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Analysis of bacterial surface interactions using microfluidic systems

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ABSTRACT

Modern microbiological research has increasingly focused on the interactions between bacterial cells and the surfaces that they inhabit. To this end, microfluidic devices have played a large role in enabling research of cell-surface interactions, especially surface attachment and biofilm formation. This review provides background on microfluidic devices and their use in biological systems, as well specific examples from current literature. Methods to observe and interrogate cells within microfluidic devices are described, as well as the analytical techniques that are used to collect these data.

Keywords: *microfluidics, bacteria, measurement, analysis, fluid, microchip, adhesion, biofilm*

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1. Introduction

1.1 Bacterial surface interactions

Since the advent of modern microbiology, most research on bacteria has focused on cells in liquid culture or on agar-based surfaces. Although these studies have certainly advanced our understanding of bacterial physiology and metabolism, they do not yield an accurate picture of bacterial behaviour in their natural environment. In natural systems, many bacteria tend to associate with surfaces, either through direct cell-surface adhesion, or by association with surface-anchored biofilms. Individual cell-surface interactions are governed by a wide range of factors, including extracellular structures (flagella, pili, adhesion proteins) and the physical and chemical properties of the surface (roughness, polarity, topography)[1–4]. Bacteria may also participate in single- and multi-species biofilms, which are essentially aggregates of bacteria and associated extracellular polymeric substances (EPS)[5]. Biofilms are typically initiated by attachment and aggregation of individual cells, followed by growth and maturation of a thick, viscoelastic biofilm superstructure. Biofilms have been implicated in persistent bacterial infections[6] mainly due to their unique mechanical and biochemical properties. Further, bacterial behaviour within biofilms differs greatly from that in well-mixed liquid culture, yielding novel survival strategies and even enhanced antibiotic resistance[7,8].

Surface attachment and biofilm formation have been associated with many pathogenic bacteria, as well as industrially and environmentally important bacteria. For these reasons, it is important to understand the physical and chemical parameters that govern these interactions, especially in systems that recapitulate the natural environments of these organisms. These parameters include surface structure, surface chemistry, dynamic fluid flow, nutrient and metabolite flux, temperature, and dissolved gas concentration/composition. One approach to controlling these parameters is to use flow-based fluidic systems. Biofilm experiments are commonly performed in various fluid flow chambers, ranging from parallel-plate systems to drip-flow reactors. These systems are able to control the fluid composition (including both chemical and gas) and the fluid flow parameters (velocity, viscosity and shear). This is extremely important in surface attachment and biofilm formation studies, since it has been shown that both attachment and biofilm formation are significantly affected, and even enhanced, by the presence of dynamic fluid flow[9].

By further reducing the dimensions of these systems to the micrometer length scale, additional fluid flow parameters can be controlled, and the entire scale of the experiment can be reduced (including the volume of media and reagents which are needed). These micrometer scale devices, collectively termed ''microfluidic'' devices, have unique properties as compared to larger-scale fluid flow systems. Due to their small size, fluid flow behaviour changes drastically, typically moving from the turbulent to laminar flow regime, which has a direct impact on shear forces and mixing at the surface. Further, microfluidic devices can be easily multiplexed (to perform many experiments in parallel) and can be incorporated with unique imaging and analysis techniques, such as confocal microscopy or atomic force microscopy. The purpose of this review is to explore the use of microfluidic devices in the study of bacterial surface interactions and how these devices can enable novel experimental strategies.

1.2 Microfluidics

1.2.1 Fluid flow in microfluidic devices

Microfluidic devices were first introduced in the 1990s and have quickly found utility in a wide range of chemical and biological applications[10–12]. These devices consist of a small channels (typically 100 micrometres or smaller in one dimension), which are used to manipulate samples, deliver reagents, or perform reactions in microlitre or smaller volumes. The advantages of using microfluidic devices include lower reagent consumption, faster reaction times, and greater ease of monitoring. In addition, critical parameters such as fluid flow velocity (and resulting shear forces) can be easily controlled by altering the fluid flow rate. Fluid flow profiles can also be controlled in microfluidic devices to create gradients of velocity and chemical composition. This ability to control velocity, shear and gradients enables the researcher to mimic physiological or environmental conditions which are relevant to the organisms being studied. This is far different from traditional test-tube or Petri dish based experiments that have limited relevance to natural conditions.

