Understanding the molecular interactions of lipopolysaccharides during \textit{E. coli} initial adhesion with a surface forces apparatus

Qingye Lu, Jing Wang, Ali Faghihnejad, Hongbo Zeng* and Yang Liu*

Molecular and surface interactions between two model lipopolysaccharides (LPS1 and LPS2) extracted from \textit{E. coli} strains JM109 and K12 and various solid substrates were measured using a surface forces apparatus (SFA) and a quartz crystal microbalance with dissipation (QCM-D).

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Understanding the molecular interactions of lipopolysaccharides during *E. coli* initial adhesion with a surface forces apparatus

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Lipopolysaccharides (LPS) occupy 75% of the surface of Gram-negative bacteria. This work investigates the role of LPS during bacterial adhesion to solid substrates. Two model lipopolysaccharides, LPS1 and LPS2, were examined. LPS1 from *E. coli* JM109 has a full LPS chain consisting of lipid A, core polysaccharides, and O-antigen; LPS2 from K12 has a truncated chain without the O-antigen portion. Interactions between an LPS layer prealigned on polystyrene (PS) and three different substrates (mica, PS-coated mica, and 3-aminopropyltriethoxysilane (APTES)-functionalized mica) in 0.1 M NaCl were measured using a surface forces apparatus (SFA). The PS-supported LPS showed strong adhesion to APTES, weak adhesion to mica, and strong repulsion to PS substrate. Electrostatic interaction and steric effects contribute significantly to the interactions between the LPS and different substrates. The presence of long O-antigen chains in LPS1 reduces bacterial adhesion to various substrates because of the presence of an energetic barrier during the adsorption process, which is caused by the affinity of hydrophilic neutral O-antigen chains to water and the steric entropic barrier of LPS chains on the cell membrane surface.

1. Introduction

Bacterial adhesion to solid surfaces is the first and essential step for bacterial colonization and formation of a biofilm. Understanding bacterial initial adhesion is very important for the control and application of bacterial adhesion and biofilms in the engineering, environmental, and biomedical industries. It is believed that bacterial adhesion is dependent on various intermolecular and surface interactions such as electrostatic, van der Waals, hydrophobic forces, hydrogen bonding and covalent bonding. These forces are influenced by the physicochemical properties of the substratum and the bacterial surface. The molecular mechanism of bacterial adhesion is complicated and remains unclear.

Cell surface structures such as lipopolysaccharides (LPS), extracellular polymeric substances (EPS), and bacterial nanofibers contribute to bacterial adhesion. LPS occupy 75% of the surface of Gram-negative bacteria and may play a large role in cell interactions with solid substrates. LPS are comprised of lipid A, core oligosaccharides, and the O-antigenic polysaccharide (see Fig. 1). LPS are anchored in the outer membrane of the cell by lipid A which contains hydrophobic saturated fats and negatively charged phospholipids. The core region of LPS contains approximately ten monosaccharides, which often carry phosphate and carboxylic acid groups. The O-antigen portion of LPS contains repeating units of one to eight monosaccharides. The hydrophilic portions from the core to the O-antigen extend up to 40 nm from the cell surface into the surrounding aquatic environment and mediate bacterial binding to surfaces.

![Fig. 1 Schematic of the LPS structures used in this study.](image-url)
Studies of the impact of LPS on cell adhesion and transport, especially for *E. coli*, have led to controversial results based on the approach used. Macroscopic adhesion experiments estimate the number of attached cells and provide qualitative evaluations of bacterial adhesion efficiency. Atomic force microscopy (AFM) measures intermolecular interaction forces between bacteria and substrates to determine interaction mechanisms. Three *E. coli* bacteria with various LPS chain lengths (JM109: full LPS chain; D21: LPS without O-antigen; D21f2: truncated LPS chain containing only lipid A and KDO (3-Deoxy-D-manno-oct-2-ulosonic acid) groups) have been studied. Some studies showed that bacterial adhesion capacity increased with LPS chain length (i.e., JM109 > D21 > D21f2) on negatively charged hydrophilic surfaces, while others presented a different relationship as (JM109 > D21f2 ≥ D21). It has also been reported that a long O-antigen layer decreases bacterial adhesion to hydrophobic surfaces. In another study, adhesion of JM109 to hydrophilic negatively charged surfaces decreased after LPS was removed with EDTA. To elucidate the molecular interaction mechanism and predict bacterial adhesion, several groups performed AFM measurements on intermolecular interaction forces between the AFM tip (a probe used to scan specimen surfaces) and isolated bacteria, between a bacteria-coated AFM tip and different substrate surfaces, or between an AFM tip and a bacterial lawn. Adhesion forces were observed to be positively correlated with LPS chain length. It was reported that hydrogen bonding dominates adhesion of bacteria expressing O-antigen, and electrostatic and hydrophobic forces dominate adhesion of bacteria without O-antigen expression. However, AFM failed to distinguish LPS length effects in some studies. Identical repulsion forces between bacteria and the AFM Si$_3$N$_4$ tip were obtained for three strains of *E. coli* bacteria (JM109, D21, D21f2). Therefore, the impact of LPS chains during bacterial adhesion is unclear.

The surface forces apparatus (SFA) measures a force (F) as a function of absolute surface separation (D) between two interacting surfaces. The force sensitivity is ~1 nN with an absolute distance resolution of 0.1 nm. Both attractive and repulsive forces can be measured at short- or long-range distances. The SFA technique is similar to using an atomic force microscope (AFM) to measure interactions between an AFM tip and a surface, but the SFA is more suited for measuring surface-surface interactions because the SFA collects information from tens to thousands of molecules that typically determine cell-substrate interactions. In addition, the SFA has been applied to measure lubrication and friction forces, interactions between lipid bilayer membranes, receptor-ligand interactions, and other dynamic interactions and time-dependent interfacial effects.

In this study, surface force measurements were compared with macroscopic bacterial adhesion experiments to investigate the impact of LPS chains during *E. coli* initial adhesion. To facilitate initial adhesion. To facilitate detailed procedure for preparing substrates for SFA experiments has been reported elsewhere. Briefly, a thin and back-silvered mica sheet of 1–5 μm was glued onto a cylindrical silica disk (of radius R = 2 cm) and the exposed mica surface was treated with different chemicals. The LPS layer was coated as follows: firstly, one mica surface was spin-coated with PS and vacuum dried at 23 °C overnight. APS-coated substrates were prepared by exposing freshly-cleaved mica to APS vapor in an evaporation chamber. APS droplets were put in a small glass vial which was placed near a mica substrate in an evaporation chamber. The chamber was first evacuated for a few minutes and then closed for more than three hours to allow surface deposition and reaction of APS with the mica.

