Journal of Structural Biology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Forces guiding staphylococcal adhesion

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ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 15 December 2015
Accepted 17 December 2015
Available online xxxx

Keywords:
Atomic force microscopy
Molecular forces
Bacterial adhesion
Biofilms
Nosocomial infections
Staphylococcus aureus
Staphylococcus epidermidis

ABSTRACT

Staphylococcus epidermidis and *Staphylococcus aureus* are two important nosocomial pathogens that form biofilms on indwelling medical devices. Biofilm infections are difficult to fight as cells within the biofilm show increased resistance to antibiotics. Our understanding of the molecular interactions driving bacterial adhesion, the first stage of biofilm formation, has long been hampered by the paucity of appropriate force-measuring techniques. In this minireview, we discuss how atomic force microscopy techniques have enabled to shed light on the molecular forces at play during staphylococcal adhesion. Specific highlights include the study of the binding mechanisms of adhesion molecules by means of single-molecule force spectroscopy, the measurement of the forces involved in whole cell interactions using single-cell force spectroscopy, and the probing of the nanobiophysical properties of living bacteria *via* multiparametric imaging. Collectively, these findings emphasize the notion that force and function are tightly connected in staphylococcal adhesion.

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1. Introduction

Many microbial pathogens attach to host tissues and implanted devices, leading to the formation of surface-associated communities called biofilms (Costerton et al., 1999; Kolter and Greenberg, 2006). Cells in the biofilm are protected from host defences and are resistant to antibiotics, making biofilm-associated infections difficult to eradicate. As biofilms are estimated to be involved in more than 65% of nosocomial infections, they represent a tremendous burden on our healthcare system.

The development of a biofilm is a multistep process (Fig. 1), starting with the adhesion of the microbial cells to host surfaces, polymer substrates, and protein-coated biomaterials, followed by cell aggregation and cell multiplication to form a mature biofilm in which the cells are trapped in a matrix of extracellular polymers. For most species, the complex set of molecular interactions at play during biofilm formation is poorly understood. Hence, there is a growing need for biophysical methods that can quantify the forces leading to cell adhesion and biofilm formation, with high force sensitivity and high spatial resolution.

Staphylococcus epidermidis and *Staphylococcus aureus* are nosocomial pathogens that represent a leading cause of biofilm-associated infections (Otto, 2008; Otto, 2009; Foster et al., 2014). Major players in staphylococcal biofilms are the Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs), a family of cell surface adhesins that target host extracellular proteins such as albumin, fibronectin and fibrinogen (Otto, 2009; Foster et al., 2014). While the biology of staphylococcal adhesins has been extensively investigated, little attention has been paid to their molecular forces.

Traditionally, biofilms are studied using molecular biology and genetic approaches, optical and electron microscopy, and microscopic adhesion or biofilm assays (Donelli, 2014; Ghannoum et al., 2015). These methods generally probe large ensembles of cells and molecules, and do not provide information on molecular interaction forces. By contrast, several force-measuring techniques have been developed to measure molecular forces on cell surfaces, including optical and magnetic tweezers, and atomic force microscopy (AFM) (Tanase et al., 2007; Moffitt et al., 2008; Neuman and Nagy, 2008). Among these tools, AFM is the only method that can simultaneously quantify and localize specific forces on cells, at a resolution of a few nanometers. This is an important asset as biomolecular interactions are linked to structure in living cells. In microbiology, AFM has opened up new avenues for studying the forces involved in cell adhesion and biofilm formation, down to molecular resolution (Dufrêne, 2014, 2015). In single-molecule force spectroscopy (SMFS), force-distance curves are acquired between AFM tips labeled with ligands and cell surfaces in order

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http://dx.doi.org/10.1016/j.jsb.2015.12.009
1047-8477/© 2015 Published by Elsevier Inc.

Please cite this article in press as: Herman-Bausier, P., et al. Forces guiding staphylococcal adhesion. J. Struct. Biol. (2015), http://dx.doi.org/10.1016/j.jsb.2015.12.009

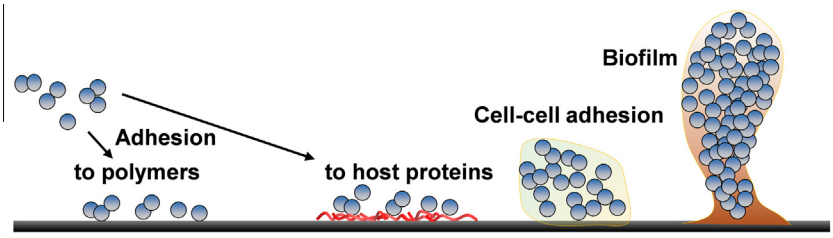

Fig. 1. Biofilm formation, a multistep process.