What is also unique about microfluidic devices is the way in which fluid flows in channels with micrometer-scale cross-section. In large cross-section channels with moderate flow conditions, fluids tend to flow turbulently, which means that there is induced physical agitation of the fluid. In micrometer scale devices, fluids

tend to exhibit laminar flow characteristics, in which there is no active mixing and diffusion-based mixing becomes dominant. In fluid mechanics, the flow behaviour of fluids can be described by the Reynold's number (R_e), a unitless measurement which takes into account the fluid density (ρ), flow velocity (V), hydraulic diameter (L), and dynamic viscosity (μ):

$$R_e = \rho VL/\mu$$

Reynold's numbers of 1000 and higher are typical for turbulent flow conditions, while those below 1000 are typical for laminar flow conditions. In most microscale systems, extremely high fluid velocities (and therefore flow rates) would be required to establish turbulent flow conditions. Thus fluid flow in microfluidic devices is primarily laminar, and the mixing of fluids within them is primarily limited by diffusion. This provides an additional level of control, and offers the ability to form chemical gradients within the channels[13]. Another important property is the fluid shear stress, which is the amount of force applied tangentially or parallel to the face of a material. Shear stress at the wall of a fluid channel may be calculated using the Poisseuille model through the following relationship, where Q is volumetric flow rate, h is channel height, and w is channel width:

$$\tau = -\left(\frac{12Q\mu}{h^2 w}\right)$$

1.2.2 Fabrication of microfluidic devices

The first generation of microfluidic devices were mainly fabricated in silicon and glass substrates using technology generated by the semiconductor industry, such as photolithographic patterning and etching. Investigations into unconventional substrate materials for biocompatible microfluidics led to interest in ceramics and hydrogels. To meet the demand for cheaper, more versatile alternatives, however, researchers began to explore the use of polymeric materials in microfluidic technology. The most commonly used method is replication, which involves methods such as hot embossing, injection moulding, and casting to transfer a pattern from a precision template or master to a polymer substrate. This master mould can be made from a variety of different materials: glass, silicon, metals, and more recently, high-aspect-ratio photoresists. The mould can then be used to hot emboss[14] or injection mould[15] devices, although physical casting is by far the most

widely used technique in the academic world. Casting involves pouring a non-crosslinked polymeric material over a moulding template, followed by crosslinking/curing, after which a soft elastomeric copy can be peeled off the mould[16]. Currently, the most popular material for casting is the silicone-based polymer, polydimethylsiloxane (PDMS)[17]. Many commercial devices are made from polymers such as polycarbonate (PC) and polymethylmethacrylate (PMMA), however, PDMS remains the most widely used casting material for research applications[18]. The use of photolithographically patterned moulds to form PDMS devices is often referred to as ''soft-lithography''[19]. An example of soft-lithography based fabrication is shown in Figure 1.

All microfluidic devices require a tight bond or seal of the channel or chamber to form an enclosed structure, and a variety of material-dependent techniques have been used to achieve reliable containment of the sample fluid. For PDMS-based microfluidic fabrication, oxygen plasma is commonly used to activate the PDMS surface. When two activated surfaces are brought in close proximity, electrostatic and covalent bonds can be formed, which

