mechanically induced unfolding of individual proteins regulates cellular processes[17,18,96]. Stem cells differentiate into specific phenotypes with extreme sensitivity to tissue-level elasticity[97]. Such examples emphasize the role that mechanical forces have in guiding cellular processes[4,5,8,98].

The principle of SMFS can be applied to probe specific interactions of single molecules on the surface of living cells (**Fig. 1b**). To do so, certain conditions must be met: (i) the attachment of the probing molecules functionalizing the AFM stylus must be much stronger than the intermolecular bond probed; (ii) the probing molecule must be in a native state; (iii) frequently occurring unspecific interactions must be suppressed or discriminated from specific ones; (iv) the interactions detected should represent those of single molecules.

Probing specific interactions of cell surfaces requires functionalizing the AFM stylus with, for example, specific ligands or chemical groups (**Fig. 1c**). Biomolecules can be covalently attached to self-assembled monolayers (SAMs) of alkanethiols that functionalize gold-coated AFM styluses, using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and *N*-hydroxysuccinimide (NHS)[32]. It is also possible to attach recombinant histidine-tagged proteins to a stylus coated with nitrilotriacetate (NTA)-terminated alkanethiols[40,41]. Another approach is to covalently anchor biomolecules or viruses on silicon styluses using amine functionalization procedures[42–44]. The amino-terminated surfaces are reacted with a crosslinker that allows the biomolecule motional freedom and prevents its denaturation[43]. Typically, crosslinkers carry two different functional ends—for example, an amine-reactive NHS group on one end, and 2-pyridyldithiopropionyl or vinyl sulfone groups (which covalently bind thiols) on the other. Heterobifunctional crosslinkers having two different amino-reactive functions require no prederivatization and minimal protein amounts (for example, 5 μg protein in 50 μl buffer)[45]. Other linkers introduced, such as poly(ethylene glycol) (PEG) spacers[29,30] and DNA molecules[46], extend the toolbox to modify and functionalize AFM styluses.

Using cells as force probes

For a number of applications it is advantageous to attach a cell to the cantilever and probe its adhesion to surfaces (**Fig. 2**). This experimental setup, which is called single-cell force spectroscopy (SCFS)[15], allows investigators to probe the adhesion between two cells[47,48]. An AFM cantilever is functionalized such that single cells can be attached (**Box 3**). The attached cell is pressed onto a target with a set force and kept stationary for a set time (**Fig. 2**). With the withdrawal of the cantilever, the force required to separate the cell from its binding partner is measured by monitoring the cantilever deflection. The resulting force-distance (F-D) curve provides a signature of the cell adhesion. With different contact forces and adhesion times, both overall cell detachment forces and the contribution of single molecules can be measured. The challenge is to interpret the force signature, because (as for SMFS measurements) various specific and nonspecific adhesion processes may occur simultaneously.

Whereas some cell surface receptors bind to many different ligands, others bind to only one or two[2,6,49]. Many different receptors may contribute to the adhesion of a cell. Comparing F-D spectra recorded in the absence and presence of specific blocking agents allows correlation of the adhesive contribution of receptors[50–53]. Surface coatings were developed to characterize specific CAMs. Nanoscopic collagen I matrices are used to probe the binding of $\alpha_2\beta_1$-integrin[54]. Other protocols use collagen IV coatings to probe interactions with integrin[53], laminin coatings to probe cellular adhesion mediated by galectins[55], fibronectin coatings to probe the binding of $\alpha_5\beta_1$-integrins[52,56], and ConA coatings to probe the binding of N-linked oligosaccharides[57].

