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Immunoimmobilization of Living *Salmonella* for Fundamental Studies and Biosensor Applications

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1. Introduction

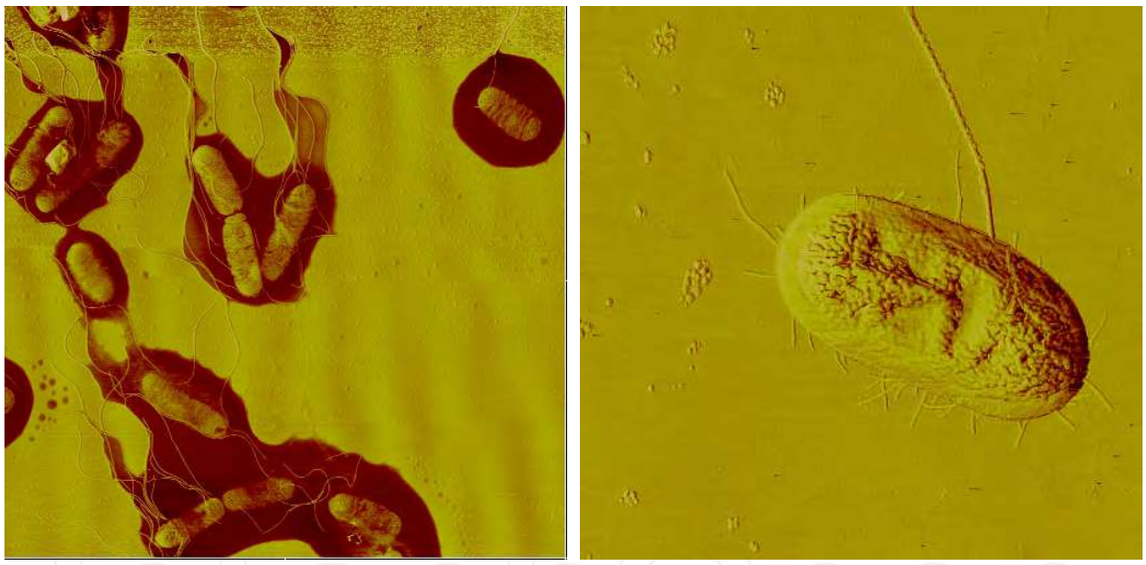
Currently, there is no technique available to probe an individual bacterium in its physiological environment for a prolonged period of time to determine its response to environmental stimuli or to conduct measurements on it. Controlling and manipulating individual bacteria will facilitate fundamental studies of such bacterial characteristics as morphology, adhesion, biomineralization and mechanical properties under physiological conditions. Access to specific individual cells will open new research frontiers in areas such as differentiation among the individual offspring of a predetermined bacterium (Arnoldi et al. 1998; Chao et al. 2011; Gao et al. 2011). In many applications, it is necessary to immobilize bacteria on flat substrates or particles. For example, there have been many reports on using living bacteria as sensors for environmental monitoring because of their low cost, fast growth, easy genetic modification and handling, and sensitivity to a wide variety of environmental stimuli (Kuang et al. 2004; Mbeunkui et al. 2002; Premkumar et al. 2002). It is often necessary to immobilize living bacteria on a designated area of a detecting surface to build a practical sensor. Reliable, controllable and efficient immobilization of bacteria is crucial for the success of pathogen detection. Typically, a captured bacterium triggers an event that converts the capturing process into a signal which is detectable by optical, electrochemical, mechanical or other means (O'Kennedy et al. 2009). The technique developed by our group appears to be very promising for these applications. Before we describe our methodology here, we will give a brief review of the previous approaches and methods for the immobilization of bacteria on material surfaces.

1.1 Nonspecific immobilization of bacteria through physical adsorption or entrapment

The majority of reported immobilization approaches utilize either the nonspecific adsorption of bacterial cells on charged surfaces by means of electrostatic forces or the physical entrapment of cells in gel or micro-holes.

1.1.1 Physical absorption

Typically, bacterial surfaces are negatively charged under physiological conditions for most Gram-positive and Gram-negative species. Thus, it is possible for bacteria to adhere to a positively charged substrate prepared by modifying the surface using positively charged polymers or silanes. Various bacteria have been attached to substrates decorated with polylysine (Rozhok et al. 2006; Rozhok et al. 2005), polyethyleneimmine (Razatos et al. 1998), amino-terminated silanes (Arnoldi et al. 1998), gelatin (Doktycz et al. 2003) and alginate (Polyak et al. 2001). However, unlike eukaryotic cells, bacterial cells are still very challenging to immobilize reliably and reproducibly under their physiological conditions using positively charged polymers. For example, none of the experiments we report here could be done using this approach. This is mostly because, in contrast to a eukaryotic cell, only a very small fraction of a bacterial cell surface can come into close enough contact with a charged substrate surface to adhere, preventing the bacterium from attaching to the surface effectively. Additionally, many bacterial species, including *Salmonella*, have a layer of capsular extracellular polymeric substances (EPS) covering their outer surface, as shown in Fig. 1A (Suo et al. 2007), which further weakens interactions with and adhesions to the substrate surface.



(a) (b)

Fig. 1. AFM phase images of *S*. Typhimurium showing the detailed structures of (A) flagella and capsular EPS (scan size: 20 μm), and (B) a single bacterium with its fimbriae and part of its flagellum (scan size: 4 μm).

1.1.2 Physical entrapment

Because bacterial motion can be significantly slowed in viscous media or micro-cavities, living bacteria can be physically entrapped in hydrogels or inside microwells. Micro- (Xu et al. 2007) and macro-contact printing (Weibel et al. 2005) have been employed to transfer live bacteria onto the surface of a nutrient-rich matrix such as agarose or hydrogel. Bacterial microarrays have also been prepared by loading individual bacterial cells into microwells (2.5 μm wide, ~3 μm deep) at the distal end of an optical fiber bundle by centrifuge (Brogan and Walt 2005). A bacterial array printed onto porous nylon has also been reported

(Heitkamp and Stewart 1996), in which cells were physically entrapped in the pores of a special nylon substrate in close contact with a nutrient medium. Akselrod et al. reported three-dimensional heterotypic arrays of living cells in hydrogels created by means of high-precision (submicron accuracy) time-multiplexed holographic laser trapping (Akselrod et al. 2006). However, this technique has limited applications in practice, as, besides the need for a trapping laser, excessive exposure to laser light may cause photodamage to the cells; furthermore, arrays are expected to merge in a few hours because of cell division. The entrapment methods suffer from slow response times, low loading rates into microwells and easy detachment from surfaces.

2. Antibody-mediated immobilization of bacteria

Another approach to bacterial immobilization takes advantage of the interaction between a receptor and an appropriate ligand on a bacterial surface. Many different types of receptors can be used for this purpose, including enzyme receptors, nucleic acid receptors, polysaccharide receptors (lectins) (Gao et al. 2010) and antibodies against bacterial surface antigens. Since receptors often recognize specific types of ligands on a bacterial surface, such immobilization could achieve a high degree of specificity and efficiency. In this chapter, we focus on antibody-mediated immobilization, referred to as immunoimmobilization. Readers can refer to a recent review for the current status of the field of immobilization using a broad spectrum of receptors (Velusamy et al. 2010b).