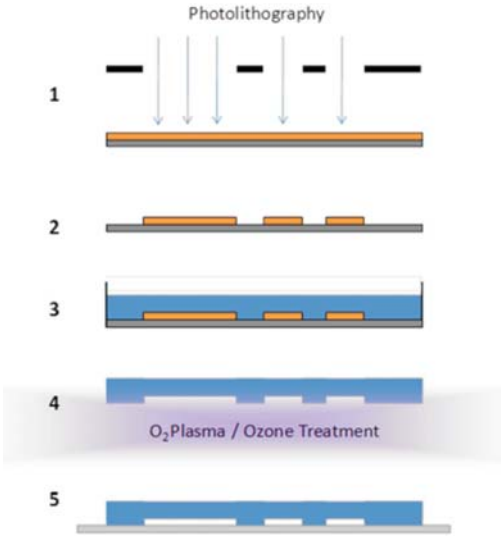

Figure 1 *Soft lithography technique for fabricating microfluidic devices.* (**1**) *Photolithographic patterning of negative photoresist,* e.g. *SU-8.* (**2**) *Resulting pattern formed in photoresist.* (**3**) *Moulding of soft polymer material,* e.g. *polydimethylsiloxane, to form microfluidic channels.* (**4**) *Plasma or ozone based treatment to prepare polymer surface for bonding.* (**5**) *Moulded polymer bonded to solid substrate,* e.g. *glass or plastic.*

are capable of withstanding high pressures. Other bonding methods include lamination, thermal bonding, ultrasonic welding, and the use of adhesives. PDMS makes an excellent fluidic seal against smooth materials (such as glass) and does not require adhesive to create an enclosed fluidic system. PDMS can also withstand high temperatures and a range of solvents, making it easy to clean and sterilize for use with bacterial cell cultures and enables experimentation under a wide range of conditions. An example of a PDMS-based microfluidic device, fabricated in the authors' laboratory is shown in Figure 2.

Fluidic attachments to microfluidic devices can be easily made by attaching polymeric or stainless steel tubing which can form a pressure-tight seal, or can be glued in place with epoxy-based adhesives[20–22]. Fluids can then be delivered to devices using a variety of methods. The most common approach is to use commercially available syringe or peristaltic type pumps. Depending on the type of pump and the size of the syringe/tubing that is used, flow rates can controlled over a range of nanolitres to microlitres per minute. Other pumping strategies include thermopneumatic[23,24], electrostatic[25–27], piezoelectric[28–30], electromagnetic[31–33], and hydrogel[34,35] based actuation. Some of these pumping systems focus on controlled direction and delivery of micro- and nanolitre solutions over long periods of time, while others seek to achieve high pumping volumes at low power.

Other considerations for microfluidic devices are the ability to control fluid flow direction and mixing within the device. Figure 3

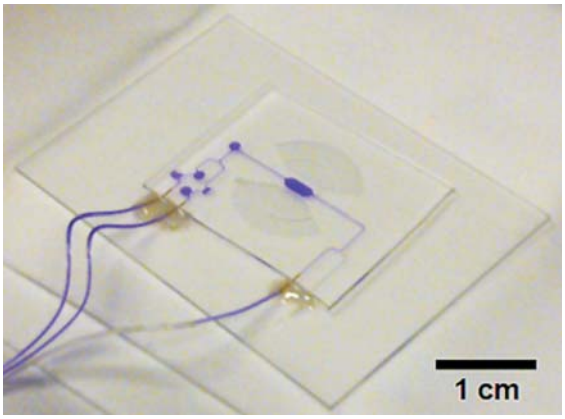

Figure 2 *A microfluidic device fabricated using soft lithography techniques. Purple dye was pumped through the device for contrast.*

shows a relatively simple microfluidic setup with in which fluid is delivered via syringe pump, and flow direction is controlled manually. To control fluid direction in more complex systems, where multiple channels are present, many devices incorporate microfluidic valves. Typical valves at the macro-scale use hydraulic, pneumatic, manual, or solenoid activation[36], most of which can be easily rescaled to microscale systems. Many of the actuation mechanisms and methods employed for microvalve construction draw upon the same principles used by microfluidic pumps[37–40]. These include microfabricated mechanical valves, temperature-control of paraffin[41], polarity/hydrophobicity controlled valves[42], microbubble valves[43], and thermally-responsive polymers[44]. As described previously, fluids in the laminar flow regime only mix by diffusion, unlike the chaotic mixing observed in turbulent systems. This can be an advantage when establishing controlled chemical gradients within a device, but can also be a problem when active mixing is needed. Mixing in microfluidic devices has been the subject of many research efforts and is too broad to be adequately covered in this review. Most mixing approaches involve either mechanical agitation, such as the use of piezo-electrics[29], gas bubbles[45,46], or magnetic microspheres[47]. Passive mixers have also been demonstrated to reduce diffusion lengths

