Interplay of Physico-Chemical and Mechanical Bacteria-Surface Interactions with Transport Processes Control Early Biofilm Growth: A Review

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Abstract

Biofilms initiate when bacteria encounter and are retained on surfaces. The surface orchestrates biofilm growth through direct physico-chemical and mechanical interactions with different structures on bacterial cells and, in turn, through its influence on cell-cell interactions. Individual cells respond directly to a surface through mechanical or chemical means, initiating "surface sensing" pathways that regulate gene expression, for instance producing extra cellular matrix or altering phenotypes. The surface can also physically direct the evolving colony morphology as cells divide and grow. In either case, the physico-chemistry of the surface influences cells and cell communities through mechanisms that involve additional factors. For instance the numbers of cells arriving on a surface from solution relative to the generation of new cells by division depends on adhesion and transport kinetics, affecting early colony density and composition. Separately, the forces experienced by adhering cells depend on hydrodynamics, gravity, and the relative stiffnesses and viscoelasticity of the cells and substrate materials, affecting mechanosensing pathways. Physical chemistry and surface functionality, along with interfacial mechanics also influence cell-surface friction and control colony morphology, in particular 2D and 3D shape. This review focuses on the current understanding of the mechanisms in which physico-chemical interactions, deriving from surface functionality, impact individual cells and cell community behavior through their coupling with other interfacial processes.

Keywords: Bacteria interactions, biofilms, cell capture, electrostatics, depletion, colony morphology, mechanosensing

1.0 Introduction

A surface is the key ingredient that enables bacteria to grow into biofilms, playing important roles, some yet to be discovered, in all steps of biofilm formation. The surface can be on a man made or natural material or on living tissue. Biofilms are commonly described as initiating with bacterial cell attachment through receptor binding or physico-chemical means and then, as illustrated in Figure 1, in time reversible interactions become irreversible, trapping cells on the surface.[1-3] In a third step, adhered cells multiply and produce extra cellular matrix, usually forming a monolayer on some region of the surface. Subsequently in a fourth step, growing cells propagate into the region above the monolayer, developing a 3-dimensional community where cells in different regions of the biofilm exhibit different functions and metabolism. Infection is spread when pieces of the biofilm break off and relocate to different surface regions. In the process of biofilm development, bacterial cells transition from their free solution or "planktonic" state to surface-bound or "sessile" phenotypes, undergoing further changes. This progression occurs as cells sense and respond to cues from each other and, of great interest to scientists, from the surface environment. Indeed, physico-chemical, mechanical, and other factors, typically in combination, produce chemical and mechanical stimuli that generate phenotypic changes in both progenitor (initially adhered) and daughter cells.

One complicating feature of real biofilm growth relative to the simplified representation in Figure 1 is the different times at which different cells arrive or originate via cell division on a surface. A second complication is the variety of ways cell can contact a surface, through their appendages, envelope, and orientations, all depending on surface functionality and physical interactions. Recent advances in understanding the ability of bacteria to sense and respond to the

mechanical force has been summarized in several recent reviews.[4-8] The character of cell-surface interactions can evolve in time, further influencing chemical and mechanical interactions of individual cells and predisposing growth patterns that determine colony morphology. Daughter cells and progenitor cells with long residence times, for instance, may be more intimately adhered, of a different phenotype, or express genes differently than cells newly arriving from solution. Likewise, some progeny from end-adsorbed cells may escape into solution or adhere to a different surface region, producing a different growth pattern of the remaining cells. Therefore, even early within a developing biofilm, some cells may be experiencing changes in their adhesion at the same time they are dividing, sensing the surface, and signaling other cells to evolve the biofilm community. The transition from a two-dimensional overlayer to a three-dimensional biofilm community depends critically on these factors, for instance facilitating cell rearrangement and reorganization.[9-13] Figure 1 includes current thinking on the transition to a three dimensional community, but also shows how initial cell orientation can influence three dimensional biofilm development from the start. It is critical to understand the link between distributions of features of individual cells and population-level behavior. The nature of the populations in Step 3 and the way individual cells interact depends on the coupling of earlier physico-chemical timescales with those governing cell transport and physico-chemical cell-surface interactions with other forces.

This review will address what is currently known about how physico-chemical processes combine with those of transport (diffusion, gravity, flow, motility), and how physico-chemical interactions (van der Waals forces, electrostatic interactions, hydrogen bonding, and others) from different surface functionalities combine with other forces (from flow, gravity, cell and

biomaterial mechanics) in determining how biofilms develop. The review addresses behaviors important to Steps 1 through the start of Step 4 with some examples of coupled physico-chemical factors listed in Table I. The review includes interactions of initiating with particular bacterial structures that influence individual cell behavior, and the review also addresses how behaviors initiating with surface physical chemistry and single cell interactions further influence collective cell behavior and colony morphology.

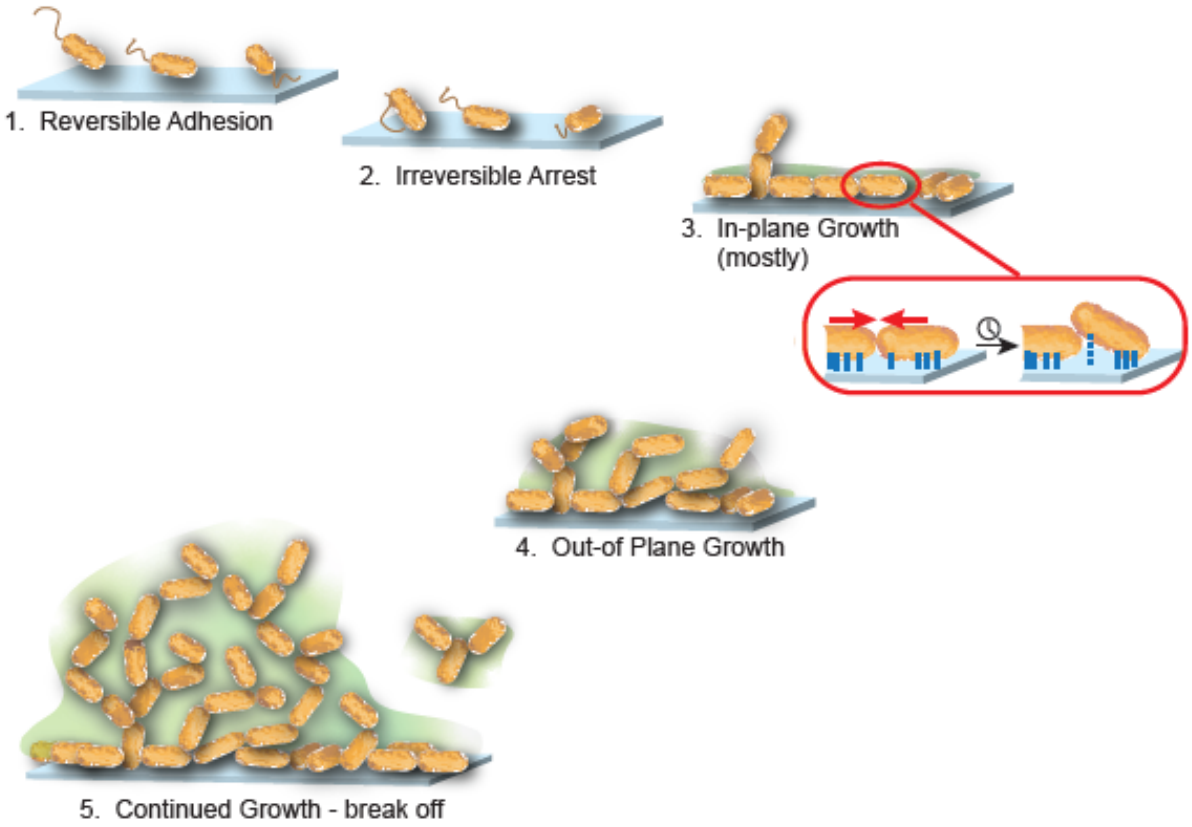

Figure 1. Steps of biofilm formation including 1) reversible adhesion of cells, which permits motion of swimmers 2) firm arrest 3) in plane growth which includes production of extracellular polymeric substances (EPS) 4) transition to a three dimensional community following build-up of forces between growing cells and partial displacement and orientation, and 5) further growth and break off of sections of the original biofilm. The schematic includes the possibility that end adsorbed capsular cells can grow off the surface from the start.

Table I. Examples of Behaviors Resulting from Coupling Physico-Chemistry Surface-Bacteria Interactions with Additional Factors

Factors in Mechanism	**Behavior**	**System(s)**	**Reference**
Adhesion rate versus convection-diffusion rate	accumulation goes through maximum with increased shear over sticky surface	*S. aureus* on collagen (ligand-receptor) -- silica microspheres heterogeneous electrostatic	Mohamed *et al*. 2000 (208) -- Kalasin et al. 2008 (60)
Adhesion rate plus gravitational settling	Ten times the numbers of captured cells on chamber floor compared with "ceiling"	*S. aureus* on glass in flow chamber	Lee *et al*. 2011 (35)
Swimming Mobility Plus Potential Adhesion	elevated near-surface cell concentration; potential for increased capture	*E. coli*	Berke *et al*. 2008 (39)
Swimming Mobility Plus Potential Adhesion	cell-surface collisions increase potential for capture	*C. crescentus*	Li and Tang 2009 (40)
Adhesion force on pili plus shear force	Cell rolling	*E. coli* on mannose-presenting surfaces via type I fimbriae	Thomas *et al.* 2002 (141); Tchesnokova *et al*. 2010 (142)
Adhesion force on envelope plus shear force	Adhered cells trapped by their ends	*E. coli* on cationic polymer	Xu et al. 2021 (30)
Adhesion force on envelope plus shear force	End adhered cells reconfigure in high shear (observed)	*E. coli* on glass, C16 monolayers	Xu et al. 2021 (30)
Adhesion force on envelope plus shear force	End adhered cells reconfigure in with time (interpretation)	*P. aerugenosa* on polyvinylidine fluoride	Marcus *et al*. 2012 (240)
Adhesion force on pili plus gravitational field	Surface orientation produces different types of twitching crawling	*P. aerugenosa* on glass	Conrad *et al*. 2011 (111); Gibiansky *et al*. 2010 (112)
Adhesion force plus cell envelope mechanics	Cells deform at varied times depending on stiffness and adhesive forces	*S. aureus* on tipless AFM cantilever against glass -- *S. aureus* on gold	Chen *et al*. 2014 (107) -- Li *et al.* 2012 (252)
Adhesion force plus interfacial remodeling	Interfacial relaxations tightly bind cells in minutes or less	*S. aureus* on cationic polymer	Fang *et al*. 2014 (241)

Adhesion force plus cell envelop mechanics	Cells more deformed on more hydrophobic surfaces with forces ~nN	*S. aureus* on thiol layers of increasing hydrophobicity	Gu *et al.* 2017 (249)
Adhesion force plus interfacial remodeling	Nanoscale roughness arrests remodeling and prevents adhesive development	*S. aureus* on sparse layer of cationic nanoparticles	Fang *et al*. 2014 (241)
Adhesion force plus flagellar forces	Tight cell circling about tethered flaggellum possible gene regulation	*P. aerugenosa* on glass adhered by flagella	Conrad *et al*. 2011 (111); Schniederberend 2019 (165)
Mechanical adhesion force without physicochemical interactions	Cells bind reversibly with low friction	*E. coli* adsorbed only via polyethylene glycol depletion	Niu *et al*. 2021 (237)
mechanical cell-cell interactions plus physico-chemical substrate interactions	Cells grow in flat ordered “crystal”	*E. coli* between agarose and glass	Dell'Arciprete *et al*. 2018 (256)
Adhesion force plus cell mechanics and repulsions	Colony transition to 3D structure	*P. aerugenosa* on glass -- *V. cholera* on polysytrene	Duvernoy *et al*. 2018 (9) -- Yan *et al.* 2016 (257)
Mechanical adhesion force plus cell interactions	Depletion aggregation enhances antibiotic tolerance via SOS stress response	*P. aerugenosa* depletion aggregated with mucin and DNA, or polyethylene glycol	Secor *et al.* 2018 (236)

2.0 Transport Processes

The rate at which cells reach a surface from the more distant solution, termed transport, is an important determinant of how rapidly cells accumulate on a surface. The accumulation rate, dependent on the combination of transport and binding kinetics, determines the residence time distribution of the adhered cells. Residence time is typically defined as the time an individual cell is adhered to a surface. With cells reaching the surface at different times after exposure of a surface to a bacteria-containing fluid, one typically thinks of distributions of cell residence times and therefore distributions of cells having different adhesion strengths and different extents of surface-sensing pathways initiated. With cell division times on the order of 20 minutes for *E. coli* in some conditions, and gene expression on this or shorter timescales, variations in surface residence times on the order of minutes or tens of minutes have the potential to influence cell- and community level bacterial behavior and potentially introduce heterogeneity. Here we briefly address mechanisms for transport of bacteria to surfaces and highlight general consequences resulting from the coupling of transport with physico-chemical interactions. Also highlighted is literature addressing the coupling of transport considerations with motility and with cell shape for capsular bacteria. On surfaces having rapid intrinsic adhesion kinetics, such as those containing cationic functionality, the influence of transport processes typically are pronounced.[14-16] However with slower intrinsic bacterial capture on anionic, hydrophilic, and some hydrophobic surfaces, the impact of transport varies, with the near-surface concentration of cells approaching that in the bulk solution, and surface processes dominating.

2.1 Diffusion

In quiescent conditions, non-motile cells reach a surface by diffusion, a transient process that becomes slower as near-surface cells are captured and late arriving cells reach the surface from further in solution. With the diffusion coefficient scaling as the inverse of an object's size, diffusion-controlled bacterial capture from a suspension containing different sized cells tends to be slow[17] and will produce surface populations weighted towards smaller cells. This may, for instance favor the capture of stationary phase over log-phase cells, cells that have just divided over those that are about to divide, or cells not expressing pili over those that do. Also, if cell capture was not initially diffusion-limited, in quiescent conditions the surface accumulation rate of cells can become diffusion limited because the flux to the surface slows progressively!

2.2 Diffusion-Convective Transport and Consequences of Cell Motion in Near-Surface Flow

Nearest a surface, a moving fluid can travel only parallel to a planar surface and so creates a shear field in which fluid velocity (parallel to the surface only) increases linearly from zero near the surface. The obvious consequence of this flow is that near-surface cells move over the surface along streamlines. When close to the surface, this motion limits the time at which material points on the cell surface are within reach of material points on the surface. This limited "contact time" can limit cell capture by receptors, such that only those receptors-ligand interactions with fast kinetics are able to trap fast moving cells. This is well known for leukocytes,[18, 19] but also important to rolling adhesion of bacteria. The kinetic limitation differs from the force criterion, in which the forces favoring capture must overcome those favoring cell movement along the surface.

While fluid motion parallel to a surface does not directly transport cells towards a surface, it can create concentration gradients of cells perpendicular to the surface, producing a diffusive-convection transport mechanism. The cell gradient from flow gives rise to a net diffusive flux of cells towards the surface.[20-22] Unlike the transient diffusion in quiescent conditions, diffusion in flow past a surface with rapid intrinsic capture kinetics can produce a constant rate of cell arrival to the interface, a "pseudo-steady-state" rate that scales as the bulk solution concentration, and in laminar slit shear chamber, also as the diffusion coefficient to the 2/3 power and the wall shear rate to the 1/3 power.[23] This behavior has been summarized in excellent reviews.[24-26] An important point is that despite the order 1 and slightly larger Peclet numbers for systems containing micron-scale particles and non-motile bacteria, diffusion-convection limitations have been confirmed to control the observed accumulations on substantially adhesive surfaces.[27-31] Several additional points relevant to cell capture are worth mentioning.

Cell capture by a combination of diffusion-convection limitations and intrinsic binding kinetics can enable bacteria to accumulate on surfaces for tens of minutes or longer.[32-35] Thus, there will result a distribution of captured cells with different surface residence times, enabling some cells to divide on the surface and experience surface conditions for several generations as new cells continue to arrive from bulk solution. The impact of this distribution on biofilm properties is unknown.

2.2.1 Transport of Motile Cells. In shear flow, the greater velocity on streamlines far from the surface imparts a rotational character (vorticity) to the flow field that causes near-surface objects to rotate. This rotation is difficult to track for free-moving spherical cells, but has an impact on

loosely adhered cells, causing them to roll.[18, 36, 37] Prior to adhesion, however, in gentle shear the rotational field can orient motile bacteria to swim against gentle shear gradients[38] or orient swimming bacteria to reach a surface more rapidly than non-motile analogs, or motile bacteria in quiescent conditions. [39, 40] This behavior occurs even in the absence of chemotactic gradients, such as a nutrient gradient produced by a dissolving food particle. Likewise, in some shear conditions, relative to bacterial hydrodynamics, swimming bacteria can become trapped in the rotational field just off the surface.[41] These factors may impact the rates at which motile bacteria ultimately reach and accumulate on a surface. While individual cells have been studied in terms of their abilities to swim upstream, the quantitative impact of combined shear and motility on interfacial flux and cell accumulation on surfaces, well characterized for non-motile cells, is an area ripe for future work.