Insights into cell surface bonds

When probing cell surfaces using force spectroscopy, the stressed bonds can unbind via different mechanisms (**Fig. 3**). As long as the cantilever's substrate and the cell surface are in contact, the adhesive bonds are not strained. While the adhesive molecule is anchored to the cell cortex (or comparable cellular structures), it is bound by quasi-elastic elements (**Fig. 3a**). As the cantilever is withdrawn from the cell surface, the force stressing the bond and its anchor increases linearly with the distance (**Fig. 3a** (i)). If the anchor strength is greater than the strength of the adhesive bond, the adhesive bond will likely unbind first. In such a case, the Bell-Evans model (**Fig. 3a** (ii))[58–60] may be applied for data analysis and interpretation. According to this model, the force at which a single bond unbinds increases as the rate at which the force on the bonds is

Box 2 AFM of cell surfaces

AFM uses short-range interactions between a sharp stylus and the specimen to generate topographs of the specimen's surface (**Fig. 1a**). The stylus is attached to a cantilever that deflects under an interacting force. A piezoelectric scanner allows high-resolution, three-dimensional positioning (~0.1 nm) of the stylus[13,14].

In force spectroscopy modalities such as single-molecule force spectroscopy (SMFS), chemical force microscopy (CFM), molecular recognition mapping (MRM) and single-cell force spectroscopy (SCFS), the cantilever deflection is recorded while the stylus and sample are approached and retracted (**Fig. 1b**). This results in a cantilever deflection-versus-displacement curve that is transformed into a force-distance (F-D) curve. The characteristic adhesion (or unbinding) force observed during stylus retraction is the key parameter that provides information on specific receptor-ligand interactions (in SMFS), on the spatial distribution of chemical interactions (in CFM), on the spatial distribution of individual receptors (in MRM) and on forces that govern cell-cell and cell-substrate interactions (in SCFS).

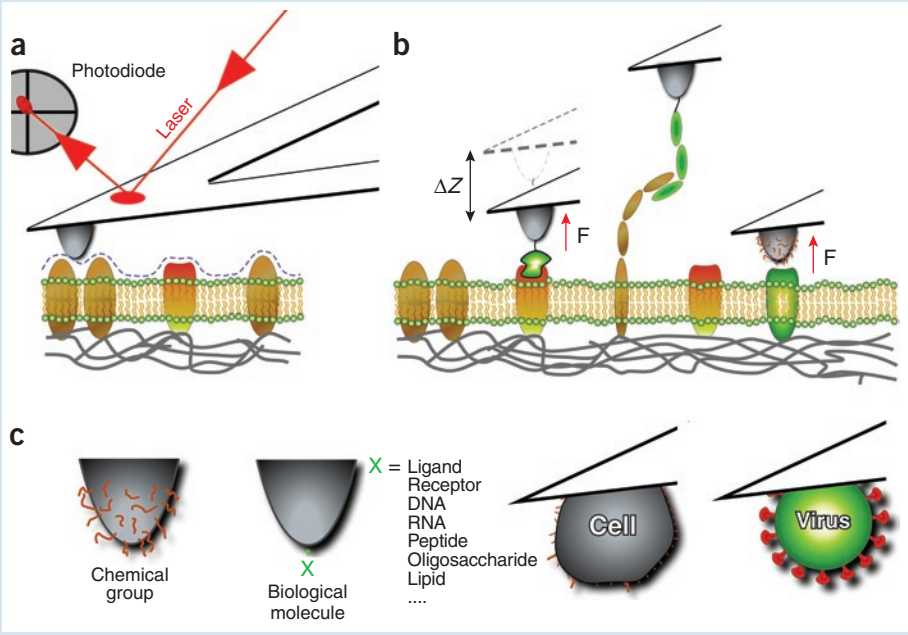

Figure 1 AFM probing surfaces of living cells. (**a**) In the imaging mode, AFM contours the topography of the cell surface (dashed line) using a sharp stylus. (**b**) The AFM stylus can be used to probe interactions of the cell surface to single-molecule resolution. Examples show a stylus that has been functionalized with a ligand to probe interactions with its cognate receptor, a stylus that carries a CAM to probe heterogeneous interactions with other CAMs, and a stylus coated with chemical groups to detect interactions of interest. (**c**) Protocols to functionalize the AFM stylus or cantilever to specifically probe chemical, biological, cellular or viral interactions are reviewed in the text.