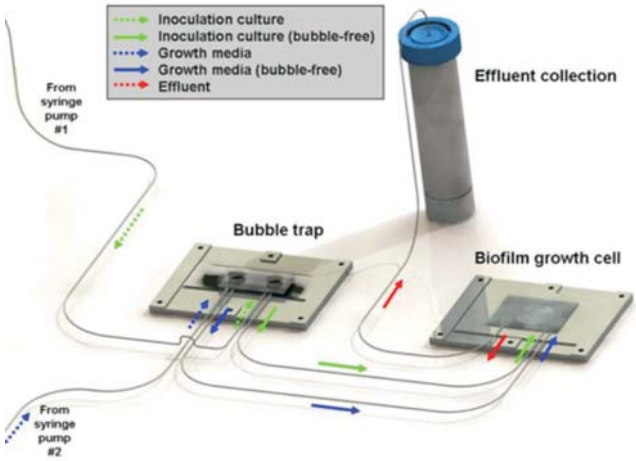

Figure 3 *An example of a microfluidic system for growing bacterial cells and biofilms. Fluid control is provided by external pumps and waste media is collected in an effluent collection tube. Note the use of a bubble trap component which is used to prevent bubbles from entering the growth chamber.*

through increased surface area and the creative manipulation of fluids by the positioning of special microfabricated structures[48–50].

2. Applications of microfluidics for studying bacterial surface interactions

The majority of bacteria in nature are associated in some way with a surface or interface, either as individual cells or as part of a biofilm consortia. Surface-adhered bacteria and the biofilms that often result have been implicated human pathogenesis, surface corrosion, and fluid system contamination. The adhesion characteristics and mechanical properties of both individual cells and surface associated-biofilms are known to be directly determined by the fluid and substratum physical and chemical properties, and are thus particularly good subjects for studies involving microfluidics. Formation of biofilms is unavoidably linked to, and often the direct the result of, the initial attachment of individual cells to a surface. For this reason many researchers investigating the formation of biofilms as pertaining to surface and fluid properties, report on the adhesion and mechanics of both single cells at the initial attachment phase, as well as of the resulting bulk biofilm. For the reasons stated previously, microfluidic devices are uniquely suited to the study of these surface interactions in both the single-cell and multi-cell regime.

2.1 Adhesion of single cells

Microfluidic systems have been employed by researchers in a variety of methods to study cell-surface interactions. The most common method is the use of microfluidic flow to deliver cells to a surface that has been physically or chemically modified while maintaining physiologically relevant shear forces. Subsequent surface adsorption/desorption of cells resulting from variations in culture preparation, surface treatment, and fluid shear is then determined indirectly by observing cell behaviour during fluid flow using passive imaging techniques such as brightfield optical microscopy, videomicroscopy, and confocal microscopy, often with the aid of fluorescent markers or dyes. Additional characterization of adhered cells, such as visualization of cell surface structure, appendages, and localization of ligands and membrane proteins, may be obtained by removal of colonized surface samples from the fluidic environment and imaging by atomic force microscopy (AFM) or scanning electron microscopy (SEM). In addition to

removal from the native liquid environment, these active imaging techniques often involve the drying, fixing or treatment of the sample, and hence, are not always indicative of the properties of living samples as existing *in situ*. The transparent nature of many microfluidic devices allows for the imaging of surface adhered cells *in situ* and in real-time during experimentation. These methods of examining single-cell surface adhesion through the passive observation of cells subject to fluid flow in a microfluidic device are straightforward and achievable with commonly available lab instrumentation; however, they are frequently indirect and qualitative in nature. Quantification of cell adhesion strength is generally achieved by determining a critical fluid shear required to remove cells from a surface under a given set of experimental parameters[51]. Shear forces are assumed based upon the results of simple fluid dynamics calculations, computational fluid dynamic simulations, or particle-flow measurements. Several representative studies from the recent literature employing microfluidic to study single-cell adhesion of bacteria in response to varying surface topography, fluid shears, or fluid chemistries are reviewed here.