2.2.2 Transport and Adhesion of Capsular Cells. A notable tumbling of non-motile capsular bacteria results from the vorticity of near-surface flow. Tumbling of ellipsoids in shear flow is much studied through modeling, with ellipsoidal particles moving in Jeffrey orbits.[42-45] With the recent ability to synthesize regular ellipsoidal particles, these motions are only now accessible in experiments.[46, 47] Kaya and Koser demonstrated that flagella-free *E. coli* cells flowing near surfaces tumble at the expected near-surface frequencies described by theory.[48] Capsular cells undergoing these rotations as they diffuse in flow towards a surface are far more likely to adhere by their ends than the side of the cell body because the ends of the cell encounter the surface when the cell's center is further from the surface. Indeed, Xu demonstrated how *E. coli* cells captured on cationic surfaces adsorb predominantly by their ends and are trapped at varied angles relative to the surface and flow directions.[30] The impact of flow and transport on the trapped

configurations of capsular bacteria will likely have further impact on colony morphology, especially the translation from in-plane to 3-dimensional growth in Steps 3 and 4 of Figure 1.

2.3 Gravitational Settling

The influence of gravity to transport bacteria to surfaces is important in nature,[49, 50] and in assays for bacterial adhesion and biofilm formation.[51, 52] Bacteria are usually sufficiently dense that they settle under gravity. The settling flux of cells or particles down onto a horizontal surface can substantially exceed the diffusional and convection-diffusion fluxes (for the same solution concentration),[53] such that biofilms initiate with greater cell concentrations on a horizontal surface. Indeed *P. aerugenosa* biofilms found[54] and then grown[55] in the microgravity environments of the space shuttles and space station exhibited different structures than those found on Earth. Additional Earth-based microgravity studies revealed further differences in *S. aureus* biofilms relative to those grown in gravity.[56] Studies in parallel plate flow chambers, first with leukocytes,[21] and later with *S. aureus* bacteria[35, 57] confirmed the dominant effects of gravity to produce preferential cell accumulation on the floor of a parallel flow chamber rather than the ceiling or side walls. Indeed capture rates of *Streptococcus cricetus* on poly(methylmethacrylate)[58] and several coagulase-negative staphylococcal strains on a negatively charged acrylic flats[59] all substantially exceeded the mass diffusion-convection-limited rate onto the floor of the test chamber, with gravity the best explanation. In later work in a parallel flow chamber the accumulation of *S. aureus* on the chamber floor was found to exceed that on the chamber ceiling by a factor of 10, somewhat dependent on the flow rate.[35] In flow, sedimentation also dominates the cell distribution along a lower surface, producing a different longitudinal gradient than would form, for instance, from transport-limited deposition.[21, 57] By

contrast, *S. aureus* accumulation on vertically-oriented test surfaces in a parallel flow chamber is well-described by calculated diffusion-convection transport rates when adhesion is strong and the underlying capture kinetics are fundamentally rapid:[31] No cases were found to exceed the diffusion-convection-limited rate. Since imaging a vertical test surface is not suited to a conventional microscope, lateral microscopes on optical benches and other vertical specimen configurations have been built to eliminate gravitational settling.[60, 61] On lateral microscopes, even for heavy silica particles, up to 2 μm in diameter, on the most adhesive surfaces capture rates do not exceed their diffusion-convection-limited values, demonstrating the absence of gravitational settling on a vertical test surfaces of the lateral microscope configuration.[62] Thus studies aiming to quantify or minimize sedimentation effects could utilize appropriately-oriented surfaces.

3.0 Forces that Can Couple with Physico-Chemical Interactions

Separate from the issue of the numbers of cells reaching and binding to a surface over time, the forces experienced by adhering cells can result from the combination of physico-chemical interactions with forces that are hydrodynamically or mechanically imposed. The latter can further depend on the stiffness or viscoelasticity of the cell or biomaterial. Evans has laid out the mechanisms by which applied force, specifically the pulling rate, affects the time to disengage individual ligand-receptor bonds.[63, 64] The situation is far more complex in the case of multiple bonds across deformable interface. Here we lay out key features of forces that often combine with physico-chemical interactions of adhering bacteria.

3.1 Hydrodynamics.

Beyond its effects on transport of cells to a surface, shear flow past a surface containing adhered cells can push cells along a surface and contribute to the forces experienced by adhering cells. Just prior to cell capture, adhesive forces must overcome hydrodynamic forces on flowing bacteria. Conversely, a rinsing step, necessarily imposing shear flow to overcome adhesion, is critical screening assays for bacterial adhesion.[65, 66] Squires emphasizes that hydrodynamic forces are equivalent to forces of physical-chemical original and the two can be added.[67] Duffadar and Davis, for instance, add hydrodynamic forces to those from DLVO theory to predict particle rolling on heterogeneous surfaces,[68, 69] analogous to *E. coli* rolling.[36, 37]

The analytical treatment of Goldman *et al* describes the translational and rotational motion, torque, and shear force on a near-surface sphere in shearing flow.[70] Their analytical forms are widely employed in the analysis of cells that are approximated as spheres,[71-76] including estimates for the forces and torques they experience when adhered to a surface. The approximation has also been successfully applied to sphero-cylindrical or capsular bacteria, such as *E. coli*.[77] Important to note is the scaling of the shear force with the square of the sphere radius: large objects experience substantially greater hydrodynamic forces than small ones.

Xu *et al*[30] recently put forth a treatment for the force and torque on an end-adhered cylinder attached to a surface (relevant to by capsular bacteria): Forces and torque from shearing flow tend to push end-adsorbed cylinders over and down onto a surface, but the force decreases as the cylinder rotates down towards a more flat configuration. As a result, adhered cells may not tip all the way flat to the surface, depending on the adhesion strength of the cell pole. However, the

contact area, and therefore the adhesion strength, of weakly binding cells could be increased by exposure to increased shear. This fact opposes the intended effect to rinse cells from a surface as part of cell adhesion assays. It is interesting to note that especially in the case of physico-chemical interactions (van der Waals and electrostatic interactions) that can be nearly uniform relative to length scales of cell contact parallel to a surface, shearing forces may not effectively dislodge cells.[78] Normal forces pulling cells up from a surface, such as those resulting from bubble impingement can be more useful to remove adhered cells.[79-81]

3.2 Forces Associated with Adhesion and Deformability

The forces holding cells to a surface are important to biofilm formation because, in order for cells to be captured, adhesive forces must overcome those from other sources. Also, cell adhesion itself must be overcome in processes of surface cleaning and in the transition from a two dimensional cell layer to a three dimensional biofilm, in Step 4 of Figure 1.[9-13] Forces resulting from the combination of physico-chemical (or ligand-receptor) interactions and material / cell mechanics comprise potential interfacial cues that initiate mechanosensing pathways. Most studies on abiotic surfaces employ stiff surfaces or have neglected substrate deformation.

It is now recognized that substrate and cell deformation[82-84] and viscoelasticity [85] are key to the development of forces between cell and materials, for instance contributing to motility and surface transport mechanisms[29, 86-88] in Step 1 and irreversible bacteria adhesion in Step 2 of Figure 1. It may seem counterintuitive that substrate mechanics and deformation contribute to Step 1 given the weakness and reversibility of the interactions but, for example, the work of

substrate deformation clearly influences processes such as cell rolling.[87, 89, 90] Regarding accounts of the influence of substrate rigidity on irreversible cell capture, results from screening assays for bacterial adhesion, appear to be conflicting. Some report greater bacterial capture or retention on stiff surfaces[91-95] while others report greater adhesion or cell accumulation on soft[96, 97] or more viscous[85] surfaces. Some argue entirely against an effect of material stiffness.[96, 98, 99] Aside from abiotic surfaces, substrate stiffness has been implicated in bacterial adhesion to and infection of mammalian cells where mammalian cells themselves act as a soft material surface onto which bacteria adhere.[100] Sometimes language is used to suggest that cells retained differently on surfaces of different stiffnesses are "sensing" the surface properties.[92] Important to note, in many of these studies, "adhesion" is taken to mean retention of cells following a challenge such as rinsing, and cell counts are reported.

The impact of substrate deformation and mechanics must involve the physical-chemical or ligand-receptor adhesion right at the surface; however, these surface adhesive interactions can be difficult to isolate or characterize, potentially contributing to apparent discrepancies. In studies of the impact of hydrogel mechanics, stiffer hydrogels and agars might have been more adhesive simply because they are more concentrated than soft gels, presenting a greater surface density of adhesive moieties.[87, 91, 92, 94] In other works the greater adhesive character of very soft hydrogels may have resulted from partial adhesion-driven engulfment of bacteria, increasing cell-surface contact area and shielding cells from rinsing forces.[97] Separately, on non-hydrogel polymer substrates such as silicones of different crosslinking densities, the mobility of surface groups may impact the underlying thermodynamic work of adhesion, separate from material stiffness.

More complex still is the possibility that components of growth media enable the crosslinking of some surfaces.[101]

Complicating the interpretation of bacterial retention data is the fact that adhesive interactions are almost never the same as those needed to remove a cell from a surface. The measured force needed to pull two adhering materials apart (for instance the force of rinsing) is sensitive to material mechanics and provides an explanation for the observed impact of material stiffness on apparent cell adhesion or retention. The underlying reversible thermodynamic work of adhesion, related to the physico-chemical interactions across an interface, is not directly quantified in such disbonding studies. Deadhesion by application of force deforms the materials themselves, and the work of material or cell deformation is ultimately dissipated. The measured energy to separate surfaces must, as a result of deformation, exceed the thermodynamic work of adhesion by orders of magnitude, but depends on geometry, on the rate of pulling, and particularly on the stiffnesses of the materials. This behavior has been reviewed extensively in the adhesion literature.[102-106] These principles are upheld at the small length scales and mechanics bacteria themselves,[83] but their application in the quantitative analysis of bacterial adhesion, especially in relating adhesion in screening studies and on biofilm-supporting surface to substrate mechanics is an area for future work. Indeed, studies of cell deformation require sub-micron resolution, for instance relating envelope deformation to van der Waals interactions in an AFM.[107]

While forces contributed by substrate (and cell) mechanics are critical to cell capture, surface cleaning and certain assays, a related question underlies the role of adhesion in biofilm formation: What forces and deformations are experienced by cells as a result of adhesion? The

forces felt by adhering cells, transmitted from the substrate material through adhesive interactions to the cell and which might be sensed through mechanotransduction routes, must depend on physico-chemical or ligand-receptor coupling, depending on substrate mechanics. The forces at the buried contact region and those transmitted from there to other parts of the cell have generally not been quantified (they are smaller than those measured by pulling for the reasons described above.) There does, however, exist evidence for adhesion-driven cell deformation. Inaugural studies of cell deformations resulting from adhesion to rigid surfaces, are included in the physico-chemical interaction section.

Future quantitation of envelope stress and strain will enable a connection to a class of mechanosensing pathways for adhered bacteria. Mechanosensitive mechanisms have been established through studies of bacterial suspensions, for instance involving changes in osmotic pressure. Inaugural studies suggesting that the same pathways could be triggered by adhesion are now appearing, for instance a report by Carniello *et al* that suggest opening of large membrane pores in *S. aureus* when adhesion exceeds 4 nN.[108] However, higher concentrations of cyclic-di-GMP, associated with biofilm formation, were found in *P. aerugenosa* adhered to soft rather than rigid PDMS.[109] New microscopy methods employing fluorescent reporters can distinguish the impact of different PEG hydrogels on cyclic-di-GMP signaling pathways,[110] and future studies will decouple physico-chemical and mechanical effects.

3.3 Gravitational force

Separate from gravity-driven transport of bacteria onto horizontal surfaces, gravitational forces may influence cell-surface contact, adhesion and, ultimately, mechanosensing. Potential

mechanisms include increasing adhesive contact on the floor of a test chamber through material deformation, or helping to overcome electrostatic or sterically repulsive physico-chemical barriers to capture in Steps 1 and 2. Likewise gravitational forces can, through their action on the main cell body, perturb and transmit forces when cells are adhered by pili or flagella. Indeed surface orientation was found to influence bacterial motility, with the Type IV pili of *P. aeruginosa* producing a crawling motion on horizontal surfaces[111] and a walking type motion with escape on vertical surfaces.[111, 112] Gravitational and hydrodynamic forces, through their influence on cell orientation,[30] would be expected to further influence these behaviors. This is an important point since settling assays, in which cells accumulate for hours on collecting surface, are now broadly employed to screen for bacterial adhesion, biofilm formation, or the bacteria-resistance of surface treatments.[52, 113-115] Due to gravity-derived differences in cell flux to horizontal and vertical surfaces and also in the impact of gravity on the underlying interactions, cell adhesion in settlement assays can differ considerably from other measures of cell adhesion and capture. For instance in one case where PEG hydrogels were found mildly adhesive to *S. aureus* and *E. coli* in settling,[91] *S. aureus* and *E. coli* cells were slowed in their near-surface travel in gentle flow but not captured on the same PEG coatings on a vertical wall.[29, 87]

4.0 Routes for the Influence Physico-Chemical Interactions on Adhered Cell Behavior

Physico-chemical interactions, along with ligand-receptor binding, are most often viewed as the driver for the bacterial capture of Steps 1 and 2 of Figure 1. Beyond the numbers of captured cells, the states of the adhered cells are also critically important to biofilms formation. Cell orientations, residence time distributions, and features of adhesion and contact, all direct downstream steps, for instance involving mechanosensing or the development and evolution of

colony morphology. The state of the bound cells is controlled by the coupling of physico-chemically-related factors with those arising from transport and external forces.

Research over the past several decades on the physico-chemical aspects of bacterial cell adhesion has refined our understanding of how different bacterial types are captured retained on surfaces of different functionality, including those of mucosa and living cells. Most experiments address the numbers of cells retained,[17, 21, 58, 116, 117] or adhesion forces, for instance measured by atomic force microscopy.[118-123] Binding rate constants are often masked by transport or other factors. Much of these studies have been summarized in reviews, and an interpretation provided through DLVO (combining van der Waals and electrostatic interactions) and extended DLVO treatments (including specific interactions like hydrogen bonding).[15, 124-131] The comparison between bacterial and colloidal systems has been powerful in advancing an understanding of bacterial behavior, but does not address the deformability of cells, once at a surface. Also less commonly reported are the orientations of captured cells, the cell-surface contact area, and the residence time distributions of cells adhering to a surface. There are also relatively few systematic reports of which parts of gram negative cells (pili, flagella, or the cell body), dominate adhesion, with the exception of highly targeted works delving deep into specific systems. While it is understood that surfaces are often preconditioned by layers of adsorbing macromolecules, a dominant impact of the underlying surface functionality is almost always evident.

This section summarizes how different parts of bacterial cells interact with surfaces through combined physico-chemical and other forces, emphasizing adsorbed features and behaviors (mechanosensing, orientation, adhesion strength) that are critical to subsequent steps of biofilm

formation. Besides those of the cell envelope, interactions of pili and flagella are prioritized over curli because there are fewer reports involving the latter. Included are new findings for depletion-driven bacterial adhesion and results for heterogeneous surfaces that are overlooked in prior reviews.

