Review

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Sticky microbes: forces in microbial cell adhesion

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Understanding the fundamental forces involved in the adhesion of microbial cells is important not only in microbiology, to elucidate cellular functions (such as ligand-binding or biofilm formation), but also in medicine (biofilm infections) and biotechnology (cell aggregation). Rapid progress in atomic force microscopy (AFM) techniques has made it possible to measure the forces driving cell–cell and cell–substrate interactions on a single cell basis. A living cell is attached to the AFM probe, thereby enabling researchers to measure the interaction forces between the cell and a target surface. Recent advances in our understanding of the forces driving cell adhesion and biofilm formation are discussed, with a focus on pathogens. These studies provide compelling evidence that, upon contact with a surface, cell adhesion components display a variety of mechanical responses that are important for cell adhesion.

AFM: a new window on single cell adhesion

Microbial cells show remarkable adhesion properties that are of relevance to medicine and industry. The adhesion of pathogens to surfaces is the primary step leading to biofilm formation and associated infections, but cell adhesion and aggregation are also widely exploited in biotechnology for immobilizing or separating microbial cells. Cell adhesion is mediated by a multitude of molecular interactions that are specific (i.e., molecular recognition between receptors and ligands) or non-specific (i.e., hydrogen bonding, hydrophobic, van der Waals, electrostatic, and macromolecular forces) [1]. Today, the precise mechanisms by which these interactions determine cell adhesion processes are not yet fully understood. Various biophysical assays have been developed for measuring biomolecular and cellular forces, including flow-chamber experiments, the surface force apparatus, microneedles, the biomembrane force probe, optical and magnetic tweezers, and AFM [2]. These techniques cover a wide range of force strengths and length scales, ranging from weak intermolecular interactions to strong covalent bonds. While optical and magnetic tweezers are useful to manipulate biomolecules in solution, including within living cells, the biomembrane force probe can measure a wide range of forces at various biological interfaces including on cell surfaces. Currently, AFM is the only technique that is well-suited for probing forces on microbial cells, both at the single cell and single-molecule levels [3,4]. AFM complements microscopic assays traditionally used to explore the mechanisms of microbial adhesion [5,6].

Unlike other microscopy techniques, AFM measures the forces between a sharp probe ('tip') and the sample while scanning over the sample surface [2–4]. The sample is mounted on a piezoelectric scanner which ensures 3D positioning with high accuracy. AFM cantilevers and tips are made of silicon or silicon nitride using microfabrication techniques. The force is measured by the deformation of a soft cantilever; this is detected by a laser beam focused on the free end of the cantilever and reflected into a photodiode. In the early days AFM was mainly used in biology as a microscope, providing structural information on biological structures, including microbial cell walls. However, it has become clear now that AFM is a valuable tool to measure, directly and quantitatively, molecular and cellular interaction forces [2–4]. In this case the force is measured as a function of the distance between tip and sample, and the characteristic adhesion (binding) force between tip and sample during retraction is determined. In single-molecule force spectroscopy (SMFS), the tip is functionalized with bioligands to localize and force-probe specific receptors [7,8]. In single cell force spectroscopy (SCFS), the AFM tip is replaced by a single cell to measure cell–cell and cell–solid interaction forces [9–12] (Figure 1).

Advances in cell probes

Central to SCFS experiments is the preparation of the cellular probes. Over the past 15 years various protocols have been developed to attach cells to AFM cantilevers. Two crucial issues to keep in mind here are (i) to guarantee that the metabolic activity and natural surface architecture of the cells are preserved after cell immobilization, and (ii) to carefully control the number of interacting cells. For animal cells, a simple method to functionalize the cantilever with a single living cell is to use specific receptor–ligand interactions [9–12]. Briefly, a tip-less cantilever is cleaned with detergent or plasma, treated with biotinylated bovine serum albumin (BSA), and then further incubated with streptavidin and biotinylated concanavalin A lectins. Carbohydrates on the cell surface then bind to the lectin-coated cantilevers. In a seminal study, Benoit *et al.* [9] used this approach to measure the adhesion forces between individual cells of

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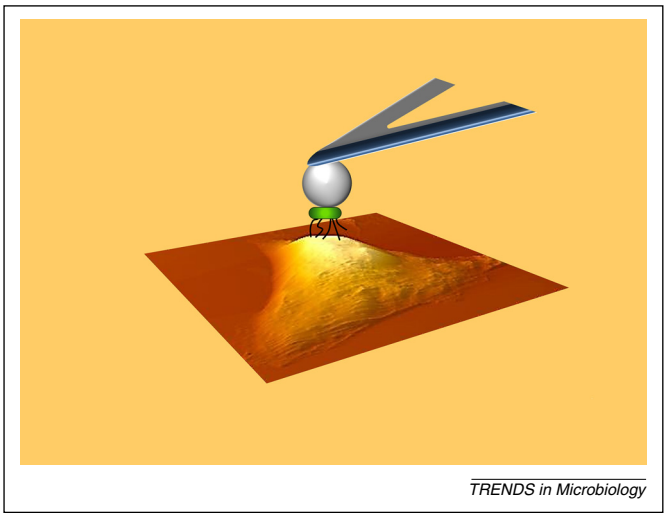

Figure 1. Measuring single cell adhesion forces. The general idea behind single cell force spectroscopy (SCFS) is to attach a single cell onto an atomic force microscopy (AFM) probe and to measure the interaction forces between the cell probe and target surfaces. Shown here is the adhesion between a piliated bacterium and a host cell.