Wang *et al*. reported on the adhesion of *Escherichia coli* cells to micro- and nano-textured surfaces formed by aluminium-induced crystallization of amorphous silicon. They found that nanopatterned surfaces with features predominantly less than $1\,\mu m^2$ exhibited higher cell attachment than micropatterned or smooth surfaces. They further investigated the method of attachment (flagellar *versus* cell body) by observing cell rotation following adhesion and correlating to the presence of engineered surface features. More recently Weaver *et al*.[52] reported on the adhesion of *Staphylococcus epidermidis* to human fibrinogen-coated surfaces under varying fluid shear forces. They found that adhesion of individual colonies is highest at low wall shear stress ($1\,dynes/cm^2$) and decays with increasing fluid shear, whereas clusters of colonies exhibit greatest adhesion at medium shear ($10\,dynes/cm^2$). Their findings suggest that increased cell-cell adhesion strength may be triggered by a critical fluid shear, a phenomenon previously unreported. In order to investigate the effect of fluid chemistry, specifically dissolved oxygen concentration, on bacterial attachment and growth, Skolimowski *et al*.[53] devised a novel microfluidic device utilizing culture chambers in conjunction with an oxygen scavenging liquid to create precise oxygen gradients. The device takes advantage of the gas-permeable nature of PDMS to allow dissolved oxygen to be drawn through the device from the culture solution to the scavenging liquid. The

ability of *Pseudomonas aeruginosa* PAO1 to attach and colonize the surfaces of the device was then correlated to predicted and measured oxygen concentration, and it was found that in regions of low oxygen, *P. aeruginosa* attachment was severely diminished, as was subsequent biofilm growth. The device created by Skolimowski *et al*. offers promise as an accessible platform to investigate the effect of dissolved gasses on the attachment and growth of bacteria.

Microfluidic attachment studies have very recently been employed to probe the role of various cell appendages in surface colonization and motility. The role of type I and type IV pili in surface attachment by *Xylella fastidiosa* was studied by De La Fuente *et al*.[51]. The fluid shear required to remove wild type cells and mutants deficient in one or both pili types was measured. They observed an interesting interplay between the adhesion strength of each pili type, and the length and concentration of those pili on the cell surface. Bahar *et al*.[54]. expanded on this study, investigating attachment, twitching motility, and subsequent biofilm forming ability of *Acidovorax citrulli*. The behaviour of *A. citrulli* mutants deficient in production of type IV pili or polar flagellum was compared to that of wild type strains. It was determined that the presence of functional type IV pili was crucial for the attachment, surface twitching, and biofilm formation, whereas the presence of polar flagella was not. In addition to bacterial studies, experiments involving microfluidics have been employed to investigate the attachment behaviour of eukaryotic mammalian cells, such as human fibroblasts[55,56], and glial cells[57].

Many other methods have been employed to investigate the cell-surface adhesion forces of individual bacteria in static fluid environments, including fluid gauging, micromanipulation, microcantilevers, and optical trapping. Thorough discussion of these methods is beyond the scope of this review and has been discussed elsewhere[58]. Arguably the most powerful and promising analytical technique for quantification of cell-surface or cell-cell adhesion is force spectroscopy, performed by an atomic force microscope (AFM). Cells may be fixed (either to a substrate or the AFM probe itself) and the force of adhesion, between cell and probe or probe/cell complex and surface, directly measured by retracting the probe[59–62]. This method offers high spatial and force resolution and may be performed in static liquid environments. Until very recently, it has not, however, been incorporated into fluidic systems to provide *in situ* measurement of cells adhered and cultured under flow conditions.

2.2 Mechanical properties of single cells

Recent research pertaining to mechanics of single bacterial cells has generally focused on the nano- and microscale behaviour of individual surface structures and proteins[63,64]. Studies utilizing microfluidics to investigate single-cell mechanical properties are few. The majority found in the literature involve the mechanical characterization of eukaryotic cells, such as studies to determine the mass of HeLa cells[65], the morphological and physiological effect of fluid shear on human endothelial cells[66] and of the protozoan *Vorticella*[67]. These cell types are generally soft and show noticeable physical deformation under physical shear or chemical stress. In contrast, the cell wall renders individual bacterium mechanically robust, and while physical stiffness of a cell may change in response to fluid environment, the study of the mechanical properties of individual bacterium within a fluid flow devices has been limited.