4.1 Pili

Pili or fimbria, ~7 nm in diameter and extending up to several microns from bacterial cells, serve multiple functions in capture, adhesion, and surface sensing. Type I and type IV pili play key roles in cell adhesion[132] and signaling and can transmit forces, enabling mechanosensing for a variety of Gram negative bacteria. Pili can be the first part of a bacterial cell to encounter a surface, dominating initial capture.[132, 133] Further, the negatively charged bacterial envelopes of many bacteria[134] are repelled from the negative charge on most surfaces, increasing the reliance on pili for bacterial capture and surface sensing. This electrostatic effect favoring the importance of pili is expected to be diminished in environments of increased salt concentration.[135]

Among the best-characterized interactions of pili are those between the type I fimbriae of *E. coli* and mannose groups on mammalian cells. FimH protein on the tips of type I fimbriae binds mannose through a catch-bond, which becomes stronger with applied tension.[136-139] This stabilizes *E. coli* attachment in flow while facilitating cell rolling and the spread of infection.[36, 37, 140, 141] It has been recently found that on surfaces with submicron-structure, type I pili reduce *E. coli* adhesion, perhaps through steric means or loss of the catch-bond benefit in the low-shear environment of microscopic crevaces.[142] Though the FimH-mannose interactions have been implicated in urinary tract infections,[143-145] some uropathogenic *E. coli* strains present FimH

mutants with reduced catch-bond sensitivity, suggesting that the flow environment itself may influence how bacteria express adhesins.[146] Also interesting, type I pili inhibited adhesion of *E. coli* on model hydrophilic surfaces but were associated with slowly developing adhesion on hydrophobic surfaces.[147]

Another important system coupling physico-chemical and mechanical interactions is the type IVa pili found on many bacteria and studied extensively in *P. aeruginosa*. Cycles of contractions and relaxation produce an irregular twitching motion, enabling cells to "crawl" over glass surfaces in a confining 3 μm high chamber[148] or on glass or plastic slides beneath an agar pad.[149, 150] These crawling cells often travel in aligned associated groups.[150] It is now understood that when a pilus makes contact with the surface, adhesion is rapidly established, causing the pilus to retract within seconds, driven by a motor at the base of the pilus where it attaches to the cell.[151] Forces generated by adhered pili can exceed 100 pN,[152, 153] with higher levels of force observed for higher agarose concentrations in the confining nutrient pad, presumably because this increases the friction between the cells and glass.[154] Pili are capable of holding fast in flow situations and can even enable cells to tread upstream.[155, 156] It has been observed that crawling occurs on many surfaces in confinement, including hydrophobic slides[150] On cationic chitin-based surfaces in type IVa pili facilitate surface sensing and biofilm formation of *Vibrio cholera* and *Vibrio parahaemolyticus*.[157, 158] In dynamic force spectroscopy (AFM) studies, strong pili adhesion was found on hydrophobic monolayers, but due to a greater number of contacts and specific interactions, even stronger pili adhesion was found on mucins.[159] These findings suggest a diversity of physico-chemical interactions of type IVa pili and other which are sufficient for adhesion, crawling, surface sensing, and other functions related to biofilm formation.

The use of the confinement or compression via an agar pad is common in studies of pili-driven crawling mobility.[148-150, 154] Normal forces resulting from the concentration of the agar pad have been found to critically influence pili pulling forces on glass, focusing attention on the adhesion of the pili tips. One role of the agar pad is to push capsular cells to a tipped-over configuration. The resulting additional forces involving the cell envelope are associated with signaling that initiates biofilm formation.[160, 161] In the absence of the agar pad, Pseudomonas exhibit standing/walking motility on glass via type IV pili.[112] This finding emphasizes the importance of external forces and cell orientation. Imposed forces such as those from shear in the range of tens of mPa can drive surface motility in ways that are opposed by pili and may couple to non-specific interactions or surface roughness.[162] While studies of pilus adhesion have revealed various motion types, it is evident that bacterial motion depends on other forces and would also be influenced by surface functionality, laying fertile ground for future work.

4.2 Flagella

Flagella are established mechanosensors of a cell's local environment. The torque needed to rotate flagella is increased by increased viscosity,[163] by antibodies that bind flagella,[164] by swimming near a surface,[8] and by adhesion to a surface.[165-167] Resistance to rotation drives secondary signaling processes that are associated with increased secretion of EPS[168] and phenotypic changes such as swarming,[164] and conversion to an immune-evasive filamentous structure.[169] The role of flagella in cell capture and the importance of flagella-triggered phenotype changes are similar across bacterial species.[88, 170] Thus, flagella interactions provide a second general route by which, even before cells are immobilized in Step 2, the coupling of

physico-chemical interactions and other forces in Step 1 of Figure 1 prime cells for biofilm formation.

Direct adhesion of flagellum filaments to abiotic surfaces can enable cell capture[167] and cause the cell body to flip in circles,[165, 166] increasing flagella torque and potentially initiating mechanosensing pathways. Flagella motility is associated with more efficient capture on glass at increased shear.[171] Without cell immobilization, swimming along a surface alters the swimming pattern both in plane and normal to a fixed surface, indicating that the flagellum is experiencing elevated mechanical and hydrodynamic resistance, and may be priming cells for later transition to a surface-bound state. For instance cells swimming in plane rotate clockwise near rigid surfaces[77, 172] and counterclockwise near fluid-air interfaces.[173] Likewise, near surfaces treated to resist bacterial capture, free swimming bacteria swim in, collide, and leave near surface region repeatedly, increasing the dynamic surface residence time substantially.[29] The established sensitivity of this process to the stiffness of a surface is one way swimming bacteria can respond to surface stiffness, potentially selectively up-regulating secondary pathways near surfaces that produce the greater flagellar torque.

Beyond mechanical sensitivity at interfaces, flagella adhere differently on surfaces of different functionalities and textures in ways important to cell immobilization. *E. coli,* exposed to textured surfaces and given several hours to establish contact, ultimately exhibited greater adhesion than controls lacking flagella, due to the ability of moving flagella to reach crevices and regions of the surface not accessible to the cell body.[174] Flagella not attached to bacterial cells adhered more extensively and strongly to model planar surfaces of increasing hydrophobicity,[175] though some

isolated flagella also adhered to immobilized mucins, suggesting possible hydrogen bonding.[176, 177] This trend was sometimes but not always borne out for swimming cells, suggesting flagella-surface adhesion can be dominated by other interactions, for instance involving the cell body or by hydrodynamics.[178, 179] On oil-infused polymer surfaces, which generally resist bacterial capture, studies with mutant strains suggest a key role of flagella in the capture of *E.coli*.[180]

From these studies, it is clear that flagella do much more than enable bacteria to swim. The mechanosensing function for adhered cells is critical to biofilm formation, and these pathways may be initiated on weakly adhesive and nonadhesive surfaces priming cells for adhesion in advance.

4.3 Cell Envelope

Early studies of bacterial adhesion on surfaces of different functionality did not usually distinguish interactions of the cell body from those of appendages, a non-issue for most gram positive bacteria which lack appendages. Modern works targeting the cell envelope also employ gram negative cells engineered without appendages. The membranes of an adhered cell envelope may sense the interfacial pH and experience changes in potential, [181] motivating focus, in this review, on charged surfaces. Bacterial adhesion on hydrophobic surfaces, important and much-studied, has been summarized in excellent reviews.[178, 182-184] Often bacterial adhesion to hydrophobic surfaces is characterized as fast and strong, relative to that on hydrophilic surfaces.[66, 116, 185, 186] However the hydrophilic control surface often employed is that of glass, which carries and can be dominated by its negative charge. Interactions of the cell envelope and

a surface, highly sensitive to biomaterial and bacterial cell mechanics, may trigger mechanosensing pathways, motivating our focus on binding tightness and timescales for its development. Also important to mechanosensing are new reports of surface-dependent cell orientation and depletion aggregation and adhesion detailed below.

4.3.1 Anionic surfaces. Bacterial capture on negative surfaces must overcome the electrostatic repulsion originating with the negative charge of the bacteria surface. Such anionic functionalities include dissociated teichoic acid on gram positive bacteria[187] and carboxyl and phosphate groups in the peptidoglycan and lipopolysaccharide cell walls of *E. coli*.[188] Interactions favoring adhesion by the cell body on negative surfaces can include hydrogen bonding, electrostatic attractions of cationic functionality present in low levels in the heterogeneous cell envelope, hydrophobic interactions with portions of the outer membrane, or specific receptor binding such as that involving fibronectin and fibrinogen binding proteins on *S. aureus*. With adhesive interactions typically shorter range than electrostatic repulsion, cell adhesion on negative surfaces is controlled by a balance of forces and often strengthens with increases in ionic strength.[129, 189, 190] Indeed, smaller cell-surface separations have been reported with increased ionic strength.[135, 191] A kinetic barrier originating from electrostatic repulsions and opposing capture can produce a slow transition from reversible binding to irreversible immobilization[185] that might be misinterpreted as weak adhesion in some assays:[192] Numbers of initially captured cells can be low and accumulation gradual; but once captured, cells can, in time, become as tightly bound as they access a primary minimum in the interaction.[185] On negative surfaces, a relatively slow transition of individual captured cells from a loosely to a

tightly bound state provides an opportunity for other processes requiring cell mobility, including reorienting of cells, discussed below.

Once adhered to a negative surface, bacteria may respond to the local surface environment. Often the routes for surface sensing are general, for instance a sensitivity to osmolarity or pH which occurs with *E. coli* in bulk solution, via a two-component signal transduction pathway involving membrane-bound histidine kinase and a cytoplasmic response regulator.[5] Since the pH and osmolality near a surface differ from those in the free solution, cell adhesion may trigger a pH response. The pH within nanometers of negative surfaces is often several pH points below that of the bulk solution, while counter ion concentrations can be orders of magnitude higher at the surface.[193] Further, the local pH in the gap between adhered *E. coli* cells and silicon oxide surfaces is found to be lower than that further away from the adhered cells but still within nanometers of the surface.[194] Low pH responses to adhesion may alter the metabolic state of the cell and directly impact the proton-motive force at the cell membrane.[195] Additionally, when exposed to abrupt reduction in solution pH, *E. coli* alter their membrane compositions[196] and upregulate damage-preventing acid-shock proteins,[197, 198] changes which may also occur upon adsorption to negative surfaces. Such responses have not, however, been documented for adhered bacteria. Indeed, the extent to which these behaviors are seen may depend on the cell-surface contact area, and, for capsular bacteria, standing versus lying down orientations.

4.3.2 Cationic Surfaces. Cationic surfaces are of increasing interest due to their inhibitory character.[199] Some bacteriocidal surfaces are engineered to mimic solution-phase host-defense peptides by employing polymers to tether cationic functionality forward of a surface, thus

facilitating penetration, poration, and disruption of the membranes of adhering bacteria.[200, 201] Tethering cationic functionality forward of a surface is not, however, necessary for biocidal activity.[202, 203] Many materials attaching cationic functionality directly to the surface, but not extending functionality on tethers, do kill bacteria efficiently, though the cationic moieties cannot reach the bacterial membrane, for instance because the cell's lipopolysaccharide layer (LPS) is sufficiently thick to obstruct membrane-cation contact. When bacteria adhere to surfaces having flat arrangements of cationic functionality, the cell envelope is thought to be deformed,[204] and the electrostatic forces in the contact region thought to be sufficiently altered to disrupt the cell.[205] Such mechanisms require a density of accessible cationic functionality exceeding 10^{12} /cm^2.[203, 206] Bacteriocidal activity at lower overall average cation charge density has, however, been reported for surfaces with nanometric clustering (~10 nm) of cationic charge.[207]

Rapid intrinsic adhesion kinetics and firm irreversible adhesion[33, 187] are the primary signatures of bacterial adhesion on cationic surfaces. Electrostatic attractions drive quantitatively identical transport-limited capture of cocci bacteria and similarly sized negatively charged spherical microparticles, excepting gravitational effects. Quantitative parallels include increased accumulations with increases in flow and a turnover beyond a maximum in the capture rate as a function of shear.[34, 60] This suggests that, in strong flow, cells move too rapidly or that hydrodynamic forces are too large to enable capture via electrostatic attractions. Parallel behavior for capture of *S. aureus* by ligand-receptor bonds[208] demonstrates a similar coupling of hydrodynamic and strong physico-chemical attractions, perhaps due to electrostatic interactions in ligand-receptor pairing.

4.3.3 Surface Heterogeneity. Bacterial surfaces and those of natural and many man made materials are rendered heterogeneous by multiple chemical functionalities,[209-212] including those that introduce competing interactions such as cationic and anionic moieties. When heterogeneity occurs on the micron scale, bacterial flow or swimming enables cells to sample the surface until an adhesive spot is found.[213] With smaller heterogeneity length scales, both attractive and repulsive functionalities may be experienced within a single contact and the cell fate, to adhere or escape, depends on the net interaction.[31, 62] Nanoscopic heterogeneity, depending on the details of the energy landscape, can therefore also produce surface regions that are favorable for cell arrest and others that are not.[210, 212] It is true therefore, that, regardless of the length scale of heterogeneity, bacterial motion and sampling along a surface can lead to cell arrest, after periods of weak reversible adhesion, a transition from Step 1 to Step 2.

A feature common to bacterial adhesion on a variety of heterogeneous surfaces is substantially strong bacterial binding when surface characterization data suggest there should be weak or no capture at all. For instance the net charge or electrophoretic mobility reflects the net average surface charge and can miss "hot spots" that are present at substantial surface concentrations and can capture cell and microparticles in large quantities.[214, 215] Heterogeneity is also an explanation for unanticipated effects of ionic strength.[216] Quantitative studies with colloids and *S. aureus* reveal that even small amounts of heterogeneity, virtually undetectable but engineered into surfaces, can produce substantial capture.[28, 31, 62, 217] Heterogeneity on bacterial cells,[218] or variations from cell to cell (also termed heterogeneity) can also contribute to unanticipated adhesion or lack of it. Here for instance, greater cell surface heterogeneity in stationary phase is

thought to drive greater adhesion of *E. coli* onto quartz, compared with the same bacteria in log phase.[215] Overall then, the adhesive behavior of bacteria and surfaces reflects the more adhesive regions of heterogeneous surfaces, at least at the level of individual cells.

4.3.4 Depletion-Driven Adhesion. Substantial investment has been committed to the development of surfaces that prevent infection and biofilm formation by avoiding bacterial capture all together. These non-fouling layers and coatings are typically hydrophilic and uncharged, with polyethylene glycol and polyzwitterions among the most-studied of a larger group of polymers, summarized in several reviews.[219-223] Even for coatings that perform well in the lab, mostly or entirely preventing bacterial arrest on surfaces, there are reports of coating failure, for instance infections or evidence for immune cell interaction. This has been sometimes attributed to the chemical degradation of polyethylene glycol, while on the other hand it now known that the body can produce antibodies to PEG and therefore recognize it, in time.

Depletion forces present an immediate explanation for cell adhesion on non-fouling surfaces. Depletion attractions are caused by solvated molecules, micelles, polymers, or nanoparticles (including proteins) that do not adsorb to surfaces or to colloidal scale objects and cells.[224-226] Depletion forces can also result from adsorbing molecules present at substantial concentrations, which adsorb to and saturate surfaces, with an excess remaining in solution. In either case, the solvated species (depletants) experience greater entropy in free solution when surfaces or colloidal-scale objects come together. The resulting effective attraction between particles, surfaces, colloids, or cells scales as the osmotic pressure of the depletant with a range on the

order of the depletant size. These forces can produce colloidal aggregation,[227] phase transitions,[224] and deformation of soft materials and membranes.[228-231]

Though depletion-induced colloidal aggregation and deposition has been studied for decades, the behavior is broadly unexplored in the biological community. Only recently has it been demonstrated that bacterial cells aggregate in the presence of solvated synthetic polyelectrolyte, a model for the extracellular polysaccharides produced by the bacteria themselves.[232-235] It has also been demonstrated recently that depletion aggregation of bacteria can enhance antibiotic tolerance.[236] Most recently it was demonstrated by depletion forces can drive bacteria to adhere to otherwise non-adhesive engineered surfaces.[237] The appearance of aggregated and captured bacteria suggests depletion can deform the envelopes of adhering/ aggregating bacteria and may also alter the contact area for bacteria captured on soft materials. The captured bacteria are viable and multiply, but at short times can also be released from the surface by reducing the depletant concentration. Thus, while it is demonstrated that depletion interactions can influence Steps 1 and 2 of biofilm formation, the observed impact of depletion aggregation on antibiotic resistance suggests the further influence of depletion interactions in surface sensing and the pathways associated with biofilm formation.

4.3.5 Evolving binding tightness and potential cell deformation. After their capture and retention at a first set of conditions, bacterial cells undergo processes that further increase the overall adhesion strength, making it increasingly difficult to remove adhered cells with elevated levels of pulling or shear force. The increase in binding strength can exceed that needed for retention in Step 2 of Figure 1.[238, 239] Progressive increases in adhesion, without molecular binding, are

expected on negatively charged surfaces where an electrostatic barrier to adhesion is gradually overcome.[185, 240] Molecular changes in the contact region and changes in cell shape and orientation can further contribute to this evolution. Reports of time dependent bond strengthening[30] or lack of it[185] on hydrophobic surfaces depend on bacterial type and experimental details. *S. aureus* cells adhered to uniformly cationic surfaces, was partially removed when challenged with elevated shear within minutes of initial capture (at lower shear), but cells became entirely resistant to removal at high shear (1000 s^{-1}) within 5 minutes after initial surface contact.[241] On surfaces capable of developing specific interactions, bond strengthening proceeded for tens of minutes.[242] On flat surfaces with reduced net cationic charge, the relaxation time to irreversible retention increased with ionic strength, demonstrating the electrostatic driving force for the adhesive strengthening. When cationic functionality was arranged on raised nanoscopic asperities, the force needed for removal, and presumably the processes of interfacial remodeling, were arrested.[241]

In these studies the sensitivity of the binding constant to the arrangement of surface charge suggests cell deformations that may increase nanoscopic contact. In AFM studies,[243] the binding strength between a silicon-nitride tip and *S. thermophyilus* cells increased due to increasing bond numbers within 2 minutes contact, in parallel with behavior of similar bond aging in flow studies.[190] Similar bond strengthening has been found in AFM studies of bacteria on engineered surfaces[244-246] and target cells.[247] These findings suggest that beyond changes that are evident in the transition to irreversible cell capture, additional evolution of the contact area size and shape, along with further binding in that region depend on the coupling of physico-chemical functionality and interfacial mechanics, further influencing processes of biofilm formation.