Most force spectroscopy studies on living cells require chemical or biological functionalization of the AFM stylus (**Fig. 1c**). SMFS and MRM require attachment of ligands, peptides or proteins to the stylus that specifically interact with cell surface receptors. For CFM the stylus is modified with chemical groups. SCFS attaches cells or viruses to AFM cantilevers.

applied (loading rate, r_f) increases The loading rate is controllable, as it depends on the velocity at which the AFM cantilever is retracted. For most receptor-ligand bonds, their rupture force increases linearly with the logarithm of the loading rate (**Fig. 3a** (ii)). Exceptions are catch bonds, which are optimized to bind at certain loading rates[61,62]. By measuring the most probable rupture force over a range of loading rates, the unbinding rate (k_{off}), the distance to the transition state (x_u) and the free energy (ΔG_u) of the unloaded bond can be estimated. Thus, force spectroscopy can be used to explore parameters that describe the energy landscape and the reaction kinetics of cellular bonds in their native environment (**Fig. 3a** (iii)).

The mechanism of unbinding a receptor-ligand bond changes if the receptor is never anchored to the cortex or if its interaction with the cortex breaks before its adhesive bond. In such a case, the receptor can be pulled away from the cell at the tip of a membrane nanotube, also referred to as a membrane tether (**Fig. 3b**). For a wide variety of

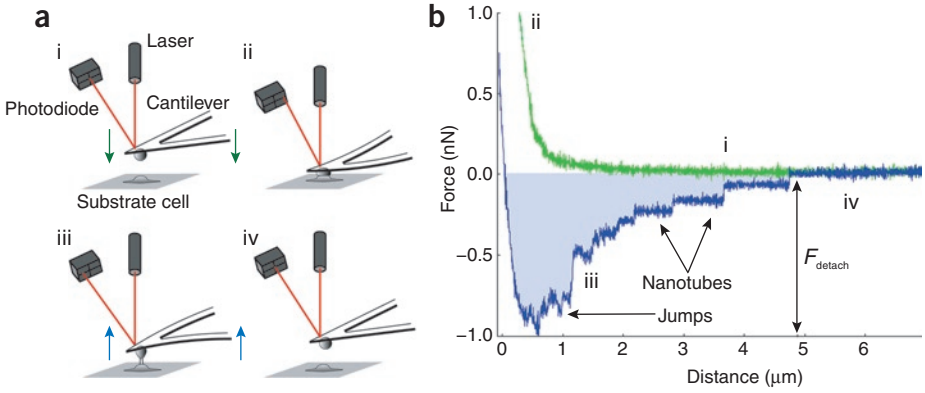

Figure 2 AFM-based single-cell force spectroscopy. (**a**) Measuring the adhesion of an AFM cantilever-bound cell. The cantilever deflection is determined using the position of a reflected laser beam and translated into an interaction force transmitted via the cell. The bound cell is brought into contact with another cell or substrate (i) and allowed to adhere (ii) before separation (iii and iv). (**b**) F-D curves recorded during approach (green) and separation (blue). The separation curve includes the forced unbinding of individual bonds (jumps) and the formation and unbinding of membrane nanotubes, and is characterized by a maximal detachment force F_{detach}. Points i–iv correspond to the events shown in **a**.