to detect, localize, and force probe individual receptors (Grandbois et al., 2000; Dupres et al., 2005; Hinterdorfer and Dufrêne, 2006). These analyses have provided molecular insights into the binding strength, affinity and specificity of staphylococcal adhesins. In single-cell force spectroscopy (SCFS), a living cell is attached on the AFM probe and force curves are obtained between the cell probe and a solid substrate or another cell (Helenius et al., 2008; Müller et al., 2009). A non-destructive SCFS assay was recently implemented, enabling the reliable and reproducible analysis of single-microbial cell adhesion forces (Beaussart et al., 2013a; Beaussart et al., 2014). A colloidal silica particle is attached to the end of a tip-less cantilever and coated with a bioinspired polydopamine wet adhesive. The sticky colloidal probe is used to pick up a single live cell. Fluorescence microscopy is used to check that the cell is properly positioned and alive. This SCFS assay has enabled the quantification of cell-substrate and cell-cell adhesive forces of staphylococci at the whole cell level. Lastly, the structural and biophysical properties of living cells have been mapped at unprecedented resolution, using newly developed multiparametric imaging (Alsteens et al., 2012; Heu et al., 2012; Alsteens et al., 2013; Chopinet et al., 2013; Dufrêne et al., 2013; Formosa et al., 2015). Force curves are recorded across the cell surface at high frequency, enabling to acquire correlated images of the structure, adhesion and mechanics of cells, including staphylococci, at much higher speed and spatial resolution than before. Here, we provide a survey of recent breakthroughs made in staphylococcal research using these modalities.

2. Fibronectin-binding proteins

There has been much progress in our use of AFM for studying the binding mechanisms of staphylococcal adhesins. Among these, fibronectin-binding proteins (FnBPs) have been the most widely investigated (Bustanji et al., 2003; Xu et al., 2008; Buck et al., 2010; Lower et al., 2010; Casillas-Ituarte et al., 2012). In early work, Bustanji and co-workers used SMFS to study the strength and dynamics of the interaction between single fibronectin (Fn) molecules and living *S. epidermidis* cells (Bustanji et al., 2003). The strength of single Fn-FnBP bonds was found to be ~100 pN and varied with the loading rate, as expected for specific receptor-ligand bonds. Dynamic SMFS data were consistent with macroscopic observations showing that Fn-dependent bacterial infections are influenced by the blood velocity. Surprisingly, several studies have reported much larger adhesion forces for Fn-FnBP interactions, up to 6 nN depending on the strain investigated (Xu et al., 2008; Buck et al., 2010; Casillas-Ituarte et al., 2012). This can be explained by the fact that Fn was attached at high density on the tip, meaning multiple Fn-FnBPs bonds were probed in parallel. It is therefore important to control the tip chemistry for proper interpretation of SMFS data. In a clinical context, the activity of the transcription factor SigB was shown to promote strong Fn-*S. aureus* bonds, an effect suggested to help host tissue colonization by small-colony variants isolated from cystic fibrosis patients (Mitchell et al., 2008). Fn-FnBP adhesion forces for *S. aureus* clinical isolates were consistent with a multivalent bond consisting of multiple proteins in parallel, and the bond lifetime was longer for bloodstream isolates from patients with an infected device (Casillas-Ituarte et al., 2012). These isolates showed a distinct force signature and had specific single amino acid polymorphisms in FnBP proteins (Lower et al., 2011). Molecular dynamics simulations revealed that three residues in the protein form extra hydrogen bonds with Fn.

Another important role of FnBPs is the promotion of cell-cell adhesion during biofilm formation, particularly in clinically-relevant methicillin-resistant *S. aureus* (MRSA) strains. Until recently, an unsolved question was whether intercellular adhesion involves direct homophilic interactions or recognition of receptors on adjacent cells. By combining SMFS and SCFS, Herman-Bausier et al. (2015) showed that low-affinity homophilic bonds between FnBPA A domains on neighboring cells mediate cell-cell adhesion. Homophilic binding required the presence of zinc, in agreement with earlier studies showing that FnBPs and other staphylococcal adhesins mediate zinc-dependent adhesion. Low-affinity binding may be of biological significance, providing a means to the bacteria to detach and colonize new sites during biofilm formation. Such homophilic cell-cell interactions could represent a widespread strategy among staphylococci to favor biofilm accumulation.

Besides providing novel insights into the FnBP binding forces, spatially-resolved force spectroscopy has revealed the distribution of single FnBPs on staphylococcal cells. In a first study, the localization of putative FnBP proteins was studied on *S. aureus* bacteria deposited on different substrates (Lower et al., 2010). The authors suggested that the production of FnBPs may be triggered by external stimuli, such as the presence of Fn on a surface. More recently, SMFS with tips functionalized with recombinant FnBP domains was used to explore the distribution of FnBPA proteins on *S. aureus* cells, showing that the adhesin was largely exposed on the cell surface, i.e. with a surface density of ~2000 proteins/μm^2 (Herman-Bausier et al., 2015).

3. Serine-aspartate repeat proteins

The serine-aspartate repeat (Sdr) proteins have also received considerable attention. A hallmark of such adhesins is the *S. epidermidis* SdrG protein which binds with high affinity to the blood plasma protein fibrinogen (Fg) *via* the "dock, lock, and latch" (DLL) mechanism involving dynamic conformational changes (Ponnuraj et al., 2003; Bowden et al., 2008). Because this interaction promotes bacterial attachment to Fg-coated biomaterials, it is thought to play an important role in infections. SCFS revealed that SdrG mediates time-dependent attachment to Fg-coated surfaces, suggesting that stable cell adhesion requires conformational changes (Herman et al., 2014; Fig. 2). The strength of single SdrG-Fg bonds measured by SMFS was ~2 nN, thus much larger than that of other cell adhesion proteins, which is typically in the 50–400 pN range depending on the protein and on the loading rate (Fig. 2). Dynamic SMFS revealed a low dissociation rate and suggested that the SdrG-Fg bond is stable. These findings favor a

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