Adhesion-driven deformation of bacterial cells may produce envelope stresses sufficient to open mechanosenstive channels or trigger other stress response. Measurements of cell deformations resulting from adhesion are, however, extremely difficult. Nonetheless, nanoscopic height reduction of *S. aureus* on glass has been reported in mutants lacking crosslinking of their peptidoglyan envlopes.[107, 248] Cell wall deformation has also been visualized for *S. aureus* on hydrophilic and hydrophobic surfaces.[249] Surface-enhanced fluorescence imaging has enabled visualization of the contact area of labeled *E. coli* on gold.[250] Enhanced fluorescence from a large area, has related a calculated increase of adhesive contact of *S. aureus* on gold in time, enabling timescales for contact area changes without quantifying contact itself.[251, 252] Decoupling changes in envelop and shape and reorientation of capsule-shaped cells, however, requires imaging capabilities and indeed, the area of contact between bacterial cells and a surface is a quantity not yet reported.

4.3.6 Orientation of Adsorbed Rod-Shaped Bacteria. Over the last decade there has been a growing understanding of the importance of factors influencing the transition from a two-dimensional surface-bound bacterial sheet to a three dimensional bacterial community. The transition depends on the tightness and reversibility of cell binding and the extent to which progeny remain on or extend from a surface. Development of colony morphology also depends critically on the orientations of the adhered cells, and the ability of these orientations to respond to interfacial conditions. However, reports of cell orientation and its sensitivity to underlying surface chemistry are few. Marcus *et al*., for instance, provide an account, based on quartz crystal microbalance frequency data, that capsular cells on hydrophilic surfaces grew more

tightly bound in time compared with static adhesion on a hydrophobic surface.[240] This was interpreted as a reorienting of cells on the hydrophilic surface though no observations of cell orientation were made.

Jones *et al.* report binding of negative polystyrene microspheres selectively to the ends of flagella-free *E. coli* in solution, at only one end per cell, in fact.[253] They argue against an impact of surface charge heterogeneity in favor of possible cell polarity effects. The same *E. coli* were found to be captured by their ends on negatively charged glass flats. Behkam and Sitti[254] also found a preference end-adhered configurations of *E. coli* on glass, for cells arriving late from a swarm, suggesting a possible role of hydrodynamics. Indeed, given the tendency for gentle flow in most situations (often unintended) and the fact that macroscopic surfaces produce shear when exposed to moving fluid, most studies likely include some influence of cell rotation, per the Jeffrey orbits established for *E. coli* by Kaya and Koser.[48] Consistent with this, in a careful population study of flagella-free *E. coli* adhering on glass from gentle shearing flow, Xu report a distribution of cell orientations, most of which were end-adhered to glass but tipped over to different degrees.[30] The orientational distributions were found highly sensitive to the surface itself, with more vertically oriented cells on cationic surfaces and the cell orientations on hydrophobic monolayers similar to those on glass. Most notably the distribution of cell orientations on the cationic surfaces, with angles reported both in the flow direction and normal to the surface, closely matched those reported for negatively charged silica rods of similar size and shape to *E. coli*.[27] This suggests physico-chemical and hydrodynamic factors, and not a cell polarity effect driving orientation on cationic surfaces. Indeed, as a result of cell rotations in

shear flow, there is a substantially greater chance that rod-shaped objects encounter a surface near its ends.

Beyond the initial orientation, the ability of captured rod-shaped bacteria to reconfigure is also critical to bacterial colony morphology. Xu demonstrated that, even though most cells were held by their ends, substantial increases in shear did not reorient of *E. coli* captured on cationic surfaces because adhesion was strong.[30] On hydrophobic and hydrophilic surfaces, increases in flow caused cells to tip over towards the surface in the flow direction, a process that was at least partially reversible. These findings on the reconfigurability of adsorbed *E. coli* cells are consistent with current general understanding of binding strength on hydrophobic, anionic and cationic surfaces.

4.4 Consequences of Coupled Physico-Chemical Interactions on Biofilm Development

The impact of forces from different extents and types of cell-surface contact regulates the behavior of individual cells that ultimately form a biofilm, for instance controlling the production of matrix polymers. While investigations have focused on matrix composition and on the pathways themselves, the connection of this regulation to the timing and nature of cell-surface contact is a substantial endeavor requiring close collaboration between microbiologists and interfacial specialists. The nature of cell adhesion to surfaces has, however, a direct and easily observed impact on colony morphology, as a result of cell orientation, cell interactions, and adhesion strength.[13, 255, 256] Particularly interesting questions involve the nature of cell division on surfaces, for capsular cells where growth is directional and imparts differences between old and new cell poles.[9] Indeed the patterns in colony growth have recently been shown to dependent on

the distribution of adhesins over the surface of micron sized cells.[9] This prompts the study of model systems presenting adhesive functionality in distributions with nanoscale heterogeneity.[28]

It was recently demonstrated that the surface growth of *V. cholera*, producing colonies of order 10,000 from a single adhered precursor, is controlled by the physical character of the environment, producing dense versus branched morphologies for cells proliferating on the bottoms of 96 well plates.[257] Cells grew in flat branched patterns on these surfaces until they became crowded, at which point a competition between cell-cell interactions and cell-surface adhesion controlled the transition to a 3D structure. These observations demonstrate the importance of the cell adhesion strength beyond its role in initial cell capture. Studies of the impact of surface chemistry to control adhesion strength and potentially manipulate biofilm morphology have not been reported. We note, however, the ability of cells to divide in-plane requires the preservation of flat adhered conformations. Findings on the influence of captured cell orientation suggest that cell reconformations may not be necessary to achieve 3D colonies on strongly adhesive surfaces that stabilize end-trapped conformations of adhered cells.[30]

5.0 Conclusions and Opportunities

Despite the continued and growing interest in biofilm formation and its control through surface design, the mechanisms connecting surface interactions to biofilm properties and function remains remain at the scientific forefront. Decades of research has evolved guidelines for cell capture on different classes of surfaces and provided screening assays to evaluate surfaces of innovative design. Here assessments focused on cell numbers and biofilm mass, mostly aimed to avoid rather than understand biofilm formation. Research at the current frontier addresses how bacteria sense their environment mechanically and chemically. Ongoing work has barely begun to address how specific biochemical pathways, established for planktonic cells, can be triggered by surface attachment. The exciting field of bacterial mechanosensing is making great headway in identifying how signals from cell appendages or the cell envelope produce cellular change, yet there are many unanswered questions. Foremost among these is the extent to which different chemical surface functionalities and surface mechanics influence these pathways and whether different pathways are triggered on surfaces relative to analogous conditions in bulk suspension. An understanding of cell deformation and the forces experienced by cells in a surface environment can be calculated or measured in AFM but buried interfaces are not broadly accessible in experiments targeting conditions relevant to various applications.

Also an area of up and coming research is the study of how bacteria-surface interactions lead to the formation of a biofilm community in which cells interact with each other in specific ways. While past work has prioritized factors related to quorum sensing, the potential mechanisms are much broader and must include biological and system timescales for transport and cell growth, and other factors like the extent of cell-surface contact and near-surface cell orientation. Here,

competition between cell-surface and cell-cell interactions can direct the transition from a two dimensional cell layer to various three dimensional structures. With an understanding of the interplay of these different factors, we will be in a better position to defeat the propagation of harmful biofilms while at the same time engineering biofilms as tools, which advance new technologies in energy, health, and sustainability sectors.

Acknowledgement: This work was supported by NSF 1848065.

References

1. Joo, H. S.; Otto, M. Molecular Basis of In Vivo Biofilm Formation by Bacterial Pathogens. *Chemistry & Biology* **2012**, *19*, 1503-1513. DOI: 10.1016/j.chembiol.2012.10.022

2. O'Toole, G. A. To build a biofilm. *Journal of Bacteriology* **2003**, *185*, 2687-2689. DOI: 10.1128/jb.185.9.2687-2689.2003

3. Costerton, J. W.; Stewart, P. S.; Greenberg, E. P. Bacterial biofilms: A common cause of persistent infections. *Science* **1999**, *284*, 1318-1322. DOI: 10.1126/science.284.5418.1318

4. Gordon, V. D.; Wang, L. Y. Bacterial mechanosensing: the force will be with you, always. *Journal of Cell Science* **2019**, *132*. DOI: 10.1242/jcs.227694

5. Kimkes, T. E. P.; Heinemann, M. How bacteria recognise and respond to surface contact. *Fems Microbiology Reviews* **2020**, *44*, 106-122. DOI: 10.1093/femsre/fuz029

6. Harper, C. E.; Hernandez, C. J. Cell biomechanics and mechanobiology in bacteria: Challenges and opportunities. *Apl Bioengineering* **2020**, *4*. DOI: 10.1063/1.5135585

7. Mordue, J.; O'Boyle, N.; Gadegaard, N.; Roe, A. J. The force awakens: The dark side of mechanosensing in bacterial pathogens. *Cellular Signalling* **2021**, *78*. DOI: 10.1016/j.cellsig.2020.109867

8. Persat, A.; Nadell, C. D.; Kim, M. K.; Ingremeau, F.; Siryaporn, A.; Drescher, K.; Wingreen, N. S.; Bassler, B. L.; Gitai, Z.; Stone, H. A. The Mechanical World of Bacteria. *Cell* **2015**, *161*, 988-997. DOI: 10.1016/j.cell.2015.05.005

9. Duvernoy, M. C.; Mora, T.; Ardre, M.; Croquette, V.; Bensimon, D.; Quilliet, C.; Ghigo, J. M.; Balland, M.; Beloin, C.; Lecuyer, S.; Desprat, N. Asymmetric adhesion of rod-shaped bacteria controls microcolony morphogenesis. *Nature Communications* **2018**, *9*. DOI: 10.1038/s41467-018-03446-y

10. Beroz, F.; Yan, J.; Meir, Y.; Sabass, B.; Stone, H. A.; Bassler, B. L.; Wingreen, N. S. Verticalization of bacterial biofilms. *Nat. Phys.* **2018**, *14*, 954-+. DOI: 10.1038/s41567-018-0170-4

11. Liu, Y.; Li, B.; Feng, X. Q. Buckling of growing bacterial chains. *Journal of the Mechanics and Physics of Solids* **2020**, *145*. DOI: 10.1016/j.jmps.2020.104146

12. Maier, B., How Physical Interactions Shape Bacterial Biofilms. In *Annual Review of Biophysics, Vol 50, 2021*, Dill, K. A., Ed. 2021; Vol. 50, pp 401-417.

13. Nijjer, J.; Li, C. H.; Zhang, Q. T.; Lu, H. R.; Zhang, S. L.; Yan, J. Mechanical forces drive a reorientation cascade leading to biofilm self-patterning. *Nature Communications* **2021**, *12*. DOI: 10.1038/s41467-021-26869-6

14. Adamczyk, Z.; Warszynski, P. Role of electrostatic interactions in particle adsorption. *Advances in Colloid and Interface Science* **1996**, *63*, 41-149. DOI: 10.1016/0001-8686(95)00281-2

15. Adamczyk, Z.; Weronski, P. Application of the DLVO theory for particle deposition problems. *Advances in Colloid and Interface Science* **1999**, *83*, 137-226. DOI: 10.1016/s0001-8686(99)00009-3

16. Warszynski, P. Coupling of hydrodynamic and electric interactions in adsorption of colloidal particles. *Advances in Colloid and Interface Science* **2000**, *84*, 47-142. DOI: 10.1016/s0001-8686(99)00015-9

17. Rijnaarts, H. H. M.; Norde, W.; Bouwer, E. J.; Lyklema, J.; Zehnder, A. J. B. Bacteria adhesion under static and dynamic conditions. *Applied and Environmental Microbiology* **1993**, *59*, 3255-3265. DOI: 10.1128/aem.59.10.3255-3265.1993

18. McEver, R. P.; Zhu, C., Rolling Cell Adhesion. In *Annual Review of Cell and Developmental Biology, Vol 26*, Schekman, R.; Goldstein, L.; Lehmann, R., Eds. 2010; Vol. 26, pp 363-396.

19. Alon, R.; Chen, S. Q.; Fuhlbrigge, R.; Puri, K. D.; Springer, T. A. The kinetics and shear threshold of transient and rolling interactions of L-selectin with its ligand on leukocytes. *Proceedings of the National Academy of Sciences of the United States of America* **1998**, *95*, 11631-11636. DOI: 10.1073/pnas.95.20.11631

20. Adamczyk, Z. Particle Deposition from flowing suspensions. *Colloids and Surfaces* **1989**, *39*, 1-37. DOI: 10.1016/0166-6622(89)80176-3

21. Munn, L. L.; Melder, R. J.; Jain, R. K. Analysis of cell flux in the parallel plate flow chamber: Implications for cell capture studies. *Biophysical Journal* **1994**, *67*, 889-895. DOI: 10.1016/s0006-3495(94)80550-8

22. Roosjen, A.; Boks, N. P.; van der Mei, H. C.; Busscher, H. J.; Norde, W. Influence of shear on microbial adhesion to PEO-brushes and glass by convective-diffusion and sedimentation in a parallel plate flow chamber. *Colloids and Surfaces B-Biointerfaces* **2005**, *46*, 1-6. DOI: 10.1016/j.colsurfb.2005.08.009

23. Leveque, M. A. Les Lois de la Transmission de Chaleur par Convection. *Ann. Mines* **1928**, *13*, 201-299.

24. Adamczyk, Z. Particle adsorption and deposition: role of electrostatic interactions. *Advances in Colloid and Interface Science* **2003**, *100*, 267-347. DOI: 10.1016/s0001-8686(02)00062-3

25. Busscher, H. J.; van der Mei, H. C. Microbial adhesion in flow displacement systems. *Clinical Microbiology Reviews* **2006**, *19*, 127-+. DOI: 10.1128/cmr.19.1.127-141.2006

26. Yang, G.; Wang, B.; Vu, K.; Tawfiq, K.; Chen, G. Role of Bacterial Adhesion in Their Subsurface Deposition and Transport: A Critical Review. *Reviews of Adhesion and Adhesives* **2015**, *3*, 216-252. DOI: 10.7569/raa.2015.097305

27. Shave, M. K.; Balciunaite, A.; Xu, Z.; Santore, M. M. Rapid Electrostatic Capture of Rod-Shaped Particles on Planar Surfaces: Standing up to Shear. *Langmuir* **2019**, *35*, 13070-13077. DOI: 10.1021/acs.langmuir.9b01871

28. Shave, M. K.; Kalasin, S.; Ying, E.; Santore, M. M. Nanoscale Functionalized Particles with Rotation-Controlled Capture in Shear Flow. *Acs Applied Materials & Interfaces* **2018**, *10*, 29058-29068. DOI: 10.1021/acsami.8b05328

29. Shave, M. K.; Xu, Z.; Raman, V.; Kalasin, S.; Tuominen, M. T.; Forbes, N. S.; Santore, M. M. Escherichia coli Swimming back Toward Stiffer Polyetheylene Glycol Coatings, Increasing Contact in Flow. *Acs Applied Materials & Interfaces* **2021**, *13*, 17196-17206. DOI: 10.1021/acsami.1c00245

30. Xu, Z.; Niu, W. A.; Rivera, S. L.; Tuominen, M. T.; Siegrist, M. S.; Santore, M. M. Surface Chemistry Guides the Orientations of Adhering E. coli Cells Captured from Flow. *Langmuir* **2021**, *37*, 7720-7729. DOI: 10.1021/acs.langmuir.1c00764

31. Kalasin, S.; Dabkowski, J.; Nusslein, K.; Santore, M. M. The role of nano-scale heterogeneous electrostatic interactions in initial bacterial adhesion from flow: A case study with Staphylococcus aureus. *Colloids and Surfaces B-Biointerfaces* **2010**, *76*, 489-495. DOI: 10.1016/j.colsurfb.2009.12.009

32. Bakker, D. P.; Busscher, H. J.; van der Mei, H. C. Bacterial deposition in a parallel plate and a stagnation point flow chamber: microbial adhesion mechanisms depend on the mass transport conditions. *Microbiology-Sgm* **2002**, *148*, 597-603. DOI: 10.1099/00221287-148-2-597