Box 3 Converting a cell into a probe

Several methods have been developed to attach cells to cantilevers. The most common is the use of concanavalin A (ConA)[15], a lectin that binds mannose residues that are covalently attached to most cell surface proteins. ConA can be adsorbed onto plasma-cleaned cantilevers or attached via a streptavidin-biotin linker[50,55]. Alternatively, another lectin (wheat germ agglutinin[48]) or extracellular matrix proteins (such as collagen and fibronectin[99]) can be used to functionalize the cantilever for cell attachment. Occasionally, cells have been grown on cantilevers[100]. Recently, cantilevers coated by complementary DNA strands allowed investigators to pick up and drop the cell after use[46].

cells, these nanotubes are of physiological relevance, as they facilitate intracellular attachment and communication[1,63]. The nanotube extension force (F_t) depends on plasma membrane properties and extension velocity (V) (**Fig. 3b**) and does not reflect the strength of the receptor-ligand bond tethering the nanotube[1,58]. As nanotube length has very little effect on extension force, cell membranes establish constant force clamps that can be used to measure the lifetime (l_n) of receptor-ligand bonds under force (**Fig. 3b** (i))[57]. According to the Bell model[64], measuring the mean lifetime of a bond at different forces (extension velocities) allows the lifetime at equilibrium (l_{off}) and distance to the transition state (x_u) to be determined (**Fig. 3b** (ii)). Again, although the Bell model describes many biological bonds, there are exceptions (such as catch bonds). By analyzing nanotube extension forces, plasma membrane properties such as the extent of anchoring to the cytoskeleton and viscosity can also be characterized[65,66]. With weaker anchoring of the membrane and the cytoskeleton, the probability of pulling nanotubes rises, whereas the force required to extract nanotubes decreases[1,57,65]. By comparing the number of bond ruptures to the number of nanotube extension events, changes in receptor-cortex anchoring strength were revealed[67].

Case studies

Here we present case studies in which the above AFM modalities have answered pertinent cellular questions.

Localizing cell surface receptors *in vivo*. SMFS-based molecular recognition mapping (MRM) identifies and localizes molecules on cell surfaces[68,69]. AFM styluses functionalized with specific biomolecules are used to generate adhesion force histograms and maps by recording an array of F-D curves. For each F-D curve the unbinding force is assigned to a location of the cell surface. Gray-shaded maps that locate unbinding forces of surfaces can range from 50 pN to 400 pN, depending on the interaction probed, the number of interacting molecules and the loading rate[70]. In one of the first applications to cells, the AFM stylus was functionalized with *Helix pomatia* lectin (HPL) to map adhesive forces to a mixed population of group A and group C red blood cells[71]. The high specificity of HPL to *N*-acetylgalactosamine–terminated glycolipids of group A red blood cells allowed investigators to distinguish them from others.

Determining how cells guide the assembly of cell surface receptors into nanoscopic domains to control their functions remains a major task in cell biology[2,6]. Applying MRM to endothelial cells, mycobacteria and lactic bacteria revealed that cell surface receptors such as growth factor receptors[72,73], cell adhesion proteins[74] and drug receptors[75] cluster