Four different surfaces: mica, PS-coated mica, APS-coated mica, and LPS-coated PS were prepared for SFA experiments. A detailed procedure for preparing substrates for SFA experiments has been reported elsewhere. Briefly, a thin and back-silvered mica sheet of 1–5 μm was glued onto a cylindrical silica disk (of radius R = 2 cm) and the exposed mica surface was treated with different chemicals. The LPS layer was coated as follows: firstly, one mica surface was spin-coated with PS and vacuum dried; secondly, 40 μg mL$^{-1}$ LPS in 0.1 M NaCl solution were dropped on the PS-coated mica for more than one hour for deposition in a water vapor saturated chamber; and lastly, the surface was rinsed with 0.1 M NaCl. PS-coated and APS-coated mica surfaces were prepared using the procedures described above.

2. **Materials and methodology**

2.1 **Chemicals**

Two LPS samples were obtained from Invivogen (Montréal, Québec, Canada): ultrapure LPS from *E. coli* JM109 (LPS1) and ultrapure LPS from *E. coli* K12 (LPS2). Structures of LPS1 and LPS2 are shown in Fig. 1. PS (MW ~ 10$^6$, Mw/Mn ~ 1.10) was purchased from Polysciences (Warrington, PA, USA). 3-Aminopropyltriethoxysilane (APTES) (98%) was purchased from Alfa Aesar (a Johnson Matthey Company, MA, USA). Sodium chloride (NaCl, ≥99.5%) was purchased from Sigma-Aldrich (St. Louis, MO, Canada). Mica sheets (ruby mica blocks, grade 1) were obtained from S&J Trading Inc. (Floral park, NY, USA). Aqueous solutions were prepared in 18.2 MΩ deionized water (Millipore, Mississauga, ON, Canada) and filtered through 0.2 μm filters (Nalgene, Rochester, NY, USA) prior to use. PS was dissolved in toluene (Fisher Scientific, Ottawa, Ontario, Canada) and filtered through 0.2 μm PTFE filters (Fisher Scientific, Ottawa, Ontario, Canada).

2.2 **Substrate preparation**

Three different substrates: mica, PS-coated mica, and APTES-coated mica were prepared for bacterial adhesion experiments and characterized through contact angle measurements and AFM imaging. Mica substrates (2 cm × 2 cm and 200 μm thick) were freshly cleaved in the laminar hood (Forma Laminar Airflow Workstation, Thermo Electron Corporation, Waltham, Massachusetts, USA). PS-coated mica was prepared by spin-coating and vacuum dried at 23 °C overnight. APTES-coated substrates were prepared by exposing freshly-cleaved mica to APTES vapor in an evaporation chamber. Briefly, APTES droplets were put in a small glass vial which was placed near a mica substrate in an evaporation chamber. The chamber was first evacuated for a few minutes and then closed for more than three hours to allow surface deposition and reaction of APTES with the mica.

Four different surfaces: mica, PS-coated mica, APTES-coated mica, and LPS-coated PS were prepared for SFA experiments. A detailed procedure for preparing substrates for SFA experiments has been reported elsewhere. Briefly, a thin and back-silvered mica sheet of 1–5 μm was glued onto a cylindrical silica disk (of radius R = 2 cm) and the exposed mica surface was treated with different chemicals. The LPS layer was coated as follows: firstly, one mica surface was spin-coated with PS and vacuum dried; secondly, 40 μg mL$^{-1}$ LPS in 0.1 M NaCl solution were dropped on the PS-coated mica for more than one hour for deposition in a water vapor saturated chamber; and lastly, the surface was rinsed with 0.1 M NaCl. PS-coated and APTES-coated mica surfaces were prepared using the procedures described above.
2.3 Surface characterization

Contact angle measurement. The water contact angles of LPS-coated PS, freshly cleaved mica, spin-coated PS on mica, and APTES-coated mica were characterized by a contact angle goniometer (KRUSS DSA 10, Germany) using the sessile drop method. Water droplets of 2 μL were placed on the sample surfaces. Images were captured and analyzed with the goniometer at room temperature (23 °C). The contact angle was measured and given by the goniometer based on the shape of the sessile drop, and the measurement was repeated for at least three samples of the same material with three tests for each sample.

Streaming potential measurement. The surface potentials of mica, APTES-coated mica, and PS-coated mica were determined using a streaming potential measurement apparatus with the scheme reported previously. Two sample sheets placed on Teflon gaskets were separated by a Teflon gasket and subjected to a flow of 0.1 M NaCl. The streaming potential (ΔE) was measured under regulated and constant hydrostatic pressure (ΔP) of the electrolyte. Zeta potentials were calculated based on the Helmholtz-Smoluchowski equation.

Atomic force microscopy (AFM) imaging. AFM (Agilent Technologies 5500, Agilent, Santa Barbara, CA, USA) imaging was carried out to characterize the surface structures and roughness of three substrates (mica, PS-coated mica, and APTES-coated mica), and LPS-coated PS surfaces. LPS-deposited surfaces were rinsed with water and dried in air prior to AFM measurements. Samples were imaged with a silicon tip (AppNANO, ACT-200, Si, N-type, tip radius <10 nm, resonant frequency of 318 kHz) operating in the tapping mode in air. At least three samples and more than three positions per sample were imaged to characterize each substrate.