33. Fang, B.; Gon, S.; Park, M.; Kumar, K. N.; Rotello, V. M.; Nusslein, K.; Santore, M. M. Bacterial adhesion on hybrid cationic nanoparticle-polymer brush surfaces: Ionic strength tunes capture from monovalent to multivalent binding. *Colloids and Surfaces B-Biointerfaces* **2011**, *87*, 109-115. DOI: 10.1016/j.colsurfb.2011.05.010

34. Fang, B.; Gon, S.; Park, M. H.; Kumar, K. N.; Rotello, V. M.; Nusslein, K.; Santore, M. M. Using Flow to Switch the Valency of Bacterial Capture on Engineered Surfaces Containing Immobilized Nanoparticles. *Langmuir* **2012**, *28*, 7803-7810. DOI: 10.1021/la205080y

35. Li, J. Y.; Busscher, H. J.; Norde, W.; Sjollema, J. Analysis of the contribution of sedimentation to bacterial mass transport in a parallel plate flow chamber. *Colloids and Surfaces B-Biointerfaces* **2011**, *84*, 76-81. DOI: 10.1016/j.colsurfb.2010.12.018

36. Anderson, B. N.; Ding, A. M.; Nilsson, L. M.; Kusuma, K.; Tchesnokova, V.; Vogel, V.; Sokurenko, E. V.; Thomas, W. E. Weak rolling adhesion enhances bacterial surface colonization. *Journal of Bacteriology* **2007**, *189*, 1794-1802. DOI: 10.1128/jb.00899-06

37. Thomas, W. E.; Nilsson, L. M.; Forero, M.; Sokurenko, E. V.; Vogel, V. Shear-dependent 'stick-and-roll' adhesion of type 1 fimbriated Escherichia coli. *Molecular Microbiology* **2004**, *53*, 1545-1557. DOI: 10.1111/j.1365-2958.2004.04226.x

38. Kaya, T.; Koser, H. Direct Upstream Motility in Escherichia coli. *Biophysical Journal* **2012**, *102*, 1514-1523. DOI: 10.1016/j.bpj.2012.03.001

39. Berke, A. P.; Turner, L.; Berg, H. C.; Lauga, E. Hydrodynamic attraction of swimming microorganisms by surfaces. *Physical Review Letters* **2008**, *101*. DOI: 10.1103/PhysRevLett.101.038102

40. Li, G. L.; Tang, J. X. Accumulation of Microswimmers near a Surface Mediated by Collision and Rotational Brownian Motion. *Physical Review Letters* **2009**, *103*. DOI: 10.1103/PhysRevLett.103.078101

41. Rusconi, R.; Guasto, J. S.; Stocker, R. Bacterial transport suppressed by fluid shear. *Nat. Phys.* **2014**, *10*, 212-217. DOI: 10.1038/nphys2883

42. Jeffery, G. B. The motion of ellipsoidal particles in a viscous fluid. *Proc. R. soc. Lond. Ser. A-Contain. Pap. Math. Phys. Character* **1922**, *102*, 161-179. DOI: 10.1098/rspa.1922.0078

43. Rosen, T.; Lundell, F.; Aidun, C. K. Effect of fluid inertia on the dynamics and scaling of neutrally buoyant particles in shear flow. *Journal of Fluid Mechanics* **2014**, *738*, 563-590. DOI: 10.1017/jfm.2013.599

44. Kim, D. K.; Hyun, J. Y.; Kim, S. C.; Kim, H. S.; Lee, S. Y. Inertial effects on cylindrical particle migration in linear shear flow near a wall. *Microfluid. Nanofluid.* **2016**, *20*. DOI: 10.1007/s10404-016-1742-1

45. Okagawa, A.; Cox, R. G.; Mason, S. G. Kinetics of Flowing Dispersions 6. Transient Orientation and Rheological Phenomena of Rods and Disks in Shear Flow *Journal of Colloid and Interface Science* **1973**, *45*, 303-329. DOI: 10.1016/0021-9797(73)90271-3

46. Mukhija, D.; Solomon, M. J. Translational and rotational dynamics of colloidal rods by direct visualization with confocal microscopy. *Journal of Colloid and Interface Science* **2007**, *314*, 98-106. DOI: 10.1016/j.jcis.2007.05.055

47. Gunes, D. Z.; Scirocco, R.; Mewis, J.; Vermant, J. Flow-induced orientation of non-spherical particles: Effect of aspect ratio and medium rheology. *J. Non-Newton. Fluid Mech.* **2008**, *155*, 39-50. DOI: 10.1016/j.jnnfm.2008.05.003

48. Kaya, T.; Koser, H. Characterization of Hydrodynamic Surface Interactions of Escherichia coli Cell Bodies in Shear Flow. *Physical Review Letters* **2009**, *103*. DOI: 10.1103/PhysRevLett.103.138103

49. Bellou, N.; Garcia, J. A. L.; Colijn, F.; Herndl, G. J. Seasonality combined with the orientation of surfaces influences the microbial community structure of biofilms in the deep Mediterranean Sea. *Deep-Sea Research Part Ii-Topical Studies in Oceanography* **2020**, *171*. DOI: 10.1016/j.dsr2.2019.104703

50. Azam, F.; Malfatti, F. Microbial structuring of marine ecosystems. *Nature Reviews Microbiology* **2007**, *5*, 782-791. DOI: 10.1038/nrmicro1747

51. Sommer, S.; Ekin, A.; Webster, D. C.; Stafslien, S. J.; Daniels, J.; VanderWal, L. J.; Thompson, S. E. M.; Callow, M. E.; Callow, J. A. A preliminary study on the properties and fouling-release performance of siloxane-polyurethane coatings prepared from poly(dimethylsiloxane) (PDMS) macromers. *Biofouling* **2010**, *26*, 961-972. DOI: 10.1080/08927014.2010.531272

52. Patel, P.; Callow, M. E.; Joint, I.; Callow, J. A. Specificity in the settlement - modifying response of bacterial biofilms towards zoospores of the marine alga Enteromorpha. *Environmental Microbiology* **2003**, *5*, 338-349. DOI: 10.1046/j.1462-2920.2003.00407.x

53. Adamczyk, Z.; Siwek, B.; Zembala, M. Kinetics of colloid particles deposition under the influence of gravity. *Bulletin of the Polish Academy of Sciences-Chemistry* **1996**, *44*, 113-122.

54. Kim, W.; Tengra, F. K.; Young, Z.; Shong, J.; Marchand, N.; Chan, H. K.; Pangule, R. C.; Parra, M.; Dordick, J. S.; Plawsky, J. L.; Collins, C. H. Spaceflight Promotes Biofilm Formation by Pseudomonas aeruginosa. *Plos One* **2013**, *8*. DOI: 10.1371/journal.pone.0062437

55. McLean, R. J. C.; Cassanto, J. M.; Barnes, M. B.; Koo, J. H. Bacterial biofilm formation under microgravity conditions. *Fems Microbiology Letters* **2001**, *195*, 115-119. DOI: 10.1016/s0378-1097(00)00549-8

56. Singh, S.; Vidyasagar, P. B.; Kulkarni, G. R. Investigating alterations in the cellular envelope of Staphylococcus aureus in simulated microgravity using a random positioning machine. *Life Sciences in Space Research* **2021**, *30*, 1-8. DOI: 10.1016/j.lssr.2021.04.001

57. Li, J. Y.; Busscher, H. J.; van der Mei, H. C.; Norde, W.; Krom, B. P.; Sjollema, J. Analysis of the contribution of sedimentation to bacterial mass transport in a parallel plate flow chamber Part II: Use of fluorescence imaging. *Colloids and Surfaces B-Biointerfaces* **2011**, *87*, 427-432. DOI: 10.1016/j.colsurfb.2011.06.002

58. Hogt, A. H.; Dankert, J.; Feijen, J. Adhesion of coagulase-negative Staphylococci to methacrylate polymers and copolymers. *Journal of Biomedical Materials Research* **1986**, *20*, 533-545. DOI: 10.1002/jbm.820200409

59. Vandermei, H. C.; Brokke, P.; Dankert, J.; Feijen, J.; Busscher, H. J. Influence of Electrostatic Interactions on the Deposition Efficiencies of Coagulase-Negative staphylococci to Collector Surfacess in a Parallel Plate Flow Chamber. *Journal of Dispersion Science and Technology* **1992**, *13*, 447-458. DOI: 10.1080/01932699208943327

60. Kalasin, S.; Santore, M. M. Hydrodynamic crossover in dynamic microparticle adhesion on surfaces of controlled nanoscale heterogeneity. *Langmuir* **2008**, *24*, 4435-4438. DOI: 10.1021/la8000202

61. Synetar, E. A.; Brych, O. I. The dynamics of biofilm overgrowth of Enterococcus faecalis. *Visnyk of Dnipropetrovsk University-Biology Medicine* **2015**, *6*, 146-150. DOI: 10.15421/021526

62. Duffadar, R.; Kalasin, S.; Davis, J. M.; Santore, M. M. The impact of nanoscale chemical features on micron-scale adhesion: Crossover from heterogeneity-dominated to mean-field behavior. *Journal of Colloid and Interface Science* **2009**, *337*, 396-407. DOI: 10.1016/j.jcis.2009.05.046

63. Evans, E.; Ritchie, K. Dynamic strength of molecular adhesion bonds. *Biophysical Journal* **1997**, *72*, 1541-1555. DOI: 10.1016/s0006-3495(97)78802-7

64. Merkel, R.; Nassoy, P.; Leung, A.; Ritchie, K.; Evans, E. Energy landscapes of receptor-ligand bonds explored with dynamic force spectroscopy. *Nature* **1999**, *397*, 50-53. DOI: 10.1038/16219

65. Coffey, B. M.; Anderson, G. G., Biofilm Formation in the 96-Well Microtiter Plate. In *Pseudomonas: Methods and Protocols*, Filloux, A.; Ramos, J. L., Eds. 2014; Vol. 1149, pp 631-641.

66. Faille, C.; Jullien, C.; Fontaine, F.; Bellon-Fontaine, M. N.; Slomianny, C.; Benezech, T. Adhesion of Bacillus spores and Escherichia coli cells to inert surfaces: role of surface hydrophobicity. *Canadian Journal of Microbiology* **2002**, *48*, 728-738. DOI: 10.1139/w02-063

67. Squires, T. M. Effective Pseudo-potentials of Hydrodynamic Origin. *Journal of Fluid Mechanics* **2001**, *443*, 403-413. DOI: 10.1017/s0022112001004332

68. Duffadar, R. D.; Davis, J. M. Interaction of micrometer-scale particles with nanotextured surfaces in shear flow. *Journal of Colloid and Interface Science* **2007**, *308*, 20-29. DOI: 10.1016/j.jcis.2006.12.068

69. Duffadar, R. D.; Davis, J. M. Dynamic adhesion behavior of micrometer-scale particles flowing over patchy surfaces with nanoscale electrostatic heterogeneity. *Journal of Colloid and Interface Science* **2008**, *326*, 18-27. DOI: 10.1016/j.jcis.2008.07.004

70. Goldman, A. J.; Cox, R. G.; Brenner, H. Slow Viscous Motion of a Sphere Parallel to a Plane Wall .2. Couette Flow. *Chem. Eng. Sci.* **1967**, *22*, 653-660.

71. Cugno, A.; Marki, A.; Ley, K. Biomechanics of Neutrophil Tethers. *Life-Basel* **2021**, *11*. DOI: 10.3390/life11060515

72. Robert, P.; Limozin, L.; van der Merwe, P. A.; Bongrand, P. CD8 Co-Receptor Enhances T-Cell Activation without Any Effect on Initial Attachment. *Cells* **2021**, *10*. DOI: 10.3390/cells10020429

73. Anderson, N. R.; Lee, D.; Hammer, D. A. Adhesive dynamics simulations quantitatively predict effects of kindlin-3 deficiency on T-cell homing. *Integrative Biology* **2019**, *11*, 293-300. DOI: 10.1093/intbio/zyz024

74. Shan, Y. P.; Harms, H.; Wick, L. Y. Electric Field Effects on Bacterial Deposition and Transport in Porous Media. *Environmental Science & Technology* **2018**, *52*, 14294-14301. DOI: 10.1021/acs.est.8b03648

75. Zalewska-Piatek, B.; Olszewski, M.; Lipniacki, T.; Blonski, S.; Wieczor, M.; Bruzdziak, P.; Skwarska, A.; Nowicki, B.; Nowicki, S.; Piatek, R. A shear stress micromodel of urinary tract infection by the Escherichia coli producing Dr adhesin. *Plos Pathogens* **2020**, *16*. DOI: 10.1371/journal.ppat.1008247

76. Wirtz, D.; Konstantopoulos, K.; Searson, P. C. The physics of cancer: the role of physical interactions and mechanical forces in metastasis. *Nature Reviews Cancer* **2011**, *11*, 512-522. DOI: 10.1038/nrc3080

77. Lauga, E.; DiLuzio, W. R.; Whitesides, G. M.; Stone, H. A. Swimming in circles: Motion of bacteria near solid boundaries. *Biophysical Journal* **2006**, *90*, 400-412. DOI: 10.1529/biophysj.105.069401

78. Wu, Y. T.; Zhu, H.; Willcox, M.; Stapleton, F. The Effectiveness of Various Cleaning Regimens and Current Guidelines in Contact Lens Case Biofilm Removal. *Investigative Ophthalmology & Visual Science* **2011**, *52*, 5287-5292. DOI: 10.1167/iovs.10-6785

79. Gomez-Suarez, C.; Busscher, H. J.; van der Mei, H. C. Analysis of bacterial detachment from substratum surfaces by the passage of air-liquid interfaces. *Applied and Environmental Microbiology* **2001**, *67*, 2531-2537. DOI: 10.1128/aem.67.6.2531-2537.2001

80. Pitt, W. G.; McBride, M. O.; Barton, A. J.; Sagers, R. D. AIR-WATER-INTERFACE DISPLACES ADSORBED BACTERIA. *Biomaterials* **1993**, *14*, 605-608. DOI: 10.1016/0142-9612(93)90179-6

81. Holmes, P. F.; Currie, E. P. K.; Thies, J. C.; van der Mei, H. C.; Busscher, H. J.; Norde, W. Surface-modified nanoparticles as a new, versatile, and mechanically robust nonadhesive coating: Suppression of protein adsorption and bacterial adhesion. *Journal of Biomedical Materials Research Part A* **2009**, *91A*, 824-833. DOI: 10.1002/jbm.a.32285

82. Pierrat, X.; Wong, J. P. H.; Al-Mayyah, Z.; Persat, A. The Mammalian Membrane Microenvironment Regulates the Sequential Attachment of Bacteria to Host Cells. *mBio* **2021**, *12*. DOI: 10.1128/mBio.01392-21

83. Velegol, S. B.; Logan, B. E. Contributions of bacterial surface polymers, electrostatics, and cell elasticity to the shape of AFM force curves. *Langmuir* **2002**, *18*, 5256-5262. DOI: 10.1021/la011818g

84. Touhami, A.; Nysten, B.; Dufrene, Y. F. Nanoscale mapping of the elasticity of microbial cells by atomic force microscopy. *Langmuir* **2003**, *19*, 4539-4543. DOI: 10.1021/la034136x

85. Valentin, J. D. P.; Qin, X. H.; Fessele, C.; Straub, H.; van der Mei, H. C.; Buhmann, M. T.; Maniura-Weber, K.; Ren, Q. Substrate viscosity plays an important role in bacterial adhesion under fluid flow. *Journal of Colloid and Interface Science* **2019**, *552*, 247-257. DOI: 10.1016/j.jcis.2019.05.043

86. Simsek, A. N.; Braeutigam, A.; Koch, M. D.; Shaevitz, J. W.; Huang, Y. F.; Gompper, G.; Sabass, B. Substrate-rigidity dependent migration of an idealized twitching bacterium. *Soft Matter* **2019**, *15*, 6224-6236. DOI: 10.1039/c9sm00541b

87. Kolewe, K. W.; Kalasin, S.; Shave, M.; Schiffman, J. D.; Santore, M. M. Mechanical Properties and Concentrations of Poly(ethylene glycol) in Hydrogels and Brushes Direct the Surface Transport of Staphylococcus aureus. *Acs Applied Materials & Interfaces* **2019**, *11*, 320-330. DOI: 10.1021/acsami.8b18302

88. Song, F.; Brasch, M. E.; Wang, H.; Henderson, J. H.; Sauer, K.; Ren, D. How Bacteria Respond to Material Stiffness during Attachment: A Role of Escherichia coil Flagellar Motility. *Acs Applied Materials & Interfaces* **2017**, *9*, 22176-22184. DOI: 10.1021/acsami.7b04757

89. Jadhav, S.; Eggleton, C. D.; Konstantopoulos, K. A 3-D computational model predicts that cell deformation affects selectin-mediated leukocyte rolling. *Biophysical Journal* **2005**, *88*, 96-104. DOI: 10.1529/biophysj.104.051029

90. Lipowsky, H. H.; Scott, D. A.; Cartmell, J. S. Leukocyte rolling velocity and its relation to leukocyte-endothelium adhesion and cell deformability. *American Journal of Physiology-Heart and Circulatory Physiology* **1996**, *270*, H1371-H1380.