The large variety of bacterial surface antigens and corresponding antibodies offers a number of choices for immunoimmobilization, which could be highly specific for a given species. This approach has been used to detect *Salmonella* (Table 1) (Mantzila et al. 2008; Oh et al. 2004) and other bacterial pathogens (Byrne et al. 2009; Skottrup et al. 2008; Velusamy et al. 2010a). Efficient capturing is always desired for bacterial detection, since it will facilitate converting captured pathogens into a detectable signal and, most importantly, a higher capture efficiency will result in a higher sensitivity (lower detection limit). Extensive research has been reported on the development of new detection methods that involve converting an already captured pathogen into an output signal by optical, electrochemical, mechanical or other means (O'Kennedy et al. 2009). However, there has been little study of how to enhance the capture efficiency. In fact, poor immobilization of bacterial cells is often observed. For example, only 2% surface coverage of the bacteria was achieved for a sensor using *E. coli* to monitor environmental toxicity (Premkumar et al. 2001).

In order to achieve reliable and efficient immunoimmobilization, the substrate should be decorated with a dense layer of an antibody which targets the most abundant antigen on the bacterial surface. This requirement draws attention to the two most critical aspects in immunoimmobilization: optimization of the surface chemistry to maximize the antibody density on the substrate surface and selection of an antibody which targets the appropriate bacterial surface antigen such as fimbriae (Fig. 1B).

2.1 Surface chemistry

The surface chemistry for linking antibody molecules is similar to that which has been popularly used to prepare protein microarrays and protein-modified resin (Hermanson et al. 1992). However, in order to achieve a high immobilization efficiency, a substrate for

bacterial immobilization should have a larger number of antibody molecules on the surface, with the paratope of each antibody molecule pointing away from the substrate. It would also be desirable for the antibody molecules to have sufficient freedom of movement to orient themselves in a proper binding direction towards the bacterial antigens.

As shown in Fig. 2A, thiolated tethers including 16-mercaptohexadecanoic acid (MHA) and 11-mercapto-undecanoic acid (MUA) have been commonly used to activate gold and silver surfaces. The carboxyl terminal of MHA and MUA can link to amino groups after activation. Silanes with an active terminal are widely used for silicon oxide, silicon nitride, glass, indium tin oxide (ITO), aluminum, titanium and steel surfaces. In Fig. 2, two popular silanes are shown, aminopropyltriethoxylsilane (APTES) (Fig. 2B) and (3-glycidoxypropyl)-trimethoxysilane (GOPTMS) (Fig. 2C). APTES is further modified with *N*-(3-maleimidopro-pionyloxy)succinimide (BMPS), a short cross-linker, to provide an active maleimido terminal to link to the cysteine residue of antibodies. The glycidyl terminal can react with amino or hydroxyl groups of an antibody. Another popularly used linkage is the biotin-avidin (streptavidin/neutravidin) system (Fig. 2E), which involves the covalent linking of biotin to antibodies followed by the binding of biotin-labeled antibodies to an avidin layer on the substrate (Taitt et al. 2004). Antibodies can also be linked to substrate surfaces through protein A/G/L (Fig. 2D), which specifically binds to the Fc region of IgG (Choi et al. 2008; Gao et al. 2006).

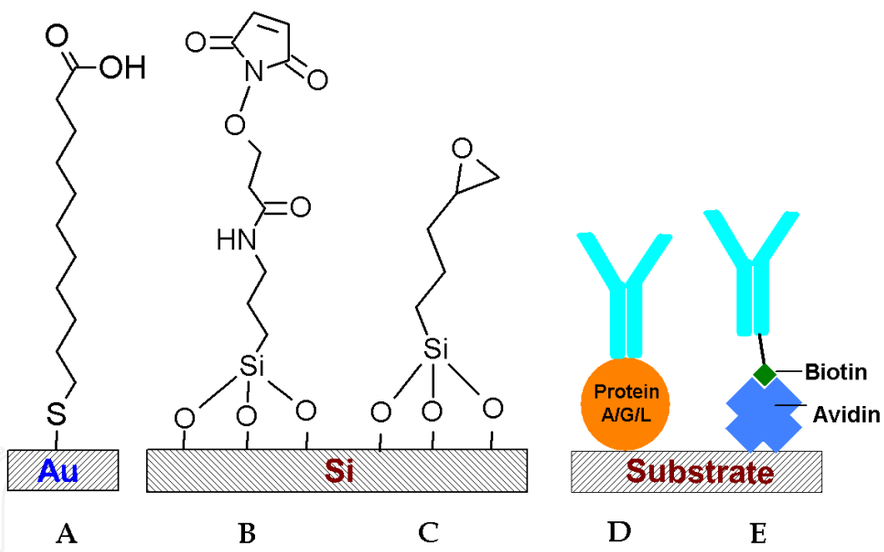


Fig. 2. Chemistry of antibody linkage

Many short cross-linkers are commercially available for linking antibodies; some examples are shown in Fig. 2. An antibody attached to a substrate through one of these cross-linkers forms a dense two-dimensional (2-D) monolayer on the substrate in which the paratopes of the individual antibody molecules are randomly oriented and the individual antibody molecules have very limited freedom of movement for reorientation. A short cross-linker works well with a high-purity antibody against a bacterial surface antigen: this gives a fairly high expression level, and a satisfactory immobilization of bacteria can be achieved. However, when the antibody is not pure or has a low affinity, or when the bacterial surface antigen has a

low expression level, it becomes necessary to boost the antibody binding probability by aligning the antibody molecules so that their paratopes point away from the substrate and using long cross-linkers to obtain the necessary degree of freedom of movement.

When linked through flexible tether molecules with lengths varying from tens to hundreds of nanometers, the antibody molecules will form a three-dimensional (3-D) network on the substrate surface. Such tethers provide the necessary degree of freedom of movement for an antibody to access a larger fraction of the bacterial surface and increase the immobilization efficiency. These tethers can be constructed from brush polymers, dendrimers (Han et al. 2010), certain peptides and block copolymers. Because of their flexibility and polydispersity, these highly branched tethers will maximize the loading of antibody molecules onto the substrate surface in a 3-D network, hence increasing the number of antibody molecules per unit area to more than can be linked by short tethers forming a 2-D network.

Poly(ethylene glycol) (PEG) has been widely used for surface modification since the early 1990's, and a variety of PEG-based cross-linkers are now commercially available. The aqueous solubility and flexibility of PEG make these linkers ideal for antibody molecules. It should be noted that most available PEG cross-linkers can only link one antibody molecule to a terminal, so it is expected that surfaces modified by such PEG cross-linkers will be covered by only one monolayer of antibody.