2.4 Quartz crystal microbalance with dissipation (QCM-D)

Adhesion of LPS to PS surfaces was studied using a QCM-D (Q-Sense E4, Biolin Scientific, Sweden). The PS coating was prepared by spin-coating the Au sensors with three drops of PS solution (0.5% in toluene) at 2000 rpm (Spin Coater WSW-400A-6NPP/LITTE, Laurell Technologies Corporation, North Wales, PA, USA) and vacuum dried. Prior to each experiment, the PS-coated sensor was equilibrated by pumping LPS-free background PA, USA) and vacuum dried. Prior to each experiment, the PS-coating was treated with Syto 9 (5 μM, Invitrogen, Burlington, ON, Canada) for 15 min to stain bacteria. After staining, the attached cells were counted within one hour under a fluorescence microscope (Leica DMRXA microscope, Nikon Digital Camera – DXM 1200, Nikon ACT-1 software 2.70). Five images, each corresponding to a surface area of 0.00348 cm², were taken randomly from different positions for each substrate. The number of adhered cells was obtained by counting the bright spots on the fluorescence images and normalized to the surface area. Bacterial adhesion experiments were repeated for at least four times.

2.5 Bacterial culture

Two kinds of E. coli strains were examined. E. coli JM109 expresses a full LPS chain that consists of lipid A, core polysaccharides, and O-antigen (LPS1). E. coli K12 produces a truncated LPS chain that extends from lipid A to the outer core polysaccharides (LPS2). The structures of LPS1 and LPS2 are shown in Fig. 1. E. coli JM109 was kindly provided by Dr Mavanur Suresh (Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Canada).

Bacteria were grown in Luria-Bertani (LB) broth (Miller) at 37 °C on a rocking incubator (Excella E24 incubator shaker series, New Brunswick Scientific, Edison, New Jersey, USA) for 20 h. Cells were harvested by centrifugation (Avanti J-20L, Beckman Coulter, CA, USA) at 3000 g at 4 °C for 10 min. The growth medium was decanted and pellets were vortexed (Genie 2, Fisher Scientific Inc., IL, USA) and resuspended in 0.1 M NaCl. The centrifugation-resuspension process was repeated three times to remove traces of growth media. Prior to bacterial adhesion tests, cell suspensions in 0.1 M NaCl were diluted to approximately 10⁷ CFU/mL as determined by an optical density (OD) of 0.1 using a UV/visible spectrophotometer (Varian, Inc., CA, USA) at a wavelength of 600 nm.

2.6 Scanning electron microscope (SEM) imaging

An SEM (Hitachi S2500, Japan) was used to examine bacterial surface structure. Harvested bacteria suspended in 0.1 M NaCl were fixed with 2.5% glutaraldehyde for 30 min and further fixed with 1% OsO₄ for 30 min. After fixation, bacteria were dehydrated in 50%, 70%, 90%, and 100% ethanol, followed by critical point drying at 31 °C for 5 min. The samples were sputter-coated with gold (Edwards, Model S150B, U.K.) to a targeted thickness of 30 nm, and examined with the SEM at an accelerating voltage of 8 kV.

2.7 Bacterial adhesion

Bacterial initial adhesion was examined with a static soaking method by putting targeted substrates in a bacterial suspension. Each substrate was soaked in 3 mL of an E. coli JM109 or K12 suspension (10⁶ CFU/mL in 0.1 M NaCl) for 30 min at 23 °C. Then the substrates were removed and dipped in 0.1 M NaCl to remove unbound cells. Substrates with attached bacteria were treated with Syto 9 (5 μM, Invitrogen, Burlington, ON, Canada) for 15 min to stain bacteria. After staining, the attached cells were counted within one hour under a fluorescence microscope (Leica DMRXA microscope, Nikon Digital Camera – DXM 1200, Nikon ACT-1 software 2.70). Five images, each corresponding to a surface area of 0.00348 cm², were taken randomly from different positions for each substrate. The number of adhered cells was obtained by counting the bright spots on the fluorescence images and normalized to the surface area. Bacterial adhesion experiments were repeated for at least four times.

2.8 Surface force measurement

Interaction forces and normal force–distance profiles between surfaces were acquired using a surface force apparatus (Surforce LLC, Santa Barbara, CA) to elucidate the intermolecular interaction mechanism. The detailed setup for SFA experiments has been reported. A schematic of the setup for studying LPS interactions is shown in Fig. 2. Two thin mica sheets (1–5 μm) were glued onto cylindrical silica disks (radius R = 2 cm), and surfaces were mounted in the SFA chamber in a crossed-cylinder configuration. The interaction force F between the curved mica surfaces was measured as a function of absolute surface separation distance D. The absolute surface separation was monitored in real-time using multiple beam interferometry employing fringes of equal chromatic order (FECO). The forces measured...
roughly correspond to a sphere of radius $R$ approaching a flat surface based on the Derjaguin approximation: $F(D) = 2\pi RW(D)$, where $F(D)$ is the force between the two curved surfaces and $W(D)$ is the interaction energy per unit area between two flat surfaces.\(^{22}\) The measured adhesion or “pull-off” force $F_{\text{ad}}$ is related to the adhesion energy per unit area $W_{\text{ad}}$ by $F_{\text{ad}} = 2\pi RW_{\text{ad}}$ for rigid (undeformable) surfaces with weakly adhesive interactions, and by $F_{\text{ad}} = 1.5\pi RW_{\text{ad}}$ (used in this study) for soft deformable surfaces with strong adhesive contact.\(^{26}\)

To evaluate intermolecular interaction mechanisms, two SFA configurations were tested. Symmetrical configuration experiments were carried out by injecting 10$\,\mu$g mL$^{-1}$ LPS in 0.1 M NaCl solution between two mica surfaces (Fig. 2a). Asymmetrical configuration experiments were performed to study the interaction forces between LPS-coated PS and mica, PS-coated mica or APTES-coated mica surfaces in 0.1 M NaCl solutions (Fig. 2b–d). During SFA measurements, the two surfaces were first brought close to reach a “hard wall”, defined as the surface-surface separation at which the thickness of the confined polymers becomes asymptotic with increasing normal load or pressure, and then the surfaces were separated. All SFA experiments were performed at 23 °C.

3. Results

3.1 Contact angle measurement

Contact angle (CA) measurements were used to characterize surface hydrophobicity changes. The measured contact angles were mica 0°, LPS1-coated PS surface 34.6° (±1.1°), LPS2-coated PS surface 38.8° (±3.3°), APTES-coated mica surface 44.6° (±1.4°), and PS-coated mica surface 92.0° (±4.6°). Mica is very hydrophilic (CA = 0°) and becomes more hydrophobic after APTES deposition (CA = 44.6°), and very hydrophobic after PS coating (CA = 92.0°).