91. Kolewe, K. W.; Peyton, S. R.; Schiffman, J. D. Fewer Bacteria Adhere to Softer Hydrogels. *Acs Applied Materials & Interfaces* **2015**, *7*, 19562-19569. DOI: 10.1021/acsami.5b04269

92. Kolewe, K. W.; Zhu, J. X.; Mako, N. R.; Nonnenmann, S. S.; Schiffman, J. D. Bacterial Adhesion Is Affected by the Thickness and Stiffness of Poly(ethylene glycol) Hydrogels. *Acs Applied Materials & Interfaces* **2018**, *10*, 2275-2281. DOI: 10.1021/acsami.7b12145

93. Lu, Z.; Mondarte, E. A. Q.; Suthiwanich, K.; Hayashi, T.; Masuda, T.; Isu, N.; Takai, M. Study on Bacterial Antiadhesiveness of Stiffness and Thickness Tunable Cross-Linked Phospholipid Copolymer Thin-Film. *Acs Applied Bio Materials* **2020**, *3*, 1079-1087. DOI: 10.1021/acsabm.9b01041

94. Keskin, D.; Mergel, O.; van der Mei, H. C.; Busscher, H. J.; van Rijn, P. Inhibiting Bacterial Adhesion by Mechanically Modulated Microgel Coatings. *Biomacromolecules* **2019**, *20*, 243-253. DOI: 10.1021/acs.biomac.8b01378

95. Lichter, J. A.; Thompson, M. T.; Delgadillo, M.; Nishikawa, T.; Rubner, M. F.; Van Vliet, K. J. Substrata mechanical stiffness can regulate adhesion of viable bacteria. *Biomacromolecules* **2008**, *9*, 1571-1578. DOI: 10.1021/bm701430y

96. Straub, H.; Bigger, C. M.; Valentin, J.; Abt, D.; Qin, X. H.; Eberl, L.; Maniura-Weber, K.; Ren, Q. Bacterial Adhesion on Soft Materials: Passive Physicochemical Interactions or Active Bacterial Mechanosensing? *Advanced Healthcare Materials* **2019**, *8*. DOI: 10.1002/adhm.201801323

97. Wang, Y.; Guan, A.; Isayeva, I.; Vorvolakos, K.; Das, S.; Li, Z. Y.; Phillips, K. S. Interactions of Staphylococcus aureus with ultrasoft hydrogel biomaterials. *Biomaterials* **2016**, *95*, 74-85. DOI: 10.1016/j.biomaterials.2016.04.005

98. Jin, H. C.; Tian, L. M.; Bing, W.; Zhao, J.; Ren, L. Q. Bioinspired marine antifouling coatings: Status, prospects, and future. *Progress in Materials Science* **2022**, *124*. DOI: 10.1016/j.pmatsci.2021.100889

99. Wen, Y. C.; Guo, X. H.; Kalasin, S.; Santore, M. M. Capture of Soft Particles on Electrostatically Heterogeneous Collectors: Brushy Particles. *Langmuir* **2014**, *30*, 2019-2027. DOI: 10.1021/la404235g

100. Bastounis, E. E.; Yeh, Y. T.; Theriot, J. A. Matrix stiffness modulates infection of endothelial cells by Listeria monocytogenes via expression of cell surface vimentin. *Molecular Biology of the Cell* **2018**, *29*, 1571-1589. DOI: 10.1091/mbc.E18-04-0228

101. Kandemir, N.; Vollmer, W.; Jakubovics, N. S.; Chen, J. J. Mechanical interactions between bacteria and hydrogels. *Scientific Reports* **2018**, *8*. DOI: 10.1038/s41598-018-29269-x

102. Ahagon, A.; Gent, A. N. Effect of Interfacial Bonding on strength of adhesion. *Journal of Polymer Science Part B-Polymer Physics* **1975**, *13*, 1285-1300. DOI: 10.1002/pol.1975.180130703

103. Gent, A. N. Adhesion and strength of viscoelastic solids. Is there a relationship between adhesion and bulk properties? *Langmuir* **1996**, *12*, 4492-4496. DOI: 10.1021/la950887q

104. Gent, A. N.; Petrich, R. P. Adhesion of Viscoelastic Materials to Rigid Substrates. *Proceedings of the Royal Society of London Series a-Mathematical and Physical Sciences* **1969**, *310*, 433-&. DOI: 10.1098/rspa.1969.0085

105. Creton, C.; Kramer, E. J.; Brown, H. R.; Hui, C. Y.; Springer; Springer, Adhesion and fracture of interfaces between immiscible polymers: From the molecular to the continuum scale. In *Molecular Simulation Fracture Gel Theory*, 2002; Vol. 156, pp 53-136.
106. Creton, C.; Ciccotti, M. Fracture and adhesion of soft materials: a review. *Reports on Progress in Physics* **2016**, *79*. DOI: 10.1088/0034-4885/79/4/046601

107. Chen, Y.; Harapanahalli, A. K.; Busscher, H. J.; Norde, W.; van der Mei, H. C. Nanoscale Cell Wall Deformation Impacts Long-Range Bacterial Adhesion Forces on Surfaces. *Applied and Environmental Microbiology* **2014**, *80*, 637-643. DOI: 10.1128/aem.02745-13

108. Carniello, V.; Peterson, B. W.; van der Mei, H. C.; Busscher, H. J. Role of adhesion forces in mechanosensitive channel gating inStaphylococcus aureusadhering to surfaces. *Npj Biofilms and Microbiomes* **2020**, *6*. DOI: 10.1038/s41522-020-00141-z

109. Song, F. C.; Wang, H.; Sauer, K.; Ren, D. C. Cyclic-di-GMP and oprF Are Involved in the Response of Pseudomonas aeruginosa to Substrate Material Stiffness during Attachment on Polydimethylsiloxane (PDMS). *Frontiers in Microbiology* **2018**, *9*, 110. DOI: 10.3389/fmicb.2018.00110

110. Blacutt, J.; Lan, Z. Y.; Cosgriff-Hernandez, E. M.; Gordon, V. D. Quantitative confocal microscopy and calibration for measuring differences in cyclic-di-GMP signalling by bacteria on biomedical hydrogels. *Royal Society Open Science* **2021**, *8*. DOI: 10.1098/rsos.201453

111. Conrad, J. C.; Gibiansky, M. L.; Jin, F.; Gordon, V. D.; Motto, D. A.; Mathewson, M. A.; Stopka, W. G.; Zelasko, D. C.; Shrout, J. D.; Wong, G. C. L. Flagella and Pili-Mediated Near-Surface Single-Cell Motility Mechanisms in P. aeruginosa. *Biophysical Journal* **2011**, *100*, 1608-1616. DOI: 10.1016/j.bpj.2011.02.020

112. Gibiansky, M. L.; Conrad, J. C.; Jin, F.; Gordon, V. D.; Motto, D. A.; Mathewson, M. A.; Stopka, W. G.; Zelasko, D. C.; Shrout, J. D.; Wong, G. C. L. Bacteria Use Type IV Pili to Walk Upright and Detach from Surfaces. *Science* **2010**, *330*, 197-U50. DOI: 10.1126/science.1194238

113. Epstein, A. K.; Wong, T. S.; Belisle, R. A.; Boggs, E. M.; Aizenberg, J. Liquid-infused structured surfaces with exceptional anti-biofouling performance. *Proceedings of the National Academy of Sciences of the United States of America* **2012**, *109*, 13182-13187. DOI: 10.1073/pnas.1201973109

114. Loo, C. Y.; Corliss, D. A.; Ganeshkumar, N. Streptococcus gordonii biofilm formation: Identification of genes that code for biofilm phenotypes. *Journal of Bacteriology* **2000**, *182*, 1374-1382. DOI: 10.1128/jb.182.5.1374-1382.2000

115. Briand, J. F. Marine antifouling laboratory bioassays: an overview of their diversity. *Biofouling* **2009**, *25*, 297-311. DOI: 10.1080/08927010902745316

116. Wiencek, K. M.; Fletcher, M. Bacterial Adhesion to Hydroxyl-Terminated and Methyl Terminated Alkanethiol Self-Assembled Monolayers. *Journal of Bacteriology* **1995**, *177*, 1959-1966. DOI: 10.1128/jb.177.8.1959-1966.1995

117. Lyklema, J.; Norde, W.; Vanloosdrecht, M. C. M.; Zehnder, A. J. B. Adhesion of bacteria to polystyrene surfaces. *Colloids and Surfaces* **1989**, *39*, 175-187. DOI: 10.1016/0166-6622(89)80186-6

118. Dorobantu, L. S.; Bhattacharjee, S.; Foght, J. M.; Gray, M. R. Analysis of Force Interactions between AFM Tips and Hydrophobic Bacteria Using DLVO Theory. *Langmuir* **2009**, *25*, 6968-6976. DOI: 10.1021/la9001237

119. Harimawan, A.; Rajasekar, A.; Ting, Y. P. Bacteria attachment to surfaces - AFM force spectroscopy and physicochemical analyses. *Journal of Colloid and Interface Science* **2011**, *364*, 213-218. DOI: 10.1016/j.jcis.2011.08.021

120. Ubbink, J.; Schar-Zammaretti, P. Colloidal properties and specific interactions of bacterial surfaces. *Current Opinion in Colloid & Interface Science* **2007**, *12*, 263-270. DOI: 10.1016/j.cocis.2007.08.004

121. Vadillo-Rodriguez, V.; Busscher, H. J.; Norde, W.; de Vries, J.; van der Mei, H. C. On relations between microscopic and macroscopic physicochemical properties of bacterial cell surfaces: An AFM study on Streptococcus mitis strains. *Langmuir* **2003**, *19*, 2372-2377. DOI: 10.1021/la020658l

122. Aguayo, S.; Bozec, L., Mechanics of Bacterial Cells and Initial Surface Colonisation. In *Biophysics of Infection*, Leake, M. C., Ed. 2016; Vol. 915, pp 245-260.

123. Alam, F.; Kumar, S.; Varadarajan, K. M. Quantification of Adhesion Force of Bacteria on the Surface of Biomaterials: Techniques and Assays. *Acs Biomaterials Science & Engineering* **2019**, *5*, 2093-2110. DOI: 10.1021/acsbiomaterials.9b00213

124. Poortinga, A. T.; Bos, R.; Norde, W.; Busscher, H. J. Electric double layer interactions in bacterial adhesion to surfaces. *Surface Science Reports* **2002**, *47*, 3-32.

125. Bos, R.; van der Mei, H. C.; Busscher, H. J. Physico-chemistry of initial microbial adhesive interactions - its mechanisms and methods for study. *Fems Microbiology Reviews* **1999**, *23*, 179-230. DOI: 10.1016/s0168-6445(99)00004-2

126. Boks, N. P.; Norde, W.; van der Mei, H. C.; Busscher, H. J. Forces involved in bacterial adhesion to hydrophilic and hydrophobic surfaces. *Microbiology-Sgm* **2008**, *154*, 3122-3133. DOI: 10.1099/mic.0.2008/018622-0

127. Hori, K.; Matsumoto, S. Bacterial adhesion: From mechanism to control. *Biochemical Engineering Journal* **2010**, *48*, 424-434. DOI: 10.1016/j.bej.2009.11.014

128. Hermansson, M. The DLVO theory in microbial adhesion. *Colloids and Surfaces B-Biointerfaces* **1999**, *14*, 105-119. DOI: 10.1016/s0927-7765(99)00029-6

129. Li, B. K.; Logan, B. E. Bacterial adhesion to glass and metal-oxide surfaces. *Colloids and Surfaces B-Biointerfaces* **2004**, *36*, 81-90. DOI: 10.1016/j.colsurfb.2004.05.006

130. Ong, Y. L.; Razatos, A.; Georgiou, G.; Sharma, M. M. Adhesion forces between E-coli bacteria and biomaterial surfaces. *Langmuir* **1999**, *15*, 2719-2725. DOI: 10.1021/la981104e

131. Tuson, H. H.; Weibel, D. B. Bacteria-surface interactions. *Soft Matter* **2013**, *9*, 4368-4380. DOI: 10.1039/c3sm27705d

132. Pratt, L. A.; Kolter, R. Genetic analysis of Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type I pili. *Molecular Microbiology* **1998**, *30*, 285-293. DOI: 10.1046/j.1365-2958.1998.01061.x

133. McLay, R. B.; Nguyen, H. N.; Jaimes-Lizcano, Y. A.; Dewangan, N. K.; Alexandrova, S.; Rodrigues, D. F.; Cirino, P. C.; Conrad, J. C. Level of Fimbriation Alters the Adhesion of Escherichia coli Bacteria to Interfaces. *Langmuir* **2018**, *34*, 1133-1142. DOI: 10.1021/acs.langmuir.7b02447

134. Hayashi, H.; Seiki, H.; Tsuneda, S.; Hirata, A.; Sasaki, H. Influence of growth phase on bacterial cell electrokinetic characteristics examined by soft particle electrophoresis theory. *Journal of Colloid and Interface Science* **2003**, *264*, 565-568. DOI: 10.1016/s0021-9797(03)00418-1

135. Renner, L. D.; Weibel, D. B. Physicochemical regulation of biofilm formation. *Mrs Bulletin* **2011**, *36*, 347-355. DOI: 10.1557/mrs.2011.65

136. Thomas, W., Catch bonds in adhesion. In *Annual Review of Biomedical Engineering*, 2008; Vol. 10, pp 39-57.

137. Sokurenko, E. V.; Vogel, V.; Thomas, W. E. Catch-Bond Mechanism of Force-Enhanced Adhesion: Counterintuitive, Elusive, but ... Widespread? *Cell Host Microbe* **2008**, *4*, 314-323. DOI: 10.1016/j.chom.2008.09.005

138. Chao, Y. Q.; Zhang, T. Probing Roles of Lipopolysaccharide, Type 1 Fimbria, and Colanic Acid in the Attachment of Escherichia coli Strains on Inert Surfaces. *Langmuir* **2011**, *27*, 11545-11553. DOI: 10.1021/la202534p

139. Yakovenko, O.; Sharma, S.; Forero, M.; Tchesnokova, V.; Aprikian, P.; Kidd, B.; Mach, A.; Vogel, V.; Sokurenko, E.; Thomas, W. E. FimH forms catch bonds that are enhanced by mechanical force due to allosteric regulation. *Journal of Biological Chemistry* **2008**, *283*, 11596-11605. DOI: 10.1074/jbc.M707815200

140. Thomas, W. E.; Trintchina, E.; Forero, M.; Vogel, V.; Sokurenko, E. V. Bacterial adhesion to target cells enhanced by shear force. *Cell* **2002**, *109*, 913-923. DOI: 10.1016/s0092-8674(02)00796-1

141. Tchesnokova, V.; McVeigh, A. L.; Kidd, B.; Yakovenko, O.; Thomas, W. E.; Sokurenko, E. V.; Savarino, S. J. Shear-enhanced binding of intestinal colonization factor antigen I of enterotoxigenic Escherichia coli. *Molecular Microbiology* **2010**, *76*, 489-502. DOI: 10.1111/j.1365-2958.2010.07116.x

142. Kallas, P.; Haugen, H. J.; Gadegaard, N.; Stormonth-Darling, J.; Hulander, M.; Andersson, M.; Valen, H. Adhesion of Escherichia Coli to Nanostructured Surfaces and the Role of Type 1 Fimbriae. *Nanomaterials* **2020**, *10*. DOI: 10.3390/nano10112247

143. Mo, L.; Zhu, X. H.; Huang, H. Y.; Shapiro, E.; Hasty, D. L.; Wu, X. R. Ablation of the Tamm-Horsfall protein gene increases susceptibility of mice to bladder colonization by type 1-fimbriated Escherichia coli. *American Journal of Physiology-Renal Physiology* **2004**, *286*, F795-F802. DOI: 10.1152/ajprenal.00357.2003

144. Connell, H.; Agace, W.; Klemm, P.; Schembri, M.; Marild, S.; Svanborg, C. Type 1 fimbrial expression enhances Escherichia coli virulence for the urinary tract. *Proceedings of the National Academy of Sciences of the United States of America* **1996**, *93*, 9827-9832. DOI: 10.1073/pnas.93.18.9827

145. Mulvey, M. A.; Schilling, J. D.; Hultgren, S. J. Establishment of a persistent Escherichia coli reservoir during the acute phase of a bladder infection. *Infection and Immunity* **2001**, *69*, 4572-4579. DOI: 10.1128/iai.69.7.4572-4579.2001

146. Hung, D. L.; Raivio, T. L.; Jones, C. H.; Silhavy, T. J.; Hultgren, S. J. Cpx signaling pathway monitors biogenesis and affects assembly and expression of P pili. *Embo Journal* **2001**, *20*, 1508-1518. DOI: 10.1093/emboj/20.7.1508

147. Otto, K.; Elwing, H.; Hermansson, M. The role of type 1 fimbriae in adhesion of Escherichia coli to hydrophilic and hydrophobic surfaces. *Colloids and Surfaces B-Biointerfaces* **1999**, *15*, 99-111. DOI: 10.1016/s0927-7765(99)00050-8

148. Skerker, J. M.; Berg, H. C. Direct observation of extension and retraction of type IV pili. *Proceedings of the National Academy of Sciences of the United States of America* **2001**, *98*, 6901-6904. DOI: 10.1073/pnas.121171698

149. Burrows, L. L., Pseudomonas aeruginosa Twitching Motility: Type IV Pili in Action. In *Annual Review of Microbiology, Vol 66*, Gottesman, S.; Harwood, C. S.; Schneewind, O., Eds. 2012; Vol. 66, pp 493-520.