2.2 Antibody-antigen selection

Antibody-mediated immobilization works in a complex environment, such as growth medium, blood or a food sample, which simplifies the sample preparation. However, previous work using antibodies against whole bacterial cells often resulted in low immobilization efficiency (Premkumar et al. 2001; Rozhok et al. 2005). A general guideline for antibody selection is to use antibodies targeting antigens on the surface of the bacterium (the outer membrane of Gram-negative and the peptidoglycan layer of Gram-positive bacteria). Antigens inside bacterial cells or embedded in cell wall components usually should be avoided since it is impossible for antibody molecules to reach them in living bacteria. A systematic evaluation and comparison of the immobilization efficiencies of selected antibody-antigen pairs associated with common bacterial surface antigens is still needed.

We have evaluated the immobilization efficiencies of IgG antibodies against four different types of surface antigens of *S.* Typhimurium and *E. coli*: lipopolysaccharides (LPS), flagella, fimbriae, and a capsular protein (Suo et al. 2009a). The results show that, with the exception of the capsular protein, all the surface antigens tested can in principle be targeted to achieve some degree of immobilization and that the immobilization efficiency is correlated to multiple factors, especially to the choice of antibody-antigen pairs.

2.2.1 Method

The immobilization efficiency is defined by the number of immunoimmobilized bacteria per unit area within a specific time period for a specific concentration of bacteria in the medium. The antibody solution is deposited onto an activated silicon substrate as small droplets. Because of the specificity of the antibody-antigen interaction, bacterial cells are immobilized only inside the antibody-modified areas. Therefore, a sharp separation of the bacteria-covered areas from those which are not covered is expected. An optical image focused on the antibody-coated areas is used to determine the immobilization efficiency.

2.2.2 Anti-fimbria antibodies

Antibodies against various fimbriae have been tested in our lab, and they usually result in very efficient immobilization. We have studied the immobilization efficiency of both engineered and wild-type strains of *S.* Typhimurium and *E. coli* using antibodies against various types of fimbriae, including K88ab (F4), K88ac (F4), K99 (F5), 987P (F6), F41 and CFA/I. An example of two CFA/I-expressing strains (*S.* Typhimurium Δ*asd*::*kan*^{R} H71-pHC and *E. coli* H681-pBBScfa) being immobilized on a silicon substrate modified with anti-CFA/I is given in Fig. 3A,B. A sharp boundary can be observed separating the bacteria-covered area from the area that is not covered, indicating the high specificity and efficiency of the antibody binding. The cell coverage within the antibody-modified area approached a dense monolayer. The cell density of *E. coli* H681-pBBScfa was slightly lower than that of *S.* Typhimurium Δ*asd*::*kan*^{R} H71-pHC, because of the lower CFA/I expression level of the *E. coli* strain relative to that of the *S.* Typhimurium strain. The areas outside the antibody-modified regions (upper right-hand sides of the panels in Fig. 3) serve as a negative control for evaluaing the immobilization efficiency. Usually no cells or only sparsely attached cells were observed in these control areas. We conducted additional control experiments on similar substrates using no antibody and using an irrelevant antibody (anti-cytochrome *c*), for which no immobilization was observed.

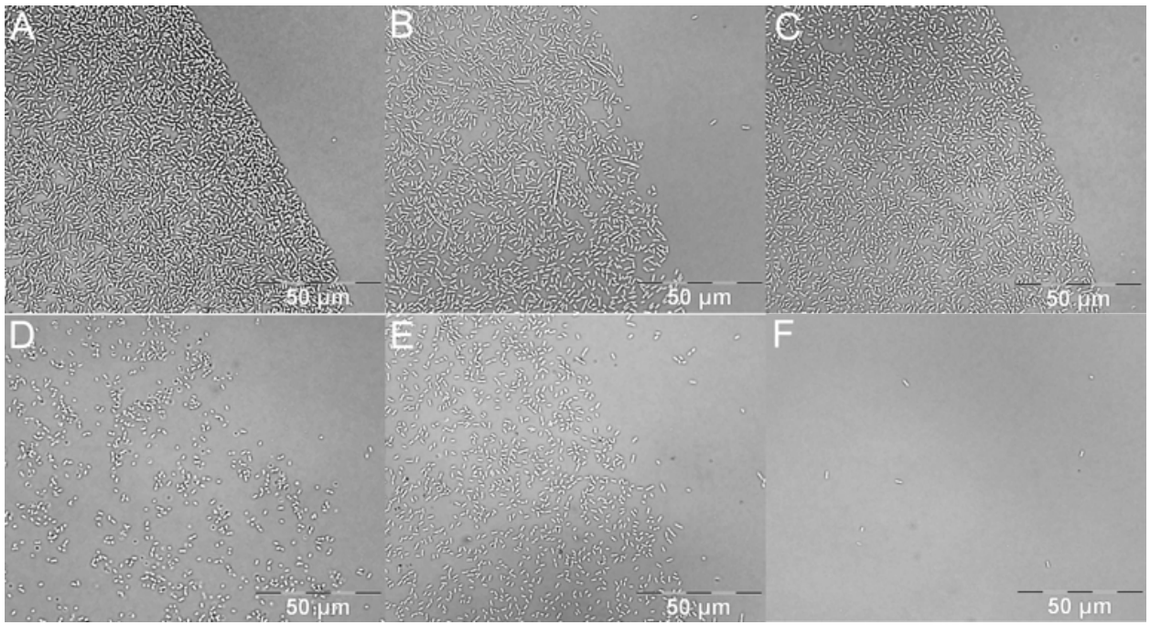


Fig. 3. Immobilization of bacteria using antibodies targeting various bacterial surface antigens: (A) *S.* Typhimurium *Δasd::kan*^{R} H71-pHC on substrate modified with anti-CFA/I, (B) *E. coli* H681-pBBScfa on substrate modified with anti-CFA/I, (C) *S.* Typhimurium *motA3::cat* H683-pTP2fliC (with flagella motion paralyzed) on substrate modified with anti-flagellin (notice that all the bacteria are lying down), (D) *S.* Typhimurium H647 (with active flagella) on substrate modified with anti-flagellin (notice that almost all the bacteria are standing up) (E) H647 on substrate modified with anti-O4 antigen (sc52224), and (F) *S.* Typhimurium *Δasd::kan*^{R} H71-pF1 on substrate modified with anti-F1.

2.2.3 Anti-flagellin

The efficiency of immobilization using anti-flagellin and anti-LPS was tested on *S.* Typhimurium H647, which expresses flagella but lacks CFA/I fimbriae. As expected, this strain could not be immobilized on substrate modified with anti-CFA/I antibody. However, H647 could be immobilized on substrates modified with anti-flagellin (Fig. 3C,D), although with a relatively low cell density as compared with CFA/I fimbriae. In spite of the fact that anti-flagellin shows a reasonably strong affinity to purified *S.* Typhimurium flagella (data not shown), the coverage density of H647 could not be improved by increasing the incubation time (Suo et al. 2009a). We speculate that the low cell coverage density of H647 is a result of the high-speed rotary motion of the flagella, which can be as high as 10,000 rpm at 35°C (Magariyama et al. 2001), hindering antibody-antigen interactions. Preliminary results showed that the immobilization efficiency could be enhanced while using the same anti-flagellin by paralyzing the flagella motion, as shown in Fig. 3C for *motA3*::*cat* H683-pTP2fliC. Notice in Fig. 3D that almost all the bacteria are standing up, most likely because of the flagella motion, as opposed to lying down when their flagella are paralyzed (Fig. 3C).