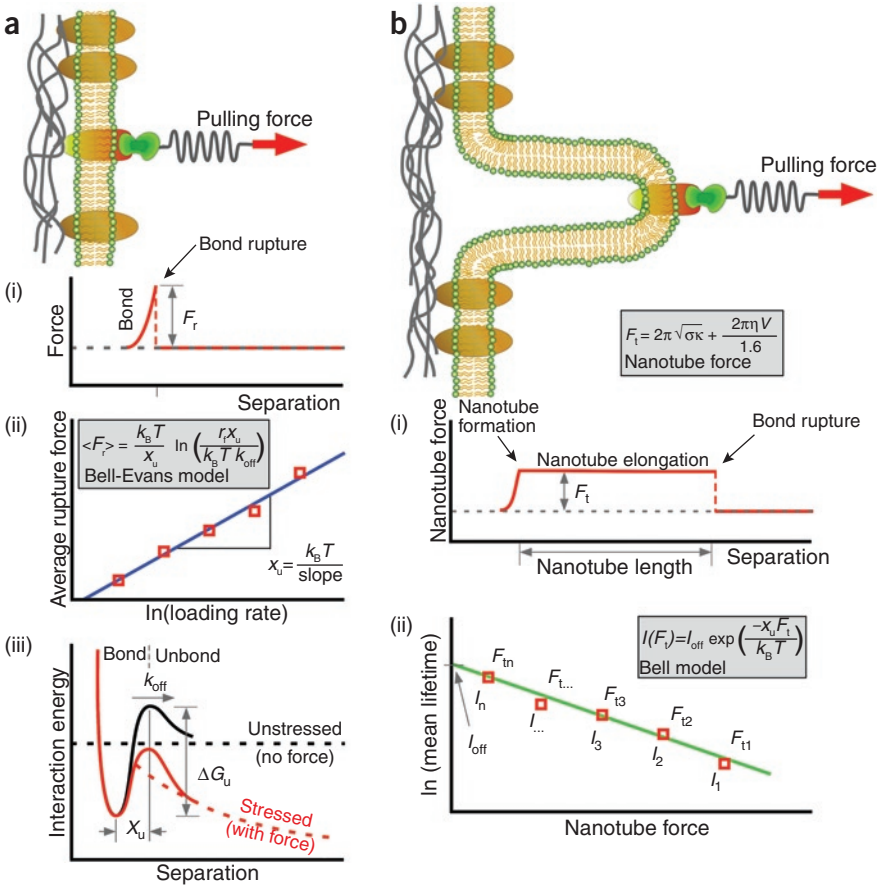

Figure 3 Force probing bonds at cell surfaces reveals their energetic and kinetic properties. (**a**) Cortex-bound adhesion molecule pulled away from the cell surface by the bond ligand. (**a** (i)) The receptor-ligand bond is mechanically stressed until it ruptures at a force F_r. (**a** (ii)) According to the Bell-Evans model[58,60], the average rupture force $\langle F_r \rangle$ of the bond linearly increases with the loading rate r_f (hypothetical data). (**a** (iii)) Interpretation of how an externally applied force lowers the unbinding barrier and increases the unbinding rate of the probed bond. (**b**) Purely membrane bound receptor being pulled from the cell surface at the tip of a membrane nanotube. The force required to extend a nanotube (F_t) depends on the extension velocity (V) and on the isotropic tension (σ), the bending rigidity (κ) and the viscosity (η) of the cell membrane[1]. (**b** (i)) As depicted, the extension force of nanotubes remains constant at constant extension velocity. Extension speed and length of the nanotube can be used to calculate the bond's lifetime. (**b** (ii)) According to the Bell model[64], measuring the bond's mean lifetime at different nanotube forces (extension velocity) can be used to determine properties of the unloaded bond as shown for hypothetical data[57].

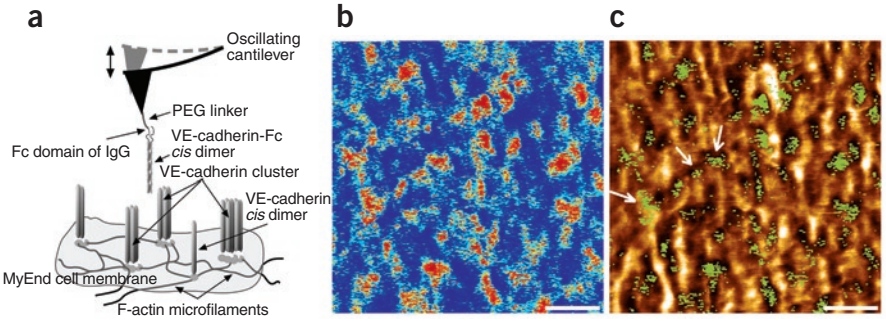

Figure 4 Localizing VE-cadherin domains on vascular endothelial cell surfaces. (**a**) Scheme of molecular recognition imaging to visualize VE-cadherin binding sites on endothelial cell surfaces. (**b**) Recognition image (512 × 512 pixels) of VE-cadherin domains. A pixel was colored red if cadherin binding of the functionalized AFM stylus changed the oscillation cantilever amplitude by ~2 nm. (**c**) Mapping the recognition image (colored green) onto the corresponding cell surface topography (brown). Scale bars, 200 nm. Adapted from ref. 76 with permission from Elsevier.