150. Semmler, A. B. T.; Whitchurch, C. B.; Mattick, J. S. A re-examination of twitching motility in Pseudomonas aeruginosa. *Microbiology-Sgm* **1999**, *145*, 2863-2873. DOI: 10.1099/00221287-145-10-2863

151. McCallum, M.; Tammam, S.; Khan, A.; Burrows, L. L.; Howell, P. L. The molecular mechanism of the type IVa pilus motors. *Nature Communications* **2017**, *8*. DOI: 10.1038/ncomms15091

152. Maier, B.; Potter, L.; So, M.; Seifert, H. S.; Sheetz, M. P. Single pilus motor forces exceed 100 pN. *Proceedings of the National Academy of Sciences of the United States of America* **2002**, *99*, 16012-16017. DOI: 10.1073/pnas.242523299

153. Merz, A. J.; So, M.; Sheetz, M. P. Pilus retraction powers bacterial twitching motility. *Nature* **2000**, *407*, 98-102. DOI: 10.1038/35024105

154. Tala, L.; Fineberg, A.; Kukura, P.; Persat, A. Pseudomonas aeruginosa orchestrates twitching motility by sequential control of type IV pili movements. *Nature Microbiology* **2019**, *4*, 774-780. DOI: 10.1038/s41564-019-0378-9

155. Shen, Y.; Siryaporn, A.; Lecuyer, S.; Gitai, Z.; Stone, H. A. Flow Directs Surface-Attached Bacteria to Twitch Upstream. *Biophysical Journal* **2012**, *103*, 146-151. DOI: 10.1016/j.bpj.2012.05.045

156. Kannan, A.; Yang, Z. B.; Kim, M. K.; Stone, H. A.; Siryaporn, A. Dynamic switching enables efficient bacterial colonization in flow. *Proceedings of the National Academy of Sciences of the United States of America* **2018**, *115*, 5438-5443. DOI: 10.1073/pnas.1718813115

157. Watnick, P. I.; Kolter, R. Steps in the development of a Vibrio cholerae El Tor biofilm. *Molecular Microbiology* **1999**, *34*, 586-595. DOI: 10.1046/j.1365-2958.1999.01624.x

158. Shime-Hattori, A.; Iida, T.; Arita, M.; Park, K. S.; Kodama, T.; Honda, T. Two type IV pili of Vibrio parahaemolyticus play different roles in biofilm formation. *Fems Microbiology Letters* **2006**, *264*, 89-97. DOI: 10.1111/j.1574-6968.2006.00438.x

159. Sullan, R. M. A.; Beaussart, A.; Tripathi, P.; Derclaye, S.; El-Kirat-Chatel, S.; Li, J. K.; Schneider, Y. J.; Vanderleyden, J.; Lebeer, S.; Dufrene, Y. F. Single-cell force spectroscopy of pili-mediated adhesion. *Nanoscale* **2014**, *6*, 1134-1143. DOI: 10.1039/c3nr05462d

160. Rodesney, C. A.; Roman, B.; Dhamani, N.; Cooley, B. J.; Touhami, A.; Gordon, V. D. Mechanosensing of shear by Pseudomonas aeruginosa leads to increased levels of the cyclic-di-GMP signal initiating biofilm development. *Proceedings of the National Academy of Sciences of the United States of America* **2017**, *114*, 5906-5911. DOI: 10.1073/pnas.1703255114

161. Jenal, U.; Reinders, A.; Lori, C. Cyclic di-GMP: second messenger extraordinaire. *Nature Reviews Microbiology* **2017**, *15*, 271-284. DOI: 10.1038/nrmicro.2016.190

162. Sharma, S.; Jaimes-Lizcano, Y. A.; McLay, R. B.; Cirino, P. C.; Conrad, J. C. Subnanometric Roughness Affects the Deposition and Mobile Adhesion of Escherichia coli on Silanized Glass Surfaces. *Langmuir* **2016**, *32*, 5422-5433. DOI: 10.1021/acs.langmuir.6b00883

163. Elgeti, J.; Winkler, R. G.; Gompper, G. Physics of microswimmers-single particle motion and collective behavior: a review. *Reports on Progress in Physics* **2015**, *78*. DOI: 10.1088/0034-4885/78/5/056601

164. McCarter, L.; Hilmen, M.; Silverman, M. Flagellar Dynamometer Controls Swarmer Cell Differentiation of *V. Parahaemolyticus*. *Cell* **1988**, *54*, 345-351. DOI: 10.1016/0092-8674(88)90197-3

165. Schniederberend, M.; Williams, J. F.; Shine, E.; Shen, C.; Jain, R.; Emonet, T.; Kazmierczak, B. I. Modulation of flagellar rotation in surface-attached bacteria: A pathway for rapid surface-sensing after flagellar attachment. *Plos Pathogens* **2019**, *15*. DOI: 10.1371/journal.ppat.1008149

166. Bennett, R. R.; Lee, C. K.; De Anda, J.; Nealson, K. H.; Yildiz, F. H.; O'Toole, G. A.; Wong, G. C. L.; Golestanian, R. Species-dependent hydrodynamics of flagellum-tethered bacteria in early biofilm development. *Journal of the Royal Society Interface* **2016**, *13*. DOI: 10.1098/rsif.2015.0966

167. Lele, P. P.; Hosu, B. G.; Berg, H. C. Dynamics of mechanosensing in the bacterial flagellar motor. *Proceedings of the National Academy of Sciences of the United States of America* **2013**, *110*, 11839-11844. DOI: 10.1073/pnas.1305885110

168. Otto, K.; Hermansson, M. Inactivation of ompX causes increased interactions of type 1 fimbriated Escherichia coli with abiotic surfaces. *Journal of Bacteriology* **2004**, *186*, 226-234. DOI: 10.1128/jb.186.1.226-234.2004

169. Justice, S. S.; Hunstad, D. A.; Cegelski, L.; Hultgren, S. J. Morphological plasticity as a bacterial survival strategy. *Nature Reviews Microbiology* **2008**, *6*, 162-168. DOI: 10.1038/nrmicro1820

170. Belas, R. Biofilms, flagella, and mechanosensing of surfaces by bacteria. *Trends in Microbiology* **2014**, *22*, 517-527. DOI: 10.1016/j.tim.2014.05.002

171. McClaine, J. W.; Ford, R. M. Characterizing the adhesion of motile and nonmotile Escherichia coli to a glass surface using a parallel-plate flow chamber. *Biotechnology and Bioengineering* **2002**, *78*, 179-189. DOI: 10.1002/bit.10192

172. DiLuzio, W. R.; Turner, L.; Mayer, M.; Garstecki, P.; Weibel, D. B.; Berg, H. C.; Whitesides, G. M. Escherichia coli swim on the right-hand side. *Nature* **2005**, *435*, 1271-1274. DOI: 10.1038/nature03660

173. Lemelle, L.; Palierne, J. F.; Chatre, E.; Place, C. Counterclockwise Circular Motion of Bacteria Swimming at the Air-Liquid Interface. *Journal of Bacteriology* **2010**, *192*, 6307-6308. DOI: 10.1128/jb.00397-10

174. Friedlander, R. S.; Vlamakis, H.; Kim, P.; Khan, M.; Kolter, R.; Aizenberg, J. Bacterial flagella explore microscale hummocks and hollows to increase adhesion. *Proceedings of the National Academy of Sciences of the United States of America* **2013**, *110*, 5624-5629. DOI: 10.1073/pnas.1219662110

175. Bruzaud, J.; Tarrade, J.; Coudreuse, A.; Canette, A.; Herry, J. M.; de Givenchy, E. T.; Darmanin, T.; Guittard, F.; Guilbaud, M.; Bellon-Fontaine, M. N. Flagella but not type IV pili are involved in the initial adhesion of Pseudomonas aeruginosa PAO1 to hydrophobic or superhydrophobic surfaces. *Colloids and Surfaces B-Biointerfaces* **2015**, *131*, 59-66. DOI: 10.1016/j.colsurfb.2015.04.036

176. Haiko, J.; Westerlund-Wikstrom, B. The Role of Bacterial Flagellum in Adhesion and Virulence. *Biology* **2013**, *2*, 1242-1267. DOI: 10.3390/biology2041242

177. Erdem, A. L.; Avelino, F.; Xicohtencatl-Cortes, J.; Giron, J. A. Host protein binding and adhesive properties of H6 and H7 flagella of attaching and effacing Escherichia coli. *Journal of Bacteriology* **2007**, *189*, 7426-7435. DOI: 10.1128/jb.00464-07

178. Berne, C.; Ellison, C. K.; Ducret, A.; Brun, Y. V. Bacterial adhesion at the single-cell level. *Nature Reviews Microbiology* **2018**, *16*, 616-627. DOI: 10.1038/s41579-018-0057-5

179. Van Houdt, R.; Michiels, C. W. Role of bacterial cell surface structures in Escherichia coli biofilm formation. *Research in Microbiology* **2005**, *156*, 626-633. DOI: 10.1016/j.resmic.2005.02.005

180. Kovalenko, Y.; Sotiri, I.; Timonen, J. V. I.; Overton, J. C.; Holmes, G.; Aizenberg, J.; Howell, C. Bacterial Interactions with Immobilized Liquid Layers. *Advanced Healthcare Materials* **2017**, *6*. DOI: 10.1002/adhm.201600948

181. Brown, D. G.; Hong, Y. Impact of the Charge-Regulated Nature of the Bacterial Cell Surface on the Activity of Adhered Cells. *Journal of Adhesion Science and Technology* **2011**, *25*, 2199-2218. DOI: 10.1163/016942411x574916

182. Krasowska, A.; Sigler, K. How microorganisms use hydrophobicity and what does this mean for human needs? *Frontiers in Cellular and Infection Microbiology* **2014**, *4*. DOI: 10.3389/fcimb.2014.00112

183. Rosenberg, M. Basic and applied aspects of microbial adhesion at the hydrocarbon-water interface. *Critical Reviews in Microbiology* **1991**, *18*, 159-173. DOI: 10.3109/10408419109113512

184. Zheng, S.; Bawazir, M.; Dhall, A.; Kim, H. E.; He, L.; Heo, J.; Hwang, G. Implication of Surface Properties, Bacterial Motility, and Hydrodynamic Conditions on Bacterial Surface Sensing and Their Initial Adhesion. *Frontiers in Bioengineering and Biotechnology* **2021**, *9*. DOI: 10.3389/fbioe.2021.643722

185. Boks, N. P.; Busscher, H. J.; van der Mei, H. C.; Norde, W. Bond-Strengthening in Staphylococcal Adhesion to Hydrophilic and Hydrophobic Surfaces Using Atomic Force Microscopy. *Langmuir* **2008**, *24*, 12990-12994. DOI: 10.1021/la801824c

186. Kimkes, T. E. P.; Heinemann, M. Reassessing the role of the Escherichia coli CpxAR system in sensing surface contact. *Plos One* **2018**, *13*. DOI: 10.1371/journal.pone.0207181

187. Formosa-Dague, C.; Feuillie, C.; Beaussart, A.; Derclaye, S.; Kucharikova, S.; Lasa, I.; Van Dijck, P.; Dufrene, Y. F. Sticky Matrix: Adhesion Mechanism of the Staphylococcal Polysaccharide Intercellular Adhesin. *Acs Nano* **2016**, *10*, 3443-3452. DOI: 10.1021/acsnano.5b07515

188. Goulter, R. M.; Gentle, I. R.; Dykes, G. A. Issues in determining factors influencing bacterial attachment: a review using the attachment of Escherichia coli to abiotic surfaces as an example. *Letters in Applied Microbiology* **2009**, *49*, 1-7. DOI: 10.1111/j.1472-765X.2009.02591.x

189. McClaine, J. W.; Ford, R. M. Reversal of flagellar rotation is important in initial attachment of Escherichia coli to glass in a dynamic system with high- and low-ionic-strength buffers. *Applied and Environmental Microbiology* **2002**, *68*, 1280-1289. DOI: 10.1128/aem.68.3.1280-1289.2002

190. Meinders, J. M.; Vandermei, H. C.; Busscher, H. J. Physico-chemical aspects of deposition of Streptococcus thermophilus B to hydrophobic aand hydrophilic substrata in a parallel plate flow chamber. *Journal of Colloid and Interface Science* **1994**, *164*, 355-363. DOI: 10.1006/jcis.1994.1177

191. Marshall, K. C.; Stout, R.; Mitchell, R. Mechanism of initial events in sorption of marine bacteria to surfaces. *Journal of General Microbiology* **1971**, *68*, 337-&. DOI: 10.1099/00221287-68-3-337

192. Adamczyk, Z.; Warszynski, P.; Szyk-Warszynska, L.; Weronski, P. Role of convection in particle deposition at solid surfaces. *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **2000**, *165*, 157-187. DOI: 10.1016/s0927-7757(99)00416-1

193. Russel, W. B.; Saville, D. A.; Schowalter, W. R., *Colloidal Dispersions*. Cambridge University Press: Cambridge, 1989.

194. Ponsonnet, L.; Boureanu, M.; Jaffrezic, N.; Othmane, A.; Dorel, C.; Lejeune, P. Local pH variation as an initial step in bacterial surface-sensing and biofilm formation. *Materials Science & Engineering C-Biomimetic and Supramolecular Systems* **2008**, *28*, 896-900. DOI: 10.1016/j.msec.2007.10.031

195. Hong, Y. S.; Brown, D. G. Variation in Bacterial ATP Level and Proton Motive Force Due to Adhesion to a Solid Surface. *Applied and Environmental Microbiology* **2009**, *75*, 2346-2353. DOI: 10.1128/aem.02671-08

196. Yuk, H. G.; Marshall, D. L. Adaptation of Escherichia coli O157 : H7 to pH alters membrane lipid composition, verotoxin secretion, and resistance to simulated gastric fluid acid. *Applied and Environmental Microbiology* **2004**, *70*, 3500-3505. DOI: 10.1128/aem.70.6.3500-3505.2004

197. Choi, S. H.; Baumler, D. J.; Kaspar, C. W. Contribution of dps to acid stress tolerance and oxidative stress tolerance in Escherichia coli O157 : H7. *Applied and Environmental Microbiology* **2000**, *66*, 3911-3916. DOI: 10.1128/aem.66.9.3911-3916.2000

198. Raja, N.; Goodson, M.; Smith, D. G.; Rowbury, R. J. Decreased DNA damage by acid and increased repair of acid-damaged DNA in acid-habituated Escherichia coli. *Journal of Applied Bacteriology* **1991**, *70*, 507-511. DOI: 10.1111/j.1365-2672.1991.tb02748.x

199. Roosjen, A.; Norde, W.; van der Mei, H. C.; Busscher, H. J. In *The use of positively charged or low surface free energy coatings versus polymer brushes in controlling biofilm formation*, 10th Dresden Polymer Meeting, Dresden, GERMANY, Apr 10-13; Dresden, GERMANY, 2005; pp 138-144.