2.3 Biofilms

A natural extension of single-cell bacterial adhesion and mechanics studies involves investigation of biofilms, as growth of a biofilm is often the result of single-cell surface attachment. Traditionally, biofilms grown under flow conditions have been generated in the laboratory using macroscale devices such as parallel plate flow chamber and rotating disc reactors[68]. These have been successful in granting insight into the behaviour of biofilms, however, do not always allow for direct measurement of morphology, mechanics, or film-surface adhesion; and when they do, it is often through a single, destructive, *ex situ* measurement performed at the end of the experiment. Microfluidics have been recently employed to obtain continuous, *in situ*, high-throughput measurements of bacterial biofilms grown under flow. Figure 4 shows an example of biofilms grown in microfluidic systems, as imaged by confocal microscopy and atomic force microscopy.

2.3.1 Biofilm surface adhesion

The ability of bacteria to form biofilms and the properties of those films are known to be highly dependent on the initial adhesion of single cells to a surface[69–71]. Many researchers investigating the particulars of single cell attachment to solid surfaces within flow systems continue their experiment and allow surface-adhered cells

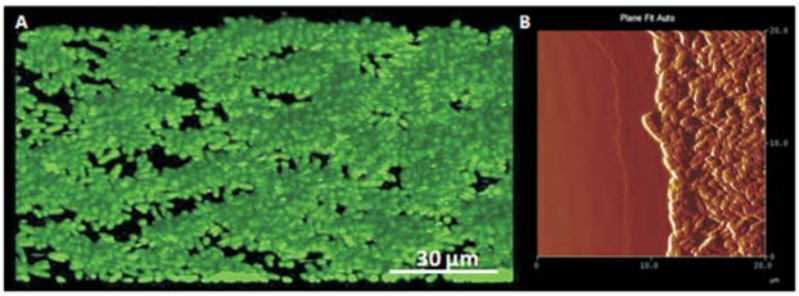

Figure 4 *Images of bacteria and biofilms grown in microfluidic systems.* (**A**) *Three dimensional reconstruction of cells/biofilm obtained using confocal microscopy.* (**B**) *Topographical reconstruction of cells/biofilm using atomic force microscopy (AFM).*

to produce biofilm. Imaging by traditional light microscopy, confocal microscopy, AFM, or SEM is then used to qualitatively assess film quantity and morphology, and correlations drawn to experimental parameters and/or the behaviour observed at initial cell adhesion stage[51,53,54,72–74]. For example, Lee *et al*.[75] developed a PDMS microfluidic device to study the effect of the enzyme dispersin B and the antibiotic rifampicin on the release of *Staphylococcus epidermidis* biofilms. Dispersin B has previously been shown to be effective at degrading the EPS matrix of *S. epidermidis* biofilms under static fluid conditions[76]. Lee *et al*. observed biofilm detachment by imaging colonized surfaces within their device via light microscopy and SEM, and also gathered indirect quantitative measurements of released bacterial cells by performing CFU counts of the fluid effluent from the device.

A few studies have used microfluidic devices to focus more directly on the interaction of grown biofilms to solid surfaces. For example, Rupp *et al*.[77] observed that the compliant viscoelastic nature of *S. aureus* biofilms allow them to resist surface detachment due to applied fluid shear, and that biofilm clusters exhibited a rolling migration behaviour which appeared to be controlled by viscoelastic tethers.

2.3.2 Biofilm mechanics

Bacterial biofilms have unique viscoelastic properties that dictate their response to shear stress environments[77–81]. These viscoelastic properties allow biofilms to survive a variety of externally applied

stresses such as turbulent and high-shear fluid flow. Micro-scale rheological studies have shown that biofilms formed by a wide range of microorganisms have certain universal properties including a common viscoelastic relaxation time[82]. This relatively long relaxation time (18 min) is associated with resistance to surface detachment, as well a type of rolling migration that allows biofilms to move along surfaces[77,82]. Studies have also shown that biofilms grown under high shear conditions are more strongly adhered to surfaces and have increased mechanical strength over those grown under low shear conditions[80]. These results suggest that biofilms can respond to environmental stresses and alter their mechanical properties, accordingly. Hence, a broad, thorough understanding of the physical properties of biofilms as a function of their environmental stimuli is required for future applications including remediation or removal.