into domains. For instance, MRM revealed the distribution and binding force of single heparin-binding hemagglutinin adhesins (HBHAs) on the surface of *Mycobacterium bovis* BCG (ref. 74). Adhesion force maps recorded with a heparin-modified AFM stylus showed that single HBHA-heparin bonds rupture at ~50 pN. HBHA was heterogeneously distributed into nanodomains that may promote adhesion to target cells by inducing the recruitment of receptors within membrane rafts. In another study, a variation of MRM named dynamic recognition imaging was used to identify cadherin binding sites on microvascular endothelial cells from mouse myocardium, and to colocalize the receptor positions with topographical features of the cell surface (**Fig. 4**)[76] and the underlying cortical cytoskeleton[73]. As mapping cell surfaces by MRM takes several minutes, cells were fixed with glutaraldehyde to avoid lateral diffusion of cadherin and increase the mechanical stability of the cell membrane. In the future, improved AFM scanning and detection mechanisms may speed up data acquisition and allow more dynamic cellular processes to be studied[70]. The ability of MRM to examine compositional changes of cell surfaces opens a wide field of applications to investigate their structure and function on the nanometer scale.

Mapping chemical groups on cell surfaces. Hydrophobic and electrostatic forces play a role in essential cellular functions such as membrane fusion and cell adhesion, but their quantitative assessment has long been challenging. Chemical force microscopy (CFM)[77] provides exciting possibilities to probe chemical groups and their interactions on the nanoscale. CFM involves modifying the AFM stylus with specific functional groups (for example, R-OH or R-CH_3) and measuring the adhesion force between the modified stylus and cell surfaces (**Fig. 5a**). A common method to functionalize CFM styluses relies on the formation of SAMs of alkanethiols on gold. Recently, CFM revealed that *Aspergillus fumigatus* conidia are strongly hydrophobic[78], a finding that is consistent with the presence of hydrophobic proteins (hydrophobins) in the rodlet layer and that directly indicates that these proteins promote cell dispersion in air and adhesion in water (**Fig. 5b**). In addition, CFM allowed investigators to observe the hydrophobic rodlet layer changing into a layer of hydrophilic polysaccharides a few hours after germination (**Fig. 5c**)[79]. In a pharmacological context, CFM was used to characterize to what extent the strong hydrophobic properties of mycobacteria are altered with antimycobacterial drug treatment[80,81].

Quantifying cell adhesion. SCFS characterizes the overall strength of cell adhesion (**Fig. 2**) that is reached when the most adhesive interactions share the load. This "maximum detachment force" typically ranges from several hundreds of piconewtons to nanonewtons (**Box 1** and **Fig. 2**) and depends on the shape and deformation properties of the cell. One basic question in developmental biology is how embryonic cells sort. The differential adhesion hypothesis (DAH) argues that in mixed populations more adhesive cells distribute centrally while less adhesive cells distribute peripherally. This hypothesis is sharply contrasted by the differential surface contraction (DSC) model, in which cortical tension (the force generated within cells parallel to their surface) drives cell sorting. To test these hypotheses, SCFS was applied to quantify the adhesion of progenitor cells from gastrulating zebrafish embryos[82]. According to the adhesive properties found, the DAH predicts a sorting behavior that is different from that observed *in vitro* and *in vivo* (**Fig. 6**). Furthermore, SCFS measurements showed that differential actomyosin-dependent cell-cortex tension regulated by Nodal/TGF-β signaling constitutes the key factor that regulates cell sorting.