2.2.4 Anti-LPS

The antibodies against *S.* Typhimurium LPS showed the anticipated results, in that only one of the four antibodies tested demonstrated efficient immobilization of H647. LPS is an important amphiphilic molecule extending out from the bacterial outer membrane. It is composed of three covalently linked domains: lipid A, core antigen (oligosaccharide) and O-antigen (polysaccharide) (Raetz 1996). The lipid A is embedded in the outer membrane lipid bilayer, and hence it is expected that it would be difficult for an antibody to recognize it in a living bacterium. Our observations support this expectation, as the antibody against lipid A failed to provide any immobilization of *S.* Typhimurium. The saccharides, including both the core antigen and O-antigen, protrude from the phospholipid bilayer of the outer membrane and, therefore, can serve as potential targets for immunoimmobilization. Our experiments also showed no successful immobilization for the antibody against the core antigen. This implies that the core antigen is shielded by the O-antigen, preventing antibody-antigen interactions.

About 67 types of O-antigens have been identified for *Salmonella* serovars (Grimont and Weill 2007). These O-antigens are long chains of polysaccharides with a total length of up to 40 repeating units, typically with three to six sugars in each repeating unit (Raetz 1996). The sugar composition and the alteration of linkages among the sugars determine the serogroup to which a specific strain belongs (Selander et al. 1996). We tested three commercially available antibodies targeting *S.* Typhimurium O-antigen (Santa Cruz Biotech. Inc., Santa Cruz, CA): sc52221, sc52223 and sc52224. The latter two antibodies (sc52223 and sc52224) are specific to the O4-antigen. Only one antibody (sc52224) showed successful immobilization (Fig. 3E). *S.* Typhymurium usually contains O-antigens 4, 5 and 12 (Grimont and Weill 2007), all of which share a common tetrasaccharide repeating unit (with different sizes) given by α-D-mannose-1→2-α-L-rhamnose-1→3-α-D-galactose trisaccharide, to which an abequose is αl,3 linked (Curd et al. 1998; Weintraub et al. 1992). So far there is not enough information on the O-antigen structure of H647, and we infer that H647 may not contain O-4 antigen. H647 is a recombinant strain constructed by complementing an *asd* mutation strain H683 with an *asd*$^+$ plasmid (Ascon et al. 1998). There are results indicating the *asd*$^+$ plasmid may interfere with the expression of cell wall components including LPS in our experiments. Based on the fact that only one of the two monoclonal O4-specific antibodies showed

positive results in immobilization experiments, we hypothesize that H647 altered its O-antigen structure to a different group, O-9 (D1), which has a repeating unit of α-D-mannose-1→2-α-L-rhamnose-1→3-α-D-galactose trisaccharide to which a tyvelose is αl,3 linked (Curd et al. 1998). This is a reasonable hypothesis considering that this structure is very similar to serogroup O-4 in that tyvelose differs from abequose only in the 3-D orientation of their OH groups at the 2 and 4 positions. Although more experiments are needed to verify this hypothesis, the results demonstrate that the antibodies targeting the O-antigen can be used for bacterial immobilization. The high specificity of anti-O-antigens can be used for rapid serotyping of *Salmonella* (Cai et al. 2005).

2.2.5 Anti-capsular protein

A polyclonal antibody raised against the proteinaceous capsular antigen (F1-antigen) was tested for immobilizing *S*. Typhimurium. F1-antigen, originally discovered for *Yersinia pestis*, can form a dense amorphous capsule that covers the bacterium (Friedlander et al. 1995; Titball and Williamson 2001). *S.* Typhimurium strain Δ*asd*::*kan*^{R} H71-pF1 was constructed to express F1-antigen as a model bacterium. The expression of F1 antigen was confirmed by immunofluorescence and Western blot analysis (Yang et al. 2007). It was expected that such a capsular proteinaceous antigen would have a relatively strong interaction with the corresponding antibody. However, Δ*asd*::*kan*^{R} H71-pF1 cells could not be immobilized on the substrate premodified with anti-F1 antigen (Fig. 3F). Most likely this is because the F1 capsule (Fig. 1), unlike CFA/I fimbriae, is not tightly bound to the bacterial cell wall and, therefore, can easily slough off from the surface of the bacterium, leading to the failure of bacterial immobilization.

2.3 Other factors

Other factors, such as the clonal type, the purity of the antibody, the antigen expression level, and the incubation time, also contribute to immobilization efficiency. The optimization of all these factors will lead to a very low detection limit. For example, we can readily detect *S*. Typhimurium or *E. coli* at a concentration of 10^3 to 10^4 CFU/ml.

3. A platform for studying an individual living bacterium

Immunoimmobilization offers access to controlling and manipulating living bacteria at the single-cell level. With this technique, it is possible to deploy a single living bacterium at a designated location, and then conduct measurements on the living bacterium with optical, mechanical, electrochemical and other tools. Combining these with PCR techniques, it is also possible to investigate the bacterial genomes of individual cells. Below, we present the preparation of bacterial micropatterns and the study of the mechanical properties of living *S*. Typhimurium.

3.1 Preparation of bacterial micropatterns

During the past decades there have been great advances in micro-electromechanical systems (MEMS) and nano-electromechanical systems (NEMS) technologies. Most of the techniques, such as microcontact printing, focused ion beam (FIB) etching, micro-plotting, e-beam lithography and dip-pen nanolithography (DPN), can be used to prepare micro- and nano-scale patterns on a flat substrate (Salaita et al. 2007).

The successful preparation of bacterial patterns relies mainly on the preparation of high-quality antibody micropatterns, which can be made using two general approaches: (1) modifying the substrate surface chemically to form a chemical micropattern in which the antibody will bind only inside or outside the modified areas and (2) depositing the antibody directly onto designated locations on an activated substrate surface. Most patterning techniques, such as FIB etching, DPN, e-beam lithography and microcontact printing, fall into one of these categories. The advantage of the first approach is the convenience of preparing patterns at nanoscale resolution (except for microcontact printing, which has a practical resolution of around 1 μm (Huck 2007)), which makes it possible to prepare single-cell arrays. However, it is difficult to represent multiple antibodies on the substrate using this approach since there are fairly limited chemical linkages available for antibody differentiation. The second approach (direct deposit approach) often refers to the microplotting method, which uses antibody solutions as ink for preparing antibody microspots on various substrates. We have tried two different microplotters whose antibody pattern sizes reached down to 25 μm in diameter. The antibody spot size is related to many factors, including the tip size, the viscosity of the protein solution, the hydrophobicity of the substrate surface and the moving speed of the tip of the microplotter (Larson et al. 2004), but generally it is very difficult to obtain spots smaller than 25 μm. The microplotting approach has a great advantage in that there is, in principle, no upper limit on the number of antibodies that can be represented in the microarray. For both approaches, it is necessary to passivate the substrate areas to prevent the nonspecific absorption of bacterial cells. In the first approach, the passivation is done by modifying the substrates using PEGlyated tethers (Lahiri et al. 1999) before patterning the surface with antibodies. In the microplotting methods, the passivation is done by post-exposing the protein micropatterns to BSA or milk proteins before exposing them to bacterial cultures.