The adsorption of LPS changes the PS surface from very hydrophobic (CA = 92.0°) to fairly hydrophilic (CA = 34.6° for LPS1-coated PS and 38.8° for LPS2-coated PS). LPS chains have long hydrophobic tails that facilitate adsorption to hydrophobic surfaces such as PS via strong hydrophobic interactions through lipid A.\(^{27}\) After LPS deposition on PS, the contact angle becomes smaller suggesting that hydrophilic polysaccharide chains are exposed outward in the solution, similar to the orientation of LPS on the bacterial outer membrane. LPS1 has a long chain of hydrophilic O-antigen groups while LPS2 does not. Lack of O-antigen may lead to more exposure of the lipid A group and result in the relatively larger contact angles for PS-supported LPS2 versus PS-supported LPS1.

3.2 Surface potential measurement

The electrical potentials of three different surfaces were measured in 0.1 M NaCl: PS-coated mica, negatively charged (zeta potential $\xi = -18 \pm 4$ mV, consistent with previous reports,\(^{28,29}\) and the origin of the surface charges of PS was discussed previously\(^{30,31}\)); mica, negatively charged ($\xi = 5 \pm 3$ mV, consistent with a previous report\(^{32}\) or extrapolated results in 0.1 M NaCl or KCl$^{32-35}$); and APTES-coated mica, positively charged ($\xi = 30 \pm 3$ mV, consistent with a previous study\(^{30}\)). Adamczyk et al.\(^{34}\) reported zeta potentials of mica: $\xi = -85$ in
0.001 M NaCl and −38 mV in 0.01 M NaCl, leading to an extrapolated $\xi$ value around zero or slightly positive in 0.1 M NaCl. Previous studies have shown that the ionic strength of the media has a significant impact on the zeta potential of mica, and most studies were performed in ionic strengths ($10^{-4}$ to $10^{-2}$ M) relatively lower than the biological condition. Streaming potential measurements at higher ionic strengths (>0.01 M) become less reliable as a result of increasing electric conductivity of the electrolyte in the stream potential flow cell; however, our measurements were consistent with the previous results and predictions.32–35

3.3 QCM-D measurement

QCM-D is an advanced technology for the study of surface interactions. It can provide sensitivity to nanograms of mass and can be applied to in situ structural arrangements.37–38 Frequency change $\Delta f$ and dissipation $\Delta D$ responses for the adsorption of LPS1 (Fig. 3a) and LPS2 (Fig. 3b) to PS surfaces were obtained from QCM-D measurements in terms of time and solution load. The frequency change $\Delta f$ reflects the amount of sample adhering to the surface, and the dissipation $\Delta D$ reflects rigidity changes on the surface. As shown in Fig. 3, adsorption occurred as soon as the LPS solution made contact with the PS surface, and reached a plateau after about 3 h for LPS1 and about 1 h for LPS2. The faster adsorption to PS of LPS2 compared to LPS1 may be accounted for the exposed lipid A region on LPS2. Bonded LPS were not desorbed upon rinsing with blank solution (0.1 M NaCl), indicating a stable adsorption of LPS to PS surfaces, probably due to strong hydrophilic interactions. Total shifts in frequency ($\Delta f$) were −95 Hz and −9 Hz (at 25 MHz, n = 5) for LPS1 and LPS2, respectively. According to the Sauerbrey approximation ($\Delta m = -17.7\Delta f$, ng cm$^{-2}$), the frequency changes correspond to an adsorbed effective mass (including coupled water) of 1680 ng cm$^{-2}$ for LPS1 and 160 ng cm$^{-2}$ for LPS2. The thickness of the adsorbed layers was estimated to be 16.8 nm (LPS1) and 1.6 nm (LPS2), assuming the adsorbed layers had a density of 1 g cm$^{-3}$. Total shifts in dissipation ($\Delta D$) of 7.8 $\times$ 10$^{-6}$ and 1.7 $\times$ 10$^{-6}$ for LPS1 and LPS2, respectively, indicate that the adsorption of LPS1 results in a less rigid film (possibly due to a considerable amount of water coupled to the long chain of hydrophilic O-antigen groups) than that obtained with LPS2.

It should be noted that the thickness of LPS adsorbed layers on PS-coated sensors are shorter than the full length for the corresponding LPS. Estimated according to the LPS components on the bacterial surface, the maximum stretched full lengths for free LPS are 38 nm for LPS1 and 8 nm for LPS2 (Fig. 1).6,8,9,39,40 This observation can be attributed to the conformational change of LPS in aqueous solution. Previous studies showed that polysaccharides can change their conformation due to bacterial growth conditions and solution chemistry, and form coiled or other compact structures.14,15,40–42 O-antigen repeat units can also vary on bacterial surfaces, leading to large variations in the LPS chain length.14,15 In this study, it is reasonable to consider the adsorbed LPS layer on PS as a single layer.

3.4 AFM measurement

AFM was used to provide information on surface roughness and surface structure. As shown in Fig. 4, the root mean square (rms) roughness values for mica, APTES-coated mica, and PS-coated mica were all less than 0.5 nm, indicating very smooth surfaces were achieved for all substrates.

LPS adsorbed on a PS surface shows particle aggregation, as shown in Fig. 5. Both topographic and phase contrast images show that nanosized aggregates were evenly distributed on the PS surface. LPS1 exhibited assemblies with lateral dimensions of 61 (±16) nm, rms roughness of 7.1 (±0.3) nm, and maximum peak height of 15.1 (±0.8) nm. Compared to LPS1, the assemblies of LPS2 were smaller and thinner, with a lateral dimension of 40 (±12) nm, rms roughness of 2.1 (±0.2) nm, and a maximum peak height of 7.1 (±0.5) nm. It is interesting that LPS patches observed on two kinds of bacterial surfaces by SEM imaging were similar, as shown in Fig. 6.