Performing mechanical measurements on biofilms is not a simple task. Mechanical properties have been measured with conventional materials testing tools, such as microindentation devices[83,84] as well as specialized rheometric devices[85], flow cells[77,80,81,86], and, to a limited extent, AFM[87–89]. Each technique provides unique physical characterization of biofilms, but not all methods yield insightful information under natural conditions or are universally adaptive to measuring response to external stimuli. For instance, microindendation and rheometric studies yield bulk properties of biofilms, but do not yield data under fluid flow or dynamic changes in environmental conditions. Additionally, these methods do not provide single-cell resolution which can be critical for evaluating EPS cohesion/adhesion. In contrast, AFM-based measurements can obtain localized mechanical data, but most studies have been performed on dehydrated or static fluid samples. These measurements do not reflect the properties of biofilms in natural environments or enable exposure to specific dynamic or chemical stimuli. Microfluidic flow cell measurements have provided intriguing data regarding biofilm properties under dynamic flow, but these methods do not directly measure mechanical properties[77,80,81,86,90]. Since biofilms exist in complex, dynamic fluid environments, it is essential to develop techniques to directly measure their mechanical properties within these environments.

In recent years, several notable studies providing quantitative mechanical analysis of bacterial biofilm utilizing microfluidics have been published. Hohne *et al.*[90] developed a novel microfluidic system to rheometrically determine both the steady-state elastic and viscoelastic relaxation properties of biofilm by using air

pressure to deform a PDMS membrane within the device on which the biofilm was grown. Finite element analysis (FEA) was used to model the combined PDMS/biofilm membrane and predict deformations resulting from applied air pressure to the back of the membrane. Their system was shown to be effective in measuring Young's moduli of soft viscoelastic material in the range 10^2–10^5 Pa.

Characterizing the mechanical properties of biofilms in a dynamic fluid environment opens vast new opportunities for more complex analyses. For example, bacteria existing in biofilms respond to intracellular signalling and external chemical stimuli. Silver ions[91] and iron salts[92] have been shown to disrupt or perturb *Staphylococcus* spp. and *Pseudomonas aeruginosa* biofilms, respectively. These compounds could have multiple effects, including electrostatic disruption of intermolecular adhesion forces (for silver ions) and repression of genes that are essential for biofilm formation (iron salts). Intercellular communication, as part of the phenomenon known as ''quorum sensing,'' has also been shown to affect biofilm formation and biofilm properties. Quorum sensing is a method by which cells regulate gene expression in response to local cell concentrations. Typically, one or more diffusible signal molecules, termed autoinducers, are produced and excreted into the environment. Once significant levels of these signalling molecules have been established (through limited diffusion, or by increases in population density), cells respond by altering gene expression. This mechanism of gene regulation has been shown to directly impact a wide range of cell behaviours, including pathogenicity, lifestyle (free-swimming to attached), and development of biofilms[93]. The well-studied biofilm forming bacterium, *Pseudomonas aeruginosa*, utilizes at least two quorum sensing pathways, which have been observed to dramatically affect both biofilm formation and the properties of the biofilm. Davies *et al*. have shown that *P. aeruginosa* lacking the *lasI* quorum sensing signal ($3OC_{12}$-HSL) produce biofilms that are flat and undifferentiated, and are sensitive to biocides and detergents[94]. These results indicate a direct role of quorum sensing in successful biofilm formation. Purevdorj *et al*. showed that quorum-sensing deficient *P. aeruginosa* can form biofilms under fluid flow conditions, but that the structure of these biofilms is significantly different from wild-type *P. aeruginosa*[80]. Quorum sensing may also be involved in regulating polysaccharide production, which is a critical component of the EPS matrix[95,96]. By genetically controlling the type and relative abundancies of alginate and glucose-rich poly-