In cancer therapy, one major strategy is to supress cancer cell adhesion, thereby suppressing tumor metastasis[83]. SCFS was applied to characterize the adhesion of leukemic cells to bone marrow stroma cells (BMSCs)[52]. The experiments showed that expression of the BCR/ABL

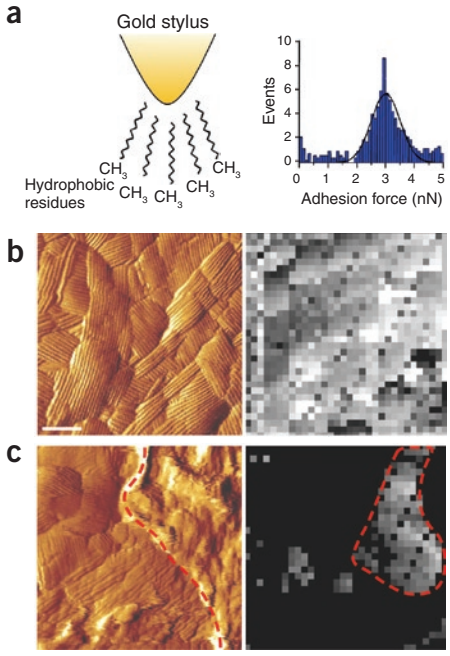

Figure 5 Mapping hydrophobic forces on live cells. (**a**) Using CFM with a hydrophobic AFM stylus, F-D curves were recorded on a single *A. fumigatus* spore to generate an adhesion force histogram. (**b**) AFM deflection image (left) of crystalline rodlet layers of hydrophobins from *A. fumigatus* spores. The right panel shows the adhesion force map (*z* range corresponds to 5 nN) obtained by recording 32 × 32 F-D curves using the hydrophobic stylus. Scale bar, 200 nm. (**c**) After germination the homogeneous chemical contrast of the hydrophobic rodlet layers (**b**) changed into a heterogeneous chemical contrast showing hydrophobic patches of rodlets (**c**, dashed curve) surrounded by hydrophilic polysaccharides. Adapted with permission from ref. 78. Copyright 2007 American Chemical Society.

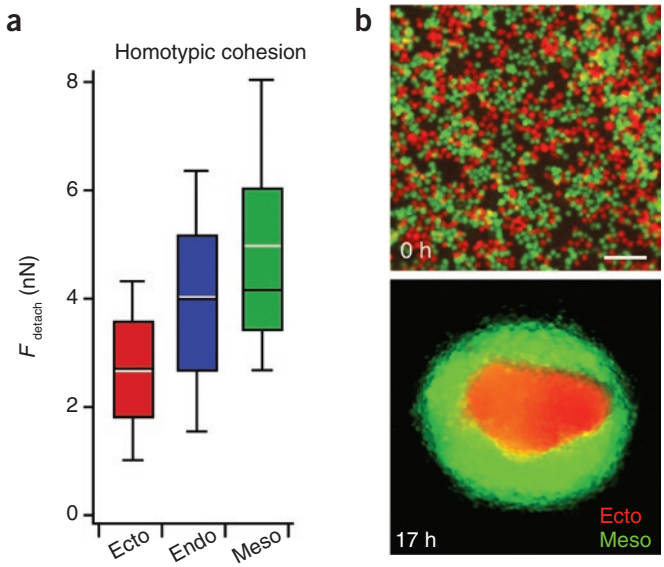

Figure 6 Quantifying the adhesion between embryonic cells by SCFS. (**a**) Detachment forces measured between individual ectoderm, mesoderm and endoderm progenitor cells from gastrulating zebrafish embryos. Ectoderm cells show the lowest cohesion; mesoderm cells show the highest cohesion. In box-whisker plots, half of the data points are within the box and 80% are within the whiskers. Black and white lines mark median and mean, respectively. (**b**) *In vitro* time-lapse experiments show that ectoderm cells (red) exhibiting the weakest adhesion sort centrally and are surrounded by mesoderm cells (green). Scale bar, 300 μm. Adapted by permission from Macmillan Publishers Ltd: *Nature Cell. Biol.* (ref. 82), copyright 2008.

tyrosine kinase, a hallmark of chronic myeloid leukemia, significantly increased the adhesion of leukemic cells to BMSCs and that inhibiting the kinase reduced the adhesion of leukemic cells to control cells. To probe which CAM was activated by BCR/ABL, SCFS was performed in the presence of specific CAM-blocking antibodies. β_1-integrins were thus shown to elevate leukemia adhesion. Unraveling such functional activation of CAMs in cancer may in the future provide a means for optimized pharmaceutical therapies.