AFM imaging in air indicated that the adsorption of LPS is likely single layered (roughness 7.1 nm for LPS1 and 2.1 nm for LPS2) and that LPS is prone to assemble on a surface in bundles of molecules rather than single molecules. The thickness of the adsorbed LPS layers was shorter than the reported length of a single LPS molecule stretched full length (LPS1 ~ 38 nm, LPS2 ~ 8 nm), which can be explained by the same factors discussed above. Further, the adsorbed LPS layer thickness from AFM studies was different from that of QCM-D measurements, 7.1 nm (AFM) versus 16.8 nm (QCM-D) for LPS1, and 2.1 nm (AFM) versus 1.6 nm (QCM-D) for LPS2. Such difference could be due to two factors. First, as AFM is imaged in air, less water in the LPS layer makes it more compact. Such effect is more obvious for LPS1 which has a long hydrophilic O-antigen portion. Hydration has less impact on LPS2 since it lacks the O-antigen portion. Second, the thickness based on QCM-D measurements is an approximate and average value arrived at by assuming a uniformly adsorbed layer with a density equal to water. This assumption is reasonable for LPS1, as a large amount of water would be entrapped by the hydrophilic chains, but it is not as reasonable for LPS2 as it has less capacity to hold water. Thus the thickness of the LPS2 layer may have been underestimated by QCM-D because of less water trapped in the layer leading to a smaller density.
QCM-D and AFM measurements indicate that the adsorbed LPS on PS was single-layered and the contact angle measurements suggest that LPS adsorption exposes the hydrophilic portion outward with a contact angle of less than 40°, indicating the LPS layer was well orientated with hydrophobic tails attached to the PS surface and hydrophilic portions exposed to the solution. Therefore, the LPS-coated PS surface can be considered to mimic LPS on bacterial cell surfaces.

3.5 SEM images

SEM was used to visualize the surface structure of bacteria. Imaging results of *E. coli* strains JM109 and K12 are shown in Fig. 6. Surfaces of JM109 and K12 are rough with lots of protrusions or bumps that can be attributed to LPS aggregation on the bacteria membrane, defined as LPS patches in Fig. 6. LPS are prone to form aggregates on the cell surface in bundles of 600–3500 molecules and covers most of the cell surface. There are several differences between JM109 and K12 surface structures. First, the assembled aggregates are relatively larger on JM109 (~34 nm in diameter) than on K12 (~17 nm in diameter), probably because LPS molecules on JM109 contain O-antigens...
while K12 LPS lack O-antigens. Second, the “dark” features were observed for both types of bacteria in Fig. 6. Similar “dark” features on bacterial surfaces have been observed by Kotra et al., who suggested the “dark” spots may represent void spaces among LPS patches where other surface components could exist. In our study, there were more visible “dark” spots on K12 compared to JM109, which may imply more exposure to the surrounding solution of other surface structures on K12 bacteria, such as outer membrane proteins. It should be noted that although SEM has been widely used to visualize microbial surface structures at high resolution and the bacterial surface structures observed in SEM evolve from naturally present structures of living bacterial surfaces, sample pre-treatment may cause certain changes on cell surface morphology. For instance, it has been reported that the SEM fixation reagent glutaraldehyde can generate cross-links on cell outer membranes. Third, the SEM image shows nanofiber structures on JM109 but not on K12. The nanofibers are ~40 nm in width and ~400 nm in length, and are most likely flagella, arrowed in Fig. 6. The growth of flagella on E. coli is dependent on environmental conditions. Our SEM results agree with previous studies that concluded that K12 bacteria lack surface flagella.

### 3.6 Surface forces measurements

SFA experiments (Fig. 2) were carried out to investigate the intermolecular interaction mechanism between LPS and different substrates during bacterial adhesion. The surface interaction force (F/R) vs. distance (D) profiles obtained during the SFA approach and separation processes are shown in Fig. 7–9. The discontinuities of the data points during the retraction processes were due to the jump-out of the two surfaces from each other when the pulling force slightly exceeded the adhesion force.

For the symmetrical configuration, 10 μg ml⁻¹ LPS in 0.1 M NaCl was injected between two mica surfaces (Fig. 2a). For LPS1 solution, the initial force measurement was taken ~10 min after injecting the solution, and an adhesion force of $F_{ad/R} = -0.70(±0.04)$ mN m⁻¹ was measured during the separation, as shown in Fig. 7a. Successive measurements showed smaller adhesion forces than the first measurement, possibly due to conformation changes of LPS1 after the previous measurement. The hard wall distance shifted from ~12 nm for the initial measurement to ~20 nm after 70 min adhesion with the adhesion force decreased to $F_{ad/R} = -0.30(±0.02)$ mN m⁻¹ as shown in Fig. 7a and 7b. For LPS2, an adhesion force of ~0.20(±0.02) mN m⁻¹ was measured after 10 min adsorption, as shown in Fig. 7c. The hard wall (1 nm) did not change after 70 min with a similar adhesion force (Fig. 7d).

According to observations in the symmetrical experiments, LPS1 adhered to mica more strongly than LPS2; adhesion forces were ~0.7 mN m⁻¹ (LPS1) and ~0.2 mN m⁻¹ (LPS2). When the adsorption time increased (to 70 min), LPS1 adhesion to mica increased while LPS2 adsorption did not. LPS has many negatively charged functional groups that will repel the negatively charged mica surface. The large O-antigen groups on LPS1 will shield the negative charges. As a result, adhesion of LPS1 to mica is more favorable than adsorption of LPS2. The layout of LPS2 on mica is possibly different from that of LPS1. For LPS2, the hard wall was only 1 nm which is much smaller than the stretched full length of LPS2, suggesting that LPS2 molecules were lying on the mica, confined by the external pressure. The adsorption of LPS2 to mica was weak and the adhesion force measured was mainly due to a bridging effect.