Once prepared, an antibody micropattern is incubated with bacterial cultures. The bacterial cells are immobilized only on the antibody-patterned areas and thus form bacterial micropatterns. In Fig. 4, micropatterns of living *S.* Typhimurium prepared using FIB, microplotting and DPN are presented as examples. As can be seen in Fig. 4A, a cellular resolution of bacterial patterning is achieved in FIB patterning. Some of the line thicknesses in the patterns, ~1 μm, are comparable to the dimensions of the bacteria, and the bacteria are concentrated along these narrow lines; very few cells are observed outside the lines. Fig. 4B shows well-defined circular patterns obtained by microplotting. Single-cell resolution is best

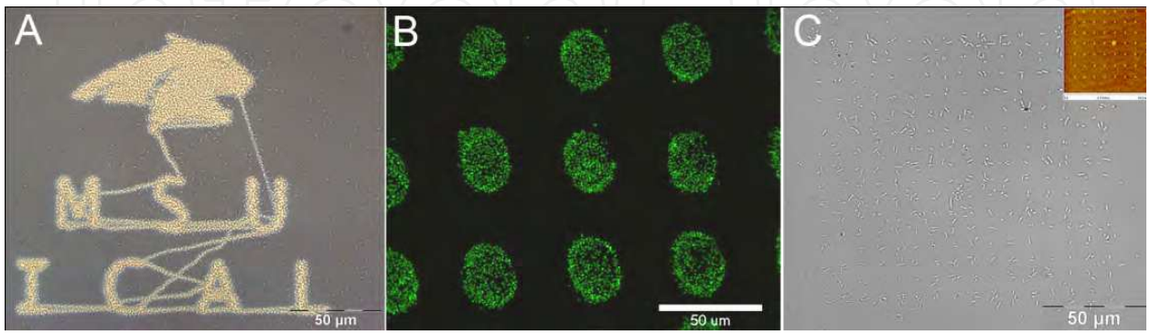


Fig. 4. Micropatterns of *S.* Typhimurium prepared by incubating the bacterial culture with antibody micropatterns fabricated using (A) FIB etching, (B) microplotting, and (C) DPN. Inset of panel C: A DPN pattern on gold substrate before antibody linking (scan size, 20 μm).

demonstrated by the microarray in Fig. 4C, which was prepared using the DPN method. An array of 16×16 antibody spots (each submicron in diameter) was placed on a chip using a sharp AFM tip. Notice that almost all the antibody spots captured at least one bacterium, most of the time multiple bacteria. This shows that immunoimmobilization, applied properly, is an extremely efficient technique.

3.2 Bacterial cells remain bioactive while immunoimmobilized

The question of whether or not immunoimmobilized microorganisms will maintain bioactivities such as cell division is highly relevant. An excessive number of antibodies surrounding a bacterium will eventually alter its behavior and perhaps even kill it. The technique used in immunoimmobilization is a fairly mild treatment of the bacterium that is immobilized. All the antibodies are bound to the surface; hence, only those bacteria that are near the surface of the substrate will interact with the antibodies. Of the many antibody molecules (~thousands per μm^2) evidence points to only a handful (tens per bacteria) interacting with the organism and keeping it bound to the surface. The evidence for this is that an immobilized bacterium still moves around with a considerable degree of freedom; in fact, when necessary it stands up, as mentioned above and also shown below, indicating that it is held to the surface by a small number of antibody-antigen pairs. This fact enables organisms to maintain their usual bioactivities, while giving rise to secondary activities triggered by the process of immobilization. We have seen evidence of such activity in the excessive production of flagella by bound bacteria as compared to that of the planktonic variety of the same bacteria (Suo et al. 2009a). Below we give some examples of the bioactivities of immobilized bacteria.

3.2.1 Immobilized bacteria are capable of division

Our results, as well as those of previous studies, indicate that immunoimmobilization does not hinder such physiological activities as the cell division (Suo et al. 2008), gene expression or bioluminescence of bacteria at the locations of their immobilization (Premkumar et al. 2001).

Immunoactivated areas exposed to low concentrations of bacteria (10^3-10^4 CFU/ml) for long periods of times (~20 hr) become populated by living bacteria for two reasons: (1) As time passes, some bacteria, even at low concentrations, are eventually captured by the antibodies within the activated areas. (2) The offspring of the captured bacteria start populating the antibody-activated areas (Fig. 5) because they too are captured at these locations following cell division, adding to the density of immunoimmobilized bacteria. Experiments with low cell concentrations are currently in progress, and the results will be published elsewhere.

It should be noted that once the surface is covered by a monolayer of live cells, the bacterial patterns on the antibody-modified areas do not change physical dimensions as a result of cell division. After the activated area is fully covered with bacteria, excess bacteria are released into the medium and become planktonic. This feature is not available for patterns prepared by embedding (Mbeunkui et al. 2002; Polyak et al. 2001; Premkumar et al. 2002; Weibel et al. 2005; Xu et al. 2007) or physical entrapment (Kuang et al. 2004). For these patterns, bacteria are held on the substrates or inside the microwells with weak forces, and the cells do not stay fixed to the patterned areas for long periods of time, causing the eventual disintegration of the pattern.

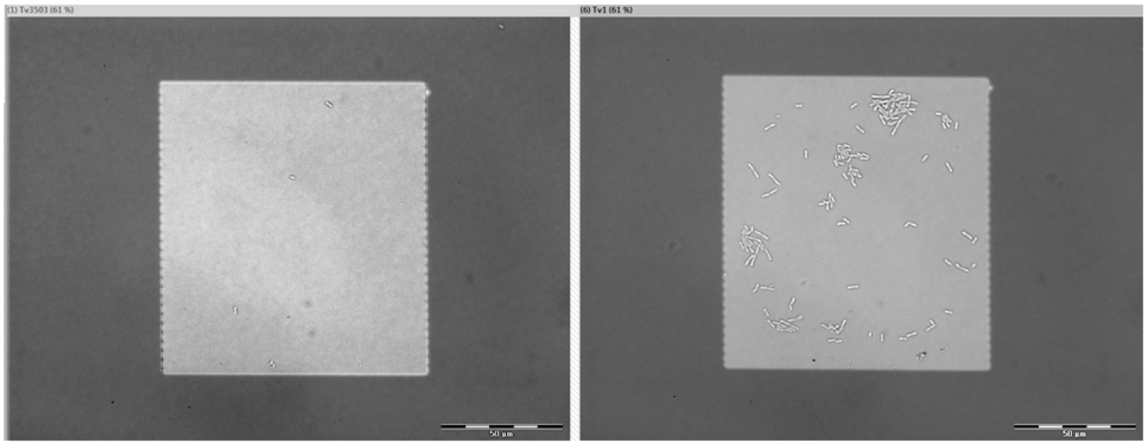


Fig. 5. Division of immobilized bacterial cells: (A) Four cells were captured inside the antibody-modified area (the bright square) when the substrate was incubated with a *S.* Typhimurium culture of 10^4 CFU/ml and (B) more cells were observed after the sample was incubated in cell-free medium for 20 hr at room temperature.