200. Kugel, A.; Stafslien, S.; Chisholm, B. J. Antimicrobial coatings produced by "tethering" biocides to the coating matrix: A comprehensive review. *Progress in Organic Coatings* **2011**, *72*, 222-252. DOI: 10.1016/j.porgcoat.2011.07.004

201. Onaizi, S. A.; Leong, S. S. J. Tethering antimicrobial peptides: Current status and potential challenges. *Biotechnology Advances* **2011**, *29*, 67-74. DOI: 10.1016/j.biotechadv.2010.08.012

202. Kaur, R.; Liu, S. Antibacterial surface design - Contact kill. *Progress in Surface Science* **2016**, *91*, 136-153. DOI: 10.1016/j.progsurf.2016.09.001

203. Murata, H.; Koepsel, R. R.; Matyjaszewski, K.; Russell, A. J. Permanent, non-leaching antibacterial surfaces - 2: How high density cationic surfaces kill bacterial cells. *Biomaterials* **2007**, *28*, 4870-4879. DOI: 10.1016/j.biomaterials.2007.06.012

204. Asri, L.; Crismaru, M.; Roest, S.; Chen, Y.; Ivashenko, O.; Rudolf, P.; Tiller, J. C.; van der Mei, H. C.; Loontjens, T. J. A.; Busscher, H. J. A Shape- Adaptive, Antibacterial-Coating of Immobilized Quaternary- Ammonium Compounds Tethered on Hyperbranched Polyurea and its Mechanism of Action. *Advanced Functional Materials* **2014**, *24*, 346-355. DOI: 10.1002/adfm.201301686

205. Colomer, A.; Perez, L.; Pons, R.; Infante, M. R.; Perez-Clos, D.; Manresa, A.; Espuny, M. J.; Pinazo, A. Mixed Monolayer of DPPC and Lysine-Based Cationic Surfactants: An Investigation into the Antimicrobial Activity. *Langmuir* **2013**, *29*, 7912-7921. DOI: 10.1021/la401092j

206. Kugler, R.; Bouloussa, O.; Rondelez, F. Evidence of a charge-density threshold for optimum efficiency of biocidal cationic surfaces. *Microbiology-Sgm* **2005**, *151*, 1341-1348. DOI: 10.1099/mic.0.27526-0

207. Fang, B.; Jiang, Y.; Nusslein, K.; Rotello, V. M.; Santore, M. M. Antimicrobial surfaces containing cationic nanoparticles: How immobilized, clustered, and protruding cationic charge presentation affects killing activity and kinetics. *Colloids and Surfaces B-Biointerfaces* **2015**, *125*, 255-263. DOI: 10.1016/j.colsurfb.2014.10.043

208. Mohamed, N.; Rainier, T. R.; Ross, J. M. Novel experimental study of receptor-mediated bacterial adhesion under the influence of fluid shear. *Biotechnology and Bioengineering* **2000**, *68*, 628-636. DOI: 10.1002/(sici)1097-0290(20000620)68:6<628::Aid-bit5>3.0.Co;2-d

209. Camesano, T. A.; Abu-Lail, N. I. Heterogeneity in bacterial surface polysaccharides, probed on a single-molecule basis. *Biomacromolecules* **2002**, *3*, 661-667. DOI: 10.1021/bm015648y

210. Simoni, S. F.; Harms, H.; Bosma, T. N. P.; Zehnder, A. J. B. Population heterogeneity affects transport of bacteria through sand columns at low flow rates. *Environmental Science & Technology* **1998**, *32*, 2100-2105. DOI: 10.1021/es970936g

211. van der Mei, H. C.; Busscher, H. J. Bacterial Cell Surface Heterogeneity: A Pathogen's Disguise. *Plos Pathogens* **2012**, *8*. DOI: 10.1371/journal.ppat.1002821

212. Chen, J. Y.; Ko, C. H.; Bhattacharjee, S.; Elimelech, M. Role of spatial distribution of porous medium surface charge heterogeneity in colloid transport. *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **2001**, *191*, 3-15. DOI: 10.1016/s0927-7757(01)00759-2

213. Utada, A. S.; Bennett, R. R.; Fong, J. C. N.; Gibiansky, M. L.; Yildiz, F. H.; Golestanian, R.; Wong, G. C. L. Vibrio cholerae use pili and flagella synergistically to effect motility switching and conditional surface attachment. *Nature Communications* **2014**, *5*. DOI: 10.1038/ncomms5913

214. Ahimou, F.; Denis, F. A.; Touhami, A.; Dufrene, Y. F. Probing microbial cell surface charges by atomic force microscopy. *Langmuir* **2002**, *18*, 9937-9941. DOI: 10.1021/la026273k

215. Walker, S. L.; Hill, J. E.; Redman, J. A.; Elimelech, M. Influence of growth phase on adhesion kinetics of Escherichia coli D21g. *Applied and Environmental Microbiology* **2005**, *71*, 3093-3099. DOI: 10.1128/aem.71.6.3093-3099.2005

216. Busscher, H. J.; van de Belt-Gritter, B.; Dijkstra, R. J. B.; Norde, W.; van der Mei, H. C. Streptococcus mutans and Streptococcus intermedius adhesion to fibronectin films are oppositely influenced by ionic strength. *Langmuir* **2008**, *24*, 10968-10973. DOI: 10.1021/la8016968

217. Song, L. F.; Johnson, P. R.; Elimelech, M. Kinetics of colloid deposition onto heterogeneously charged srufaces in porous media. *Environmental Science & Technology* **1994**, *28*, 1164-1171. DOI: 10.1021/es00055a030

218. Dorobantu, L. S.; Bhattacharjee, S.; Foght, J. M.; Gray, M. R. Atomic force microscopy measurement of heterogeneity in bacterial surface hydrophobicity. *Langmuir* **2008**, *24*, 4944-4951. DOI: 10.1021/la7035295

219. Banerjee, I.; Pangule, R. C.; Kane, R. S. Antifouling Coatings: Recent Developments in the Design of Surfaces That Prevent Fouling by Proteins, Bacteria, and Marine Organisms. *Advanced Materials* **2011**, *23*, 690-718. DOI: 10.1002/adma.201001215

220. Cheng, G.; Zhang, Z.; Chen, S. F.; Bryers, J. D.; Jiang, S. Y. Inhibition of bacterial adhesion and biofilm formation on zwitterionic surfaces. *Biomaterials* **2007**, *28*, 4192-4199. DOI: 10.1016/j.biomaterials.2007.05.041

221. Jiang, S. Y.; Cao, Z. Q. Ultralow-Fouling, Functionalizable, and Hydrolyzable Zwitterionic Materials and Their Derivatives for Biological Applications. *Advanced Materials* **2010**, *22*, 920-932. DOI: 10.1002/adma.200901407

222. Kingshott, P.; Wei, J.; Bagge-Ravn, D.; Gadegaard, N.; Gram, L. Covalent attachment of poly(ethylene glycol) to surfaces, critical for reducing bacterial adhesion. *Langmuir* **2003**, *19*, 6912-6921. DOI: 10.1021/la034032m

223. Salwiczek, M.; Qu, Y.; Gardiner, J.; Strugnell, R. A.; Lithgow, T.; McLean, K. M.; Thissen, H. Emerging rules for effective antimicrobial coatings. *Trends in Biotechnology* **2014**, *32*, 82-90. DOI: 10.1016/j.tibtech.2013.09.008

224. Poon, W. C. K. The physics of a model colloid-polymer mixture. *Journal of Physics-Condensed Matter* **2002**, *14*, R859-R880. DOI: 10.1088/0953-8984/14/33/201

225. Gotzelmann, B.; Evans, R.; Dietrich, S. Depletion forces in fluids. *Physical Review E* **1998**, *57*, 6785-6800. DOI: 10.1103/PhysRevE.57.6785

226. Zhao, K.; Mason, T. G. Assembly of colloidal particles in solution. *Reports on Progress in Physics* **2018**, *81*. DOI: 10.1088/1361-6633/aad1a7

227. Poon, W. C. K.; Pirie, A. D.; Pusey, P. N. Gelation in colloid-polymer mixtures. *Faraday Discussions* **1995**, *101*, 65-76. DOI: 10.1039/fd9950100065

228. Bickel, T. Depletion forces near a soft surface. *Journal of Chemical Physics* **2003**, *118*, 8960-8968. DOI: 10.1063/1.1568732

229. Chu, Y. S.; Dufour, S.; Thiery, J. P.; Perez, E.; Pincet, F. Johnson-Kendall-Roberts theory applied to living cells. *Physical Review Letters* **2005**, *94*. DOI: 10.1103/PhysRevLett.94.028102

230. Nam, J.; Santore, M. M. Depletion versus Deflection: How Membrane Bending Can Influence Adhesion. *Physical Review Letters* **2011**, *107*. DOI: 10.1103/PhysRevLett.107.078101

231. Zhang, Z. W. W.; Neu, B. Role of Macromolecular Depletion in Red Blood Cell Adhesion. *Biophysical Journal* **2009**, *97*, 1031-1037. DOI: 10.1016/j.bpj.2009.06.006

232. Dorken, G.; Ferguson, G. P.; French, C. E.; Poon, W. C. K. Aggregation by depletion attraction in cultures of bacteria producing exopolysaccharide. *Journal of the Royal Society Interface* **2012**, *9*, 3490-3502. DOI: 10.1098/rsif.2012.0498

233. Eboigbodin, K. E.; Newton, J. R. A.; Routh, A. F.; Biggs, C. A. Role of nonadsorbing polymers in bacterial aggregation. *Langmuir* **2005**, *21*, 12315-12319. DOI: 10.1021/la051740u

234. Schwarz-Linek, J.; Valeriani, C.; Cacciuto, A.; Cates, M. E.; Marenduzzo, D.; Morozov, A. N.; Poon, W. C. K. Phase separation and rotor self-assembly in active particle suspensions. *Proceedings of the National Academy of Sciences of the United States of America* **2012**, *109*, 4052-4057. DOI: 10.1073/pnas.1116334109

235. Schwarz-Linek, J.; Winkler, A.; Wilson, L. G.; Pham, N. T.; Schilling, T.; Poon, W. C. K. Polymer-induced phase separation in Escherichia coli suspensions. *Soft Matter* **2010**, *6*, 4540-4549. DOI: 10.1039/c0sm00214c

236. Secor, P. R.; Michaels, L. A.; Ratjen, A.; Jennings, L. K.; Singh, P. K. Entropically driven aggregation of bacteria by host polymers promotes antibiotic tolerance in Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences of the United States of America* **2018**, *115*, 10780-10785. DOI: 10.1073/pnas.1806005115

237. Niu, W. A.; Rivera, S. L.; Siegrist, M. S.; Santore, M. M. Depletion forces drive reversible capture of live bacteria on non-adhesive surfaces. *Soft Matter* **2021**, *17*, 8185-8194. DOI: 10.1039/d1sm00631b

238. Busscher, H. J.; Norde, W.; Sharma, P. K.; van der Mei, H. C. Interfacial re-arrangement in initial microbial adhesion to surfaces. *Current Opinion in Colloid & Interface Science* **2010**, *15*, 510-517. DOI: 10.1016/j.cocis.2010.05.014

239. Olsson, A. L. J.; van der Mei, H. C.; Busscher, H. J.; Sharma, P. K. Novel Analysis of Bacterium-Substratum Bond Maturation Measured Using a Quartz Crystal Microbalance. *Langmuir* **2010**, *26*, 11113-11117. DOI: 10.1021/la100896a

240. Marcus, I. M.; Herzberg, M.; Walker, S. L.; Freger, V. Pseudomonas aeruginosa Attachment on QCM-D Sensors: The Role of Cell and Surface Hydrophobicities. *Langmuir* **2012**, *28*, 6396-6402. DOI: 10.1021/la300333c

241. Fang, B.; Jiang, Y.; Rotello, V. M.; Nusslein, K.; Santore, M. M. Easy Come Easy Go: Surfaces Containing Immobilized Nanoparticles or Isolated Polycation Chains Facilitate Removal of Captured Staphylococcus aureus by Retarding Bacterial Bond Maturation. *Acs Nano* **2014**, *8*, 1180-1190. DOI: 10.1021/nn405845y

242. van der Mei, H. C.; Rustema-Abbing, M.; de Vries, J.; Busscher, H. J. Bond strengthening in oral bacterial adhesion to salivary conditioning films. *Applied and Environmental Microbiology* **2008**, *74*, 5511-5515. DOI: 10.1128/aem.01119-08

243. Vadillo-Rodriguez, V.; Busscher, H. J.; Norde, W.; de Vries, J.; van der Mei, H. C. Atomic force microscopic corroboration of bond aging for adhesion of Streptococcus thermophilus to solid substrata. *Journal of Colloid and Interface Science* **2004**, *278*, 251-254. DOI: 10.1016/j.jcis.2004.05.045

244. Zeng, G. H.; Muller, T.; Meyer, R. L. Single-Cell Force Spectroscopy of Bacteria Enabled by Naturally Derived Proteins. *Langmuir* **2014**, *30*, 4019-4025. DOI: 10.1021/la404673q

245. Prystopiuk, V.; Feuillie, C.; Herman-Bausier, P.; Viela, F.; Alsteens, D.; Pietrocola, G.; Speziale, P.; Dufrene, Y. F. Mechanical Forces Guiding Staphylococcus aureus Cellular Invasion. *Acs Nano* **2018**, *12*, 3609-3622. DOI: 10.1021/acsnano.8b00716

246. Xu, L. C.; Vadillo-Rodriguez, V.; Logan, B. E. Residence time, loading force, pH, and ionic strength affect adhesion forces between colloids and biopolymer-coated surfaces. *Langmuir* **2005**, *21*, 7491-7500. DOI: 10.1021/la0509091

247. Te Riet, J.; Joosten, B.; Reinieren-Beeren, I.; Figdor, C. G.; Cambi, A. N-glycan mediated adhesion strengthening during pathogen-receptor binding revealed by cell-cell force spectroscopy. *Scientific Reports* **2017**, *7*. DOI: 10.1038/s41598-017-07220-w

248. Chen, Y.; Norde, W.; van der Mei, H. C.; Busscher, H. J. Bacterial Cell Surface Deformation under External Loading. *mBio* **2012**, *3*. DOI: 10.1128/mBio.00378-12

249. Gu, J. H.; Valdevit, A.; Chou, T. M.; Libera, M. Substrate effects on cell-envelope deformation during early-stage Staphylococcus aureus biofilm formation. *Soft Matter* **2017**, *13*, 2967-2976. DOI: 10.1039/c6sm02815b

250. Lee, K.; Hahn, L. D.; Yuen, W. W.; Vlamakis, H.; Kolter, R.; Mooney, D. J. Metal-Enhanced Fluorescence to Quantify Bacterial Adhesion. *Advanced Materials* **2011**, *23*, H101-H104. DOI: 10.1002/adma.201004096

251. Carniello, V.; Peterson, B. W.; Sjollema, J.; Busscher, H. J.; van der Mei, H. C. Surface enhanced fluorescence and nanoscopic cell wall deformation in adhering Staphylococcus aureus upon exposure to cell wall active and non-active antibiotics. *Nanoscale* **2018**, *10*, 11123-11133. DOI: 10.1039/c8nr01669k

252. Li, J. Y.; Busscher, H. J.; Swartjes, J.; Chen, Y.; Harapanahalli, A. K.; Norde, W.; van der Mei, H. C.; Sjollema, J. Residence-time dependent cell wall deformation of different Staphylococcus aureus strains on gold measured using surface-enhanced-fluorescence. *Soft Matter* **2014**, *10*, 7638-7646. DOI: 10.1039/c4sm00584h

253. Jones, J. F.; Feick, J. D.; Imoudu, D.; Chukwumah, N.; Vigeant, M.; Velegol, D. Oriented adhesion of Escherichia coli to polystyrene particles. *Applied and Environmental Microbiology* **2003**, *69*, 6515-6519. DOI: 10.1128/aem.69.11.6515-6519.2003

254. Behkam, B.; Sitti, M. In *Towards hybrid swimming microrobots: Bacteria assisted propulsion of polystyrene beads*, 28th Annual International Conference of the IEEE-Engineering-in-Medicine-and-Biology-Society, New York, NY, Aug 30-Sep 03; New York, NY, 2006; p 3170

255. Beloin, C.; Roux, A.; Ghigo, J. M., Escherichia coli biofilms. In *Bacterial Biofilms*, Romeo, T., Ed. 2008; Vol. 322, pp 249-289.

256. Dell'Arciprete, D.; Blow, M. L.; Brown, A. T.; Farrell, F. D. C.; Lintuvuori, J. S.; McVey, A. F.; Marenduzzo, D.; Poon, W. C. K. A growing bacterial colony in two dimensions as an active nematic. *Nature Communications* **2018**, *9*. DOI: 10.1038/s41467-018-06370-3

257. Yan, J.; Sharo, A. G.; Stone, H. A.; Wingreen, N. S.; Bassler, B. L. Vibrio cholerae biofilm growth program and architecture revealed by single-cell live imaging. *Proceedings of the National Academy of Sciences of the United States of America* **2016**, *113*, E5337-E5343. DOI: 10.1073/pnas.1611494113