During asymmetrical experiments, LPS was deposited on the PS-coated mica by hydrophobic interaction to mimic the LPS on bacterial surface and the interactions between LPS-coated PS and one of the mica, PS-coated mica, and APTES-coated mica surfaces were studied (Fig. 2c–d). No adhesion or repulsion forces were measured between LPS1 and mica and a hard wall of 10 (±1) nm was reached on close approach of the samples (Fig. 8a). The force–distance profiles did not show obvious change with increasing the contact time from 1 min to 60 min (Fig. 8b). Repulsion forces were observed in interactions between PS-supported LPS1 and PS, as shown in Fig. 8c; repulsion forces decreased with increasing contact time from 1 min to 60 min (Fig. 8d). A strong adhesion force $F_{ad/R} = -1.50(±0.08)$ mN m⁻¹ (interaction energy $W_{ad} = F_{ad}d/1.5πr = -0.32$ mJ m⁻²) was obtained between LPS1 and APTES (Fig. 8e), and the adhesion force increased to $F_{ad/R} = -3.1(±0.07)$ mN m⁻¹ ($W_{ad} = 0.66$ mJ m⁻²) when contact time increased from 1 min to 60 min (Fig. 8f). The hard wall distances for the above measurements were all 10 (±1) nm. As shown in Fig. 9a–f, similar trends were obtained for LPS2. The adhesion force of LPS2 was the lowest on PS, and highest on APTES. The hard wall distance for LPS2 was 6.0 (±0.5) nm, smaller than for LPS1. Adhesion forces between PS-supported LPS2 and APTES were $F_{ad/R} = -7.4(±0.1)$ mN m⁻¹ ($W_{ad} = 1.57$ mJ m⁻²) and $F_{ad/R} = -8.5(±0.1)$ mN m⁻¹ ($W_{ad} = 1.80$ mJ m⁻²) at contact times 1 min and 60 min, respectively, stronger than those between PS-supported LPS1 and APTES. The detachment forces and hard wall distances for asymmetrical experiments were listed in Table 1.

SFA experiments showed that the hard wall distances of adsorbed LPS layers on PS were 10 nm and 6 nm for LPS1 and LPS2, respectively. Compared to the thickness values obtained from other methods, e.g., QCM-D (16.8 nm for LPS1, and 1.6 nm for LPS2), AFM (7.1 nm for LPS1, and 2.1 nm for LPS2), and the estimated stretched full length (LPS1 38 nm, LPS2 8 nm) reported previously, the values obtained from SFA measurements are within the same range and can be considered quite consistent. The variations may be attributed to conformational changes of LPS in solution and on the supporting substrates, and the surface coverage of LPS deposition on PS-coated mica. First, all the experimental thicknesses were less than the maximum stretched full length; impacts of LPS assembly, growth conditions, solution chemistry, and changes in LPS conformation can lead to smaller effective LPS length. The SFA results further confirmed that LPS were adsorbed in a single layer. Second, SFA provides important information that complements AFM and QCM-D measurements about the conformation and molecular interactions of the LPS layer. AFM was operated in air which gave smaller values for LPS thickness compared to solution conditions, while the thickness estimated from QCM-D measurement was based on the Sauerbrey approximation, assumption of uniform surface coverage and density.
3.7 Bacterial initial adhesion

The adhesion capabilities of E. coli strains JM109 and K12 which have LPS1 and LPS2 structures, respectively, were tested. Bacterial adhesion results are shown in Fig. 10. For each bacterial strain, adhesion was highest on APTES-coated surfaces and lowest on PS-coated surfaces. This observation is consistent with the SFA results, where the highest adhesion force was observed between LPS and APTES-coated surfaces and the highest repulsive force was detected between LPS and PS-coated surfaces. The number of JM109 cells that adhered to APTES was ~12 times the number of JM109 cells that adhered to mica, and 470 times the number of JM109 cells that adhered to PS. The number of K12 cells that adhered to APTES was 4.5 times the number of K12 cells that adhered to mica, and 341 times the number of K12 cells that adhered to PS. Adhesion of JM109 was ~1.4 times the adhesion of K12 on the APTES surface, ~0.6 times the adhesion of K12 on mica, and ~1.2 times the adhesion of K12 on the PS surface. According to the t-test, the adhesion efficiency of JM109 is statistically significantly higher than that of K12 on the APTES surface (two-tailed t-test, \( p = 0.0008 \)), significantly lower than that of K12 on mica surface (\( p = 0.01 \)), and has no much difference to that of K12 on PS surface (\( p = 0.5 \)).

4. Discussion

4.1 Adhesion mechanisms

Electrical double layer forces. Several kinds of intermolecular forces may be involved in the interaction of LPS and substrate surfaces during bacterial initial adhesion. The interaction forces measurement by SFA showed that the first and most important force is electrostatic force. The surface charges of the three substrate surfaces were different. In 0.1 M NaCl, the double layer Debye length was compressed to \( \kappa^{-1} = 0.304 \) nm and the electrical double layer interactions were in the short-range. The impact of surface morphology and roughness can be ignored because the substrates were all very flat with rms values of less than 0.5 nm (Fig. 3). Both types of LPS are negatively charged with phosphate and carboxyl groups. Among mica, PS, and APTES, the positively charged APTES-coated surface showed the strongest adhesion to LPS during the retracting cycles, indicating a very stable adsorption. E. coli strains JM109 and
K12 also adhered to the APTES-coated surface with the highest efficiency. Repulsion forces were observed between negatively charged LPS and negatively charged PS, consistent with AFM studies of Ong et al.\textsuperscript{16} who reported repulsion between \textit{E. coli} K12 and PS. Short-range repulsion forces arise from electrical properties of the materials being tested. Bacterial adhesion to PS was also the lowest of the substrates tested. The mica surface showed an almost neutral zeta potential in concentrated salt solution where it is neutralized by sodium ions. Thus only slight repulsion forces were observed between LPS and mica when two surfaces were placed very close to each other (within several nanometres). Bacteria also adhered to mica more strongly than they adhered to PS.

**Steric effects.** Repulsive forces between PS-supported LPS and PS may have been enhanced by steric hindrance between carbohydrate chains of the LPS molecules and polymer chains radiating from the PS surface.\textsuperscript{14,16,50,51} After bringing the two surfaces together, no adhesion forces were detected during retraction. This indicates that attractive hydrophobic forces were...
Fig. 9 Normal forces $F$ (normalized by the radius $R$ of the surfaces) measured for two approaching and separating surfaces: (a and b) PS-supported LPS2 vs. mica, (c and d) PS-supported LPS2 vs. PS, and (e and f) PS-supported LPS2 vs. APTES as a function of surface separation $D$, in 0.1 M NaCl: waiting for $t = 1$ min (a, c and e) and $t = 60$ min (b, d and f) after bringing the surfaces in contact.