3.2.2 Crowded bacteria stand up

A surprising result is that bacteria initially immobilized in a lying-down orientation take a standing-up orientation as their density increases (Fig. 6A,B). This standing-up orientation of crowded cells has also been confirmed by laser scanning confocal microscopy (LSCM) images (Fig. 6C,D). Images (A) and (B) correspond to a sample incubated in growth medium at 37°C for 3 and 15 h, respectively. Notice that all the cells that were lying down in panel (A) appear to stand up in panel (B), corresponding to the increased bacterial density (see also panel (D)). Fig. 6C shows an LSCM image of a sample incubated in growth medium for

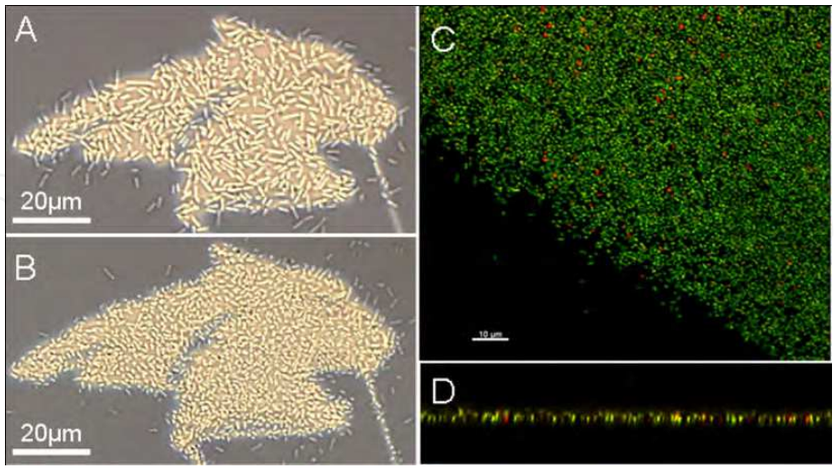


Fig. 6. Reorientation of immobilized *S.* Typhimurium cells due to surface population density. (A) Sample incubated in growth medium at 37°C for 3 h. (B) Same sample, incubated in growth medium at 37°C for 15 h. (C) LSCM image of a sample incubated in growth medium for 15 hr and then stored in PBS buffer at 4°C for 6 h and stained with viability stains. (D) Reconstituted Z-section image of the immobilized cells in (C).

15 hr and then stored in PBS buffer at 4°C for 6 h and stained with viability stains, which indicated that a majority of the cells were alive. The reconstituted Z-section image of the immobilized cells (Fig. 6D) further confirms that a majority of the cells took a standing-up orientation when crowded. Although at this time this behavior is not well understood, it might be related to the depletion of nutrients at the crowded bacterial positions and to the struggle of the bacteria to move away from their immobilized positions. This hypothesis is supported by the observation of an excess number of flagella produced by the immobilized bacteria (Suo et al, *Langmuir* 2008), presumably in an effort to free themselves from their positions.

3.2.3 Biofilm formation

The immobilized bacteria retained their cellular functions, including division, and continued to divide until a dense monolayer of bacteria filled the patterned area of the surface (Suo et al. 2008). We observed that biofilm started to form on the substrate after the initial two weeks. We incubated fifteen identical antibody-modified silicon chips with *S.* Typhimurium Δ*asd*::*kan*^{R} H71-pHC to form the initial bacterial patterns, and then all of the chips were rinsed with PBS and transferred to non-flow cell-free growth medium at room temperature. These silicon chips were prepared such that the patterned area was modified with anti-CFA/I fimbriae and the rest of the area was passivated using PEG molecules to prevent nonspecific attachment (Suo et al. 2008). After a predetermined incubation period, one silicon chip was taken out and rinsed with PBS to remove the unattached and loosely attached cells, then imaged under the optical microscope to determine the bacterial content on the substrate surface and its viability. The bacterial patterns were maintained for about two weeks, after which a bacterial biofilm started to form on the substrate, both inside the antibody-modified area and in the PEG-covered areas (Suo et al. 2008).

3.3 Investigation of mechanical properties of living *Salmonella*

We measured the physical properties of living *S.* Typhimurium using an atomic force microscope (AFM) at room temperature (Suo et al. 2009b). In a previous study of bacterial turgor pressure, a fairly gentle pressure, not enough to break the bacterial cell wall, was applied to bacterial cells (Yao et al. 2002). A fairly common belief is that a bacterium will lyse and die if its cell wall suffers severe mechanical damage, such as being punctured by an AFM tip. Our results show not only that *S.* Typhimurium cells can survive such damage, but also that the cells are still capable of self-replication.

3.3.1 Puncturing curves

A layer of *S.* Typhimurium cells in their exponential growth phase was immobilized in well-defined square patterns on a silicon wafer with ~20 cells within each square. Such immobilization lends itself to the continuous observation of the same group of cells under a light microscope before and after they are subjected to AFM probing. A sharp AFM tip was brought into contact with a live *S.* Typhimurium cell, and the loading force on the tip was increased until the tip punctured the cell wall (Fig. 7A). This was marked by a sharp decrease in the cantilever deflection value from ~150 nm to ~35 nm (inset in Fig. 7B). The tip was pushed continuously until it stopped penetrating the cell, which was interpreted as the

tip contacting the hard silicon surface underneath the organism. When the loading force reached a preset value (i.e. ~4 nN) the tip was lifted to ~2 μm above the cell surface in order to start another puncture cycle at an adjacent location on the organism. This puncturing process was repeated at a rate of 1 Hz or 2 Hz until each cell within a 7 × 7 μm² or 10 × 10 μm² area had undergone ~20 or ~40 puncturing events per μm². Each puncturing event generated a pair of force vs. displacement curves, or "puncturing curves" (Fig. 7B). These curves reveal a variety of information, including the true height of a live bacterium, its mechanical properties under physiological conditions, the pressure required to puncture the cell wall for a given tip geometry, and amount of cell deformation (indentation) before the cell wall is punctured. The inset shows the raw cantilever deflection versus displacement curves from which the puncturing curves were obtained by subtracting the cantilever deflection from the z-piezo displacement using a MatLab code developed by our group.