Dictyostelium discoideum. They showed that cell aggregation, a process engaged in the development of multicellular organisms, is mediated by specific glycoproteins.

The lectin methodology is often inappropriate for microbial cells because the cell–cantilever bond is too weak, leading to cell detachment during the measurements. Therefore, several investigators have attached cells to cantilevers using chemical fixation, glue, or drying [13–19]. In the first procedure, a cell pellet is transferred onto the cantilever and the coated probe is then treated with a drop of glutaraldehyde [13,14]. In doing so, Ong and colleagues measured hydrophobic forces between bacteria-coated probes and solid substrates [13]. Yeast cells can also be picked up and attached to tipless cantilevers using glue, thereby allowing the measurement of the interaction forces with solid substrates [15,16]. Alternatively, cantilevers can be immersed in a concentrated cell suspension, removed from the suspension, and allowed to dry [17,18]. These cell probes have been used to study the adhesion forces of pathogens including *Candida parapsilosis*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* [17,18]. Although straightforward, the above methods are invasive because they may lead to cell surface denaturation, or even cell death, raising questions about the biological relevance of the data.

Several methods are based on electrostatic interactions using poly(ethyleneimine) (PEI), poly-L-lysine, or charged silanes [19–21]. In the silane procedure, glass beads are incubated with positively charged aminopropyltriethoxysilane, and the cells are then linked to the amino-functionalized beads by spinning a cell–bead mixture. A bacteria-coated bead is then attached to a cantilever with epoxy. Lower *et al.* used this electrostatic approach to quantify the forces between living *Shewanella oneidensis* bacteria and goethite [21]. The drawback here is that multiple cells are probed together, thus making single cell analysis not possible. Hence, none of the above methods enable reliable single cell force measurements for multiple reasons (the cell–cantilever bond is too weak, cell surface is altered, multiple cells are probed).

Fluidic force microscopy (FluidFM) offers exciting opportunities for non-invasive SCFS [22–25]. This new technology uses microchanneled cantilevers with nano-sized apertures for manipulation of single living cells under physiological conditions. The hollow cantilevers are connected to a pressure controller, enabling their operation in liquid as force-controlled nanopipettes under optical control. The key benefit for SCFS is that many cells are probed in a short time-frame, meaning that statistically-relevant datasets can be obtained within a few hours. In microbiology, FluidFM has allowed researchers to quantify hydrophobic forces on *Candida albicans* cells [25]. Despite its strong potential for SCFS, FluidFM involves specific pieces of equipment that are not readily available on all microscopes.

Recently, a versatile platform was developed for reliable SCFS in microbiology (Figures 1 and 2) [26,27]. A colloidal particle is attached to the end of a cantilever [28] and coated with a bioinspired polydopamine wet adhesive (Figure 2A) [29]. The sticky colloidal probe is used to pick up a single live cell [30]. Fluorescence microscopy may be used to check that the cell is properly positioned and alive (Figure 2A, inset). Force–distance curves are then recorded on different areas of the target surface with the same cell probe, and the experiment is repeated with different surfaces and different probes to permit statistical analysis. This assay is non-destructive, enables single cell manipulation, and offers good control of the contact area, meaning that reproducible single cell analysis is possible. Yeast probes can be prepared in the same way, except that it is no longer necessary to attach a colloid to the cantilever. As a proof of concept, the method was used to analyze the specific and non-specific forces of probiotic bacteria interacting with biotic or abiotic surfaces (Figure 2B,C). While adhesion to lectins was mediated by polysaccharides (Figure 2B), binding to hydrophobic surfaces primarily involved hydrophobic proteins (Figure 2C). In the past few years SCFS has been instrumental in unraveling the forces involved in microbe–microbe, microbe–host, and microbe–substrate interactions. In the following the most recent breakthroughs in microbiological SCFS are surveyed, with an emphasis on microbial adhesins, bacterial pili, and cell–cell associations. These experiments have led to the discovery that, upon mechanical contact with a surface, many cell adhesion components display unanticipated mechanical responses which play important roles in promoting cellular interactions.

Forces driving microbial cell adhesion

Microbial adhesins: specific versus non-specific forces

Studying the binding mechanisms (e.g., specificity, binding strength, and mechanics) of adhesins is important to understanding their functional roles and offers a means to identify potential drug targets. SCFS has provided direct, quantitative information on the specific and non-specific forces of microbial adhesins. In *S. epidermidis*, several adhesins attach to biomaterial surfaces by targeting host extracellular proteins [31]. A prototype of such adhesins is the widely investigated SdrG protein which binds to the