Table 1 The detachment forces ($F_{ad}/R$) and hard wall distances ($D_h$) from asymmetrical SFA measurements

<table>
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<tr>
<th>SFA configuration</th>
<th>$F_{ad}/R$ (mN m$^{-1}$) (LPS1)</th>
<th>$F_{ad}/R$ (mN m$^{-1}$) (LPS2)</th>
<th>$D_h$ (nm) (LPS1)</th>
<th>$D_h$ (nm) (LPS2)</th>
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<tr>
<td>Mica vs. PS-supported LPS</td>
<td>0 min contact 0</td>
<td>0 min contact 0</td>
<td>10 (±1)</td>
<td>6 (±0.5)</td>
</tr>
<tr>
<td>PS vs. PS-supported LPS</td>
<td>0 min contact 0</td>
<td>0 min contact 0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>APTES vs. PS-supported LPS</td>
<td>−1.5 (±0.08)</td>
<td>−3.1 (±0.07)</td>
<td>−7.4 (±0.1)</td>
<td>−8.5 (±0.1)</td>
</tr>
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not significant in the interaction between PS-supported LPS and PS surfaces. Steric effects may also have contributed to the repulsion observed between LPS and the APTES-coated surface when samples were brought within 10 to 20 nm of each other (Fig. 8 and 9).

**Nanobubbles on hydrophobic PS surface.** Similar to steric effects, nanobubbles on the hydrophobic PS surface may have hindered interaction between LPS and PS. Nanobubbles might have been critical in the long-range repulsion observed between PS-supported LPS and PS. Discrete interfacial nanobubbles have been observed to exist at the interface of water and smooth hydrophobic solid substrates. The coalescence of nanobubbles produces an attractive long-range Laplace adhesion pressure between hydrophobic surfaces.

In this study, LPS was coated on the PS surface through strong hydrophobic interactions exposing the hydrophilic chains of LPS outward with a water contact angle of less than 40°. The PS surface is very hydrophobic and supports a thin layer of discrete nanobubbles. Therefore, the long range repulsion is probably due to the repulsion between hydrophilic LPS chains and hydrophobic nanobubbles. Repulsion decreased after the two surfaces were kept in contact for 60 min. Interactions between a hydrophobic surface and a hydrophilic surface remain controversial in the literature, and both attractive and repulsive forces have been reported for different systems. For example, Georgiou's group observed opposite forces for bacteria interacting with two different hydrophobic surfaces, attraction to octadecyltri-chlorosilane treated glass, and repulsion from PS coated silicone. The conflict between these observations has been rationalized by appealing to differences in surface charge, interfacial nanobubble nucleation, surface wetting, sample preparation, and solution chemistry.

**Theoretical models.** Interactions between LPS and different substrates are modeled theoretically by incorporating steric repulsion, double layer interaction and van der Waals forces based on classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, and nanobubble hindrance effects. As water is a good solvent for the long hydrophilic chains of LPS, the tails or loops of immobilized LPS molecules on a PS surface tend to stretch and act as a swollen brush. Interaction between a brush and a solid substrate can be described by the Alexander-de Gennes theory, and interactions between asymmetric configurations in this study (PS-supported LPS against APTES-coated mica or PS-coated mica) can be given using the Derjaguin approximation (eqn (1)),

$$F = \frac{8kT \pi L}{35s}[7\left(\frac{L}{D}\right)^{5/4} + 5\left(\frac{D}{L}\right)^{7/4} - 12]$$

where $L$ is polymer brush thickness, $s$ is the average distance between polymer grafting sites, $D$ is the distance between the two curved surfaces. The theoretically fitted results are shown in Fig. 11. The theoretical steric force curve overlaps with the experimental force profile reasonably well. For LPS against APTES-functionalized mica, the polymer brush thickness $L$ was ~28 nm. A larger brush thickness $L$ of ~50 nm was obtained for LPS against PS-coated mica; this could be due to nanobubbles of ~20 nm height on the hydrophobic PS surface. The fitted $s$
values of the LPS1 deposited on PS were 5 nm and 9 nm based on LPS1 vs. APTES and LPS1 vs. PS force–distance profiles, respectively, and the s difference might be due to the presence of the nanobubbles on the hydrophobic PS substrate which makes the fitting deviate from the brush model. An average distance \( s \approx 5 \) nm between the “grafting” sites of LPS1 indicates that the coated layer of lipopolysaccharides with long hydrophilic O-antigen chains behaves similarly to a dense brush layer in water.

The double-layer energy interactions for the interactions between two planar surfaces of different materials in a 1 : 1 electrolyte (e.g., NaCl) can be approximated by the “Hogg–Healy–Fuerstenau” (HHF) equation (eqn (2)). Thus the DLVO interaction forces, including an electrostatic double layer interaction (through the Derjaguin approximation) and van der Waals interactions for two curved surfaces, are given by eqn (3),

\[
W_{DL}(D) = \varepsilon_0 \varepsilon \kappa^{-2} \left( \frac{\Psi_1^2 e^{-2D} + \frac{\Psi_2^2 e^{-2D}}{e^{2D} - 1}}{} - \frac{2 \Psi_1 \Psi_2}{e^{2D} - 1} \right)
\]

\[
F = \frac{2 \pi \varepsilon_0 \varepsilon \kappa^{-2} \left( \frac{\Psi_1^2 e^{-2D} + \frac{\Psi_2^2 e^{-2D}}{e^{2D} - 1}}{} - \frac{A_{123}}{6D^2} \right)}{}
\]

where \( D \) is the separation distance, \( \Psi_1 \) and \( \Psi_2 \) are the surface potentials, \( R \) is the surface radius, \( \kappa \) is the inverse Debye length, \( \varepsilon_0 \) and \( \varepsilon \) are the permittivity of a vacuum and the relative dielectric permittivity of water, respectively, and \( A_{123} \) is the Hamaker constant for two surfaces 1 and 3 interacting in medium 2. The Debye length was 1 nm in 0.1 M NaCl and the electrical double layer interactions were in the short-range, \( 22 \) DLVO forces become significant only when the two surfaces are brought very close together, a situation shown in Fig. 11 and based on eqn (3). The Hamaker constant \( A_{123} \) is given by \( A_{123} = (A_{11} - A_{12})/(\sqrt{A_{11} A_{12} A_{22}}) \), where \( A_{11}, A_{22}, \) and \( A_{12} \) are the Hamaker constants for LPS1, water, and substrates (PS or APTES), respectively, in vacuum, and \( A_{11} = (5–7) \times 10^{-20} \) J, \( A_{22} = 3.7 \times 10^{-20} \) J, and \( A_{12} = (5–7) \times 10^{-20} \) J, which gives \( A_{123} \approx (3–4) \times 10^{-21} \) J. In high salt concentrations the surface potentials used in eqn (3) were approximated by measured zeta potentials: 30 mV for APTES, −18 mV for PS, and −30 mV for LPS1, where the zeta potential of LPS1 was approximated by the zeta potential of JM109. Overall, the theoretical steric force curve fits reasonably well with the experimental approaching force–distance profile. The electrostatic double layer interaction and van der Waals interactions can take effect in the close separation distance, which is not obviously shown in the experimental approaching profile. Thus the repulsion forces measured are mainly due to the steric effects at large separation, while the adhesion is normally detected during the retracting process. It should be noted that when the separation distance was reduced to below 10 nm, deviation of the experimental data from the model predictions was observed in Fig. 11. This deviation may be attributed to the presence of other forces, e.g., hydration forces, hydrophobic interaction and hydrogen bonding, at short separation distances.