When a sharp tip punctures the cell wall of a Gram-negative bacterium it tears the three-layer cell wall structure before entering the bacterial cytoplasm. The pressure required to tear, or puncture, the cell wall is determined from the puncturing curve. The distance (~800 nm) between where the tip makes contact with the bacterial surface and where it touches the substratum is the measure of the true height of the bacterium in its physiological medium. The maximum penetration of F ≈ 2 nN at ~100 nm suggests that the bacterium deformed under the pressure exerted by the sharp tip and that the bacterial surface was indented by ~100 nm just before the tip punctured the cell wall. The modulus of elasticity of the living cell at the initial contact and the turgor pressure of the organism can be determined from the early part of the loading vs. tip penetration curve, using simple models such as the Hertzian model (Yao et al. 2002) or more complicated ones (Arnoldi et al. 2000).

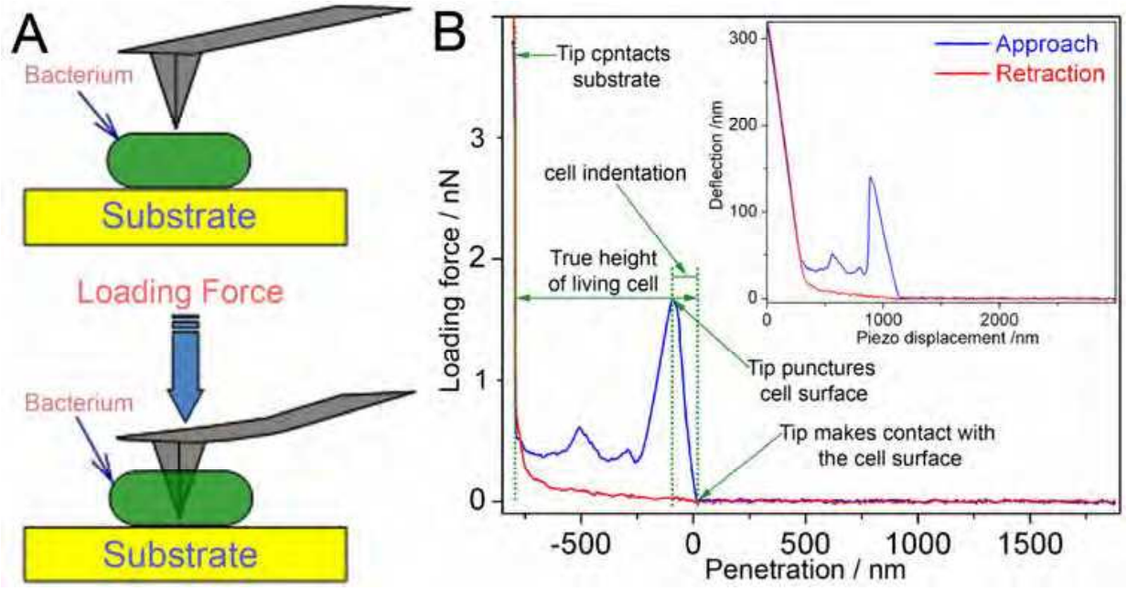


Fig. 7. (A) Schematics of puncturing experiments and (B) a typical puncturing curve

The pressure, *P*, required to penetrate the bacterial cell wall can be determined from $P = F/\pi r^2 \approx 5.0 \pm 0.8$ atm where *r* refers to the radius of the AFM tip. In our experiments, the average modulus of elasticity was calculated to be $0.4 \pm 0.2 \times 10^6$ Pa for living *S*. Typhimurium

cells in growth medium or in PBS buffer. This is comparable to results reported for *P. aeruginosa* and *E. coli* (Ingraham and Marr 1996; Yao et al. 2002).

The fine structures in the penetration range from ~250 nm through ~600 nm are ascribed to the resistance the tip experienced as it penetrated the cytoplasm of the organism. These forces are related to the tip geometry and, more importantly, to the tip aspect ratio. The force the tip experiences when it is pushed into the cytoplasm can be traced to two sources: (1) vertical resistance from the cell membranes, particularly from the peptidoglycan layer, during the initial 50 to 200 nm indentation (depending on the tip radius), until the tip overcomes this resistance and punctures the cell wall, and (2) lateral resistance as the tip tears the peptidoglycan layer. A tip with a high aspect ratio, such as a "spike tip" with a conical geometry, will give virtually no fine structures after the initial puncturing. A lower aspect ratio tip (pyramid tip) will tear the cell wall much more as it pushes into the cytoplasm and will introduce more severe cell damage than a spike tip (Suo et al. 2009b).

The common feature among all the puncturing curves is that there was very little or no resistance as the tip was pulled back from the cytoplasm of the bacteria (red line in Fig. 7B). This lack of resistance during tip retraction implies that there are only weak tip/cell wall interactions once the tip breaks the cell wall. The lipids, which are in continuous contact with the tip, offer no resistance to the motion of the tip as it is withdrawn from the cytoplasm.

3.3.2 Bacteria maintain their viability after being punctured multiple times

The size of the puncture hole while a tip penetrates a bacterium depends on the tip radius, the aspect ratio of the tip and the depth of penetration. However, there is no evidence of damage due to a puncture hole left behind from a puncturing event. It appears that puncture holes self-repair so that bacterial integrity and functionality are maintained, which is supported by the observation that punctured cells are capable of cell division, as is shown in Fig. 8. In this experiment, the colony of bacteria encircled by the blue dashed square in Fig. 8B was imaged before and after the puncturing. The data show that there was a one-to-one correspondence between the bacteria inside the blue square and the bright features in the AFM force-volume image. The light pixels in the dark background mark the locations of the punctured bacteria with a density of 20 puncturing events per square micron. Fig. 8B corresponds to the optical image acquired immediately after the puncturing experiment. We then kept the bacteria in their growth medium while taking images of the same location at one-minute intervals for 100 minutes. The effect of puncturing on the viability of *S.* Typhimurium was also studied using viability dyes. The majority of cells that had been punctured multiple times were able to divide, and there was no statistical difference found in survival rate between the punctured and the un-punctured cells, regardless of tip geometry or puncturing density varying between 20 and 40 puncturing events per μm^2.

It is also notable that the puncturing curves for dead *S.* Typhimurium cells were markedly different from those for living cells (Suo et al. 2009b). *S.* Typhimurium cells killed using glutaraldehyde did not show the force maximum associated with the puncturing event for live bacteria. Further analysis of the puncturing curves of dead cells revealed that dead bacteria shrink by about 40% and also appear to be softer, with an elastic modulus of ~0.13 ± 0.07 MPa in PBS as opposed to an elastic modulus of ~0.50 ± 0.10 MPa for living bacteria.