Following cell adhesion dynamics. At the receptor level there are several ways to regulate cell adhesion: (i) change the number of binding sites, (ii) modulate the mechanical strength of adhesive interactions[84], and (iii) modulate the load-sharing or cooperativity of adhesive interactions[5,6]. SCFS can distinguish these mechanisms because together with the cell adhesion strength, the method measures the force at which individual receptors and assemblies of such unbind.

Integrin regulation is central to many cell adhesion processes[6]. The interaction of function-associated antigen-1 (LFA-1 or $\alpha_L\beta_2$-integrin) and intracellular adhesion molecule-1 (ICAM-1) plays a crucial role in regulating leukocyte adhesion. SCFS was applied to study the mechanism by which phorbol myristate acetate (PMA), a protein kinase C activator, stimulates this leukocyte adhesion[50]. PMA-stimulated cells increased their maximum detachment force ~tenfold, while the rupture force of single $\alpha_L\beta_2$-integrin–ICAM-1 bonds (~90 pN) remained unchanged. Thus the overall leukocyte adhesion increased by binding more $\alpha_L\beta_2$-integrins and not by strengthening individual binding interactions. It was further shown that activating antibodies and the presence of Mg/EGTA strengthen $\alpha_L\beta_2$-integrin–ICAM-1 bonds[51]. This finding supports current models describing the co-existence of different binding conformations of integrin[84]. An SCFS study of $\alpha_2\beta_1$-integrin–mediated adhesion to collagen I showed that within minutes of cell-substrate contact, cells coordinate their adhesion receptors[54]. This actomyosin-dependent process results in a several-fold increase in the overall cell adhesion. Similarly, MDCK cells adhering to different ECM proteins rapidly increased their initial adhesion to an enhanced adhesion[53].

Outlook

The unique possibility of AFM to qualitatively and quantitatively characterize specific interactions of surfaces from living cells opens an enormous variety of applications. This potential is based on the versatility of AFM force detection and mapping methods and the toolbox that allows functionalizing the AFM stylus to probe given aspects of cell biological and medical applications[14,16]. Currently, the time resolution of AFM applications limits what biological questions can be addressed. Many biological interactions occur faster than the time required by the AFM to probe (~0.001–1 s) and locate (~15 min) them. Hopefully, advances in developing high-speed AFM imaging[85–87] will improve the time resolution of force spectroscopy experiments. Newcomers intending to apply AFM to probe cell surfaces should realize that even though most commercial AFMs are user friendly and well-established methods for conducting force spectroscopy experiments are available, there are no firm standards for how data are acquired and analyzed. In this review, we hope to establish the initial basis for such standards.

SCFS reveals a molecular 'force signature' of cell adhesion with a dynamic force range and sensitivity that is sufficient to characterize cell adhesion to the contribution of single receptors. This has opened the door to studies of how cells establish adhesion ranging from the attachment of single molecules to the formation of firm attachments. A great potential is in combining AFM with advanced light microscopy and spectroscopy techniques. This will allow cell surface structures to be simultaneously identified by light microscopy and probed by AFM. Impressive theoretical and experimental approaches in far field microscopy achieve resolutions of ~10 nm of fluorescently labeled structures[88]. Such multitechnological platforms will allow observation and probing of biological matter from microscopic to nanoscopic resolution (~1 nm) in order to elucidate their sophisticated functions.

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