Other mechanisms. Surface interactions between the LPS layer and the APTES surface were time-dependent and increased with contact time as shown in Fig. 8 and 9. The increase in adhesion was possibly due to rearrangement of the local LPS molecules in contact with the APTES surface, resulting in more interfacial adhesive “bonds” (electrostatic adhesion, hydrogen bonds, van der Waals adhesion, etc.) with time.

Adhesion of JM109 to APTES was slightly higher than that of K12 as shown in Fig. 10, while adhesion of LPS1 with APTES was lower than that of LPS2 from the SFA measurement (Fig. 7 and 8). These results suggest that LPS was not the only component contributing to bacterial initial adhesion. Extracellular polymeric substances (EPS), membrane-bound proteins, and flagella, may contribute to adhesion. Previous research found that nanofibers on a bacterial cell surface can contribute to the adhesion. The higher adhesion of JM109 compared with K12 on APTES may be due to flagella on the JM109 surface (shown in the SEM image in Fig. 6).

4.2 Molecular interactions of LPS1 and LPS2

The molecular structure differences between LPS1 and LPS2 are shown in Fig. 1. Both types of LPS have saccharide sections, which are easily hydrated in aqueous solutions. These hydrated saccharide segments contribute to the repulsive steric force and may also shield the surface charges of the deposited LPS layer leading to hydration force. The major structural difference between the two LPS is the long hydrated neutral O-antigen chain on LPS1 which shields the negative charges on the polycarbohydrates. Electrostatic interactions between LPS (or bacteria) and substrate surfaces decrease as the length of the neutral O-antigen increases. The presence of long O-antigen chains in LPS can reduce the adhesive capability of bacteria to substrates because the affinity of hydrophilic O-antigen chains to water and the steric entropic barrier of LPS chains on the bacterial surface increase the energy of the adsorption process. These factors can explain our SFA results. Adhesion to APTES was stronger for LPS2 than for LPS1 because of the stronger electrostatic attractions between APTES and LPS2. On the negatively charged PS surface, repulsion forces were slightly stronger for LPS2 than for LPS1, this might be due to the more exposure of negative charges on LPS2 in addition to the nanobubble effects on the PS substrate. Mica is nearly neutral in 0.1 M NaCl, which leads to weak electrostatic interactions between mica and LPS, thus no obvious adhesion force difference was observed between LPS1 and LPS2.

In terms of bacteria, LPS with an O-antigen also lead to less exposure of other components (e.g., EPS and membrane-bound proteins) on bacterial surface. Although the interaction forces were almost the same for the two kinds of LPS against mica, the bacterial adhesion efficiency is higher for K12 than JM109 on mica. This observation can be explained by the presence of other components such as EPS and membrane-bound proteins, which may play more significant roles in bacteria with shorter LPS chains. LPS plus O-antigen can lead to less exposure of other components on bacterial surfaces than LPS alone. Previous studies showed that the exposure degree of surface proteins depends on LPS composition, and decreases with longer LPS chains. SEM imaging showed that K12 has more exposed “dark” features or void spaces between LPS patches, which would likely lead to more exposure of other surface components, such as outer membrane proteins. In addition, the presence of flagella on JM109 surfaces may impact JM109 adhesion and lead...
to the higher adhesion capacity of JM109 compared with K12 on APTES surfaces.

4.3 Techniques for bacterial adhesion studies

Molecular interaction mechanisms of bacterial initial adhesion are affected by many factors, including substrate properties, EPS, LPS, and solution chemistry. Macroscopic adhesion techniques provide qualitative evaluation of bacterial initial adhesion. Force probe techniques such as AFM and SFA can measure intermolecular interactions during bacterial initial adhesion. Although the SFA has been widely applied to investigate molecular interactions in bilayers, membranes and proteins, few studies have addressed bacterial adhesion and biofilm formation using this technique. SFA can provide precise distance information in situ and in real time during the force measurement. Surface-surface interactions measured by an SFA can be compared between artificial and natural systems, e.g., a bacterial surface interacting with a substrate can be modeled by SFA measurements of PS-supported LPS adherence to the substrate. Our present study of LPS adhesion to several substrates suggests that macroscopic and microscopic adhesion characterization techniques can be well combined with molecular and nanoscale force probe techniques to elucidate the contribution of various surface components and environmental factors to bacterial adhesion.

5. Conclusions

Molecular and surface interactions between two model lipopolysaccharides (LPS1 and LPS2) extracted from E. coli strains JM109 and K12 and three different substrates (mica, PS, and APTES) were measured using an SFA and a QCM-D. Due to strong hydrophobic interactions between lipid A and PS, a layer of LPS was successfully coated onto PS substrates forming a model surface to mimic the LPS on bacterial surfaces. AFM, QCM-D, and contact angle measurements confirmed the adsorption of LPS to PS surfaces. LPS was shown to play an important and positive role in bacterial initial adhesion. A PS-supported LPS layer adhered strongly to APTES, weakly to mica, and was repelled by a PS substrate. Electrostatic interactions and steric effects contributed significantly to LPS and bacterial adhesion to the substrates. Although LPS has been shown to play a major role, the SFA force measurements together with the macroscopic bacterial adhesion tests indicate that components other than LPS contribute to bacterial adhesion.

Acknowledgements

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