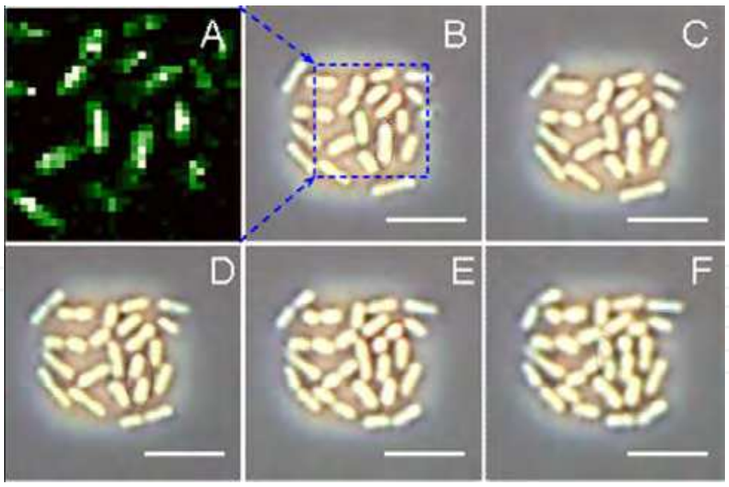


Fig. 8. Viability test: *S.* Typhimurium cells divide after being punctured multiple times. (A) An AFM force-volume image with bright spots showing the locations of the punctured bacteria. These spots have a one-to-one correlation with the cells shown in the blue dashed square of the optical image in panel B. Panels B-F show time-lapse images of the same group of bacteria taken 0, 25, 50, 75, and 100 min after the puncturing experiment, respectively. Cell division is highlighted by red, olive, and black ovals. Scale bar: 5 μm. Reprinted from Suo et al, *Langmuir* 2009 with permission from ACS

A model has been proposed to explain how bacteria survive the harsh damage done to their cell walls by an AFM tip. A detailed description of this model can be found elsewhere (Suo et al. 2009b). Briefly, the survival of punctured cells is owed to the remarkable features of the phospholipid bilayer that surrounds the cytoplasm of each bacterium. Phospholipid bilayers, at temperatures above the glass transition temperature, are composed of highly dynamic, fluid-like phospholipid molecules decorated with cross-membrane proteins (Osborn et al. 1972; Voet and Voet 1995). We believe that phospholipid molecules, including cross-membrane proteins with their hydrophobic regions intersecting the bilayers, undergo a reconfiguration (in a ns time frame) in response to AFM tip penetration (in a ms time frame). As the tip moves into or out of the cytoplasm, the fast-responding lipid membrane and membrane proteins are always in close contact with the tip surface and continuously seal against leakage into or out of the cytoplasmic and perhaps even the periplasmic regions regardless of the tip geometry. Apparently, a torn peptidoglycan layer is not a serious threat to cell viability.

4. Immunosensors for rapid detection of bacterial species

The rapid detection of microbial pathogens at a contamination site is critical for preventing the spread of the disease-causing microorganisms before the disease becomes epidemic. Diseases caused by water- and food-borne pathogens have been a serious threat to public health. A comprehensive examination of all the pathogen detection studies conducted over a period of 20 years showed that 38% were related to the food industry, 18% to clinical analysis, 16% to water and the environment and 27% to other areas (Lazcka et al. 2007), which emphasizes the importance of biosensor applications in these fields. *Salmonella* and *E. coli* were the most commonly detected pathogens in the aforementioned samples, with percentages of 33% and 27%, respectively (Turner 2011).

Bacterial pathogens are generally detected and identified using either a polymerase chain reaction (PCR) or antibody-based techniques (Velusamy et al. 2010a). The PCR approach offers an accurate determination of pathogens at the genomic level, but requires a proper design of primers targeting specific genes (Alexa et al. 2001; Holoda et al. 2005; Malorny et al. 2009). Antibody-based assays focus on the detection of bacterial antigens, and it is possible to detect multiple pathogens in a single assay using microarray techniques (Cai et al. 2005; Choi et al. 2008). Antibody-based techniques usually involve two events: capturing of the targeted pathogen on the sensor surface and follow-up signal generation. Efficient capturing is always desired, since it will facilitate converting captured pathogens into a detectable signal and, most importantly, a higher capture efficiency will result in a higher sensitivity (lower detection limit). Extensive research has been reported on the development of new detection methods that involve converting an already captured pathogen into an output signal by optical, electrochemical, mechanical or other means (O'Kennedy et al. 2009). During the past decade considerable advances were made in detecting pathogens by coupling immunological techniques with chemical and electronic actuators and techniques based on chemoluminescence (Wolter et al. 2008), electrochemical impedance (Geng et al. 2008), surface plasmon resonance (SPR) (Zordan et al. 2009), quartz crystal microbalance (QCM) (Adanyi et al. 2006; Boujday et al. 2008; Hirst et al. 2008) and wave guides (Adanyi et al. 2006). However, there has been little study of how to enhance the capture efficiency.

There have been many reports on the detection of *Salmonella* using antibody-based methods in the past decade, and in Table 1 we summarize these reports. The traditional detection of *Salmonella* spp. in food industries using differential growth media usually takes days to weeks (Amaguafia and Andrews 2000; Eaton et al. 2005). Detection based on immunoimmobilization or other antibody-based methods requires much less time, as evidenced by Table 1, showing typical assay times for these methods between 20 min and 6 hr. Such assays significantly reduce the time and effort needed for *Salmonella* detection and can possibly be used as on-site analysis in the critical initial stage of food poisoning.

As shown in Table 1, there have been many efforts to sense bacteria captured on an antibody-modified surface, ranging from the rather "traditional" method (ELISA) to new techniques such as microcantilevers. Most reports claim a detection limit close to 10^3 cells/ml, which could produce meaningful measurements in real applications, considering the infectious dose for human salmonellosis is around 10^3 CFU (Blaser and Newman 1982). Many different antibodies have been used, including commercial antibodies and antibodies prepared by the authors. It is desired for the readers to know the source, specificity, clonal type and purity of the antibodies used. However, it is not uncommon for inadequate information to be provided in this respect. For example, the term "anti-*Salmonella*" is often used without further specification.

Microarrays prepared using multiple antibodies are particularly efficient in the detection and identification of *Salmonella*. Cai et al. constructed a 8×15 model array for the identification of 20 common *Salmonella* serovars and evaluated the use of 117 target and 73 nontarget *Salmonella* strains (Cai et al. 2005). A total of 35 polyclonal antibodies against O-antigens or H-antigens (flagella) were used for serotyping *Salmonella* strains. Microarrays have been prepared using antibodies targeting multiple bacterial species. Choi et al. prepared a microarray containing four monoclonal antibodies against *E. coli* O157:H7, *S.* Typhimurium, *Yersinia enterocolitica*, and *Legionella pneumophila* (Choi et al. 2008). One problem with an antibody microarray is that generally the signal intensity of each antibody spot varies as a function of both the antibody concentration and the strain. This is actually

	Sensing element	Method	Detection limit	Medium	Assay time	Ref.
S. Infantis, S. Berta, S. enteritidis, S. Thompson, S. Typhimurium, S. Agona, S. Braenderup, S. Heidelberg, S. Dublin	paramagnetic beads coated with specific antibodies	immunomagnetic separation and