saccharides, *P. aeruginosa* may therefore be able to alter the mechanical properties of their biofilms[96]. These important studies indicate that chemical cues in the liquid environment and intercellular signalling can greatly affect biofilm physical properties. Extending these advances and enabling improved, spatially-resolved and quantitative understanding of biofilm responses requires implementation of a novel measurement technology that can perform mechanical measurements (viscoelastic, elastic, and adhesive) while simultaneously altering the dynamic and chemical environment surrounding the biofilm. The implementation of such an approach is a prime focus of our work on the development of an integrated AFM/confocal microfluidic system for the growth and analysis of bacterial biofilms (Figure 5).

To this end, we have developed PDMS microdevices (manuscript submitted) that have the unique ability to allow for the growth of bacterial biofilms and *in situ* analysis by AFM and confocal microscopy under dynamic fluid conditions. A dilute cell culture is used to inoculate the device. Following cell attachment to the glass floor, sterile media is flowed through to encourage the growth of biofilm within a central reactor chamber measuring approximately 2 mm wide, 10 mm long, and 100 μm high (which is shown in Figure 2, above). At all times, the cell activity within reactor

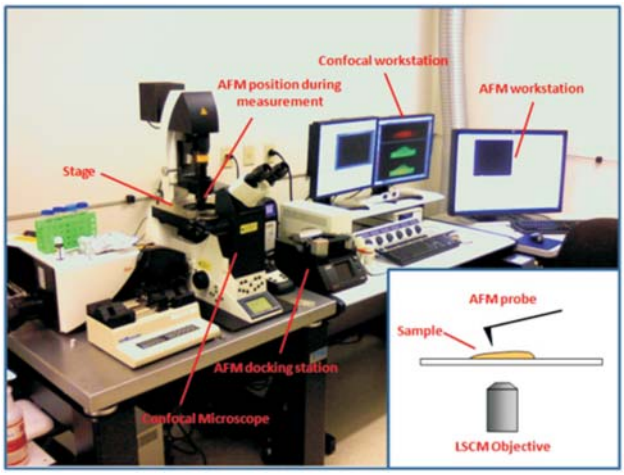

Figure 5 *Integrated atomic force microscope (AFM)/laser scanning confocal microscope (LSCM) for dual mechanical and optical interrogation of samples. The inverted LSCM performs optical imaging of samples in microfluidic devices, while the AFM can be used to make mechanical measurements (adhesion, modulus* etc.*) on cells or biofilms within the devices.*

chamber may be observed through the glass by inverted optical or confocal microscopy. Additionally, at any point a portion of the PDMS device roof may be removed to allowing the sample to be analyzed by AFM force spectroscopy. A key advantage of this system is its ability to maintain control over fluid conditions at all stages of film growth (cell attachment, film maturation, and dispersal), as well as both prior to and during AFM analysis. We have performed characterization studies confirming the ability of the system to precisely measure the elasticity of reference materials (PDMS of varying preparations and polyacrylamide hydrogels) and grown *Pseudomonas aeruginosa* biofilms.

3.0 Summary and outlook

Microfluidic devices are powerful platforms for exploring the interactions between bacterial cells and the surfaces that they inhabit. This review has attempted to summarize the most current methods of fabricating these devices and their use in answering basic questions about the physical, chemical and biochemical nature of cells at this interface. Importantly, microfluidic systems offer a unique approach towards recapitulating environmental or physiological parameters in a tractable, laboratory-scale system. Although these devices cannot reproduce exact replicas of natural systems, they offer an unprecedented level of control over fluid dynamics, chemical concentrations, gradients, and surface exposure. Understanding how these individual parameters affect cellular behaviour, either individually, or with consortia, is paramount to developing mitigation strategies for disease or retention strategies for biotechnological applications. Perhaps beyond the scope of this review is the future use of microfluidics for highly-parallel, high-throughput analyses. Microfluidics have made a huge impact in the fields of analytical chemistry and biochemistry, and have spurred the commercialization of multiple instruments for the pharmaceutical and biomedical industries. There is little doubt that microfluidics have also made a large impact on microbiological research and that they will continue to play a role in the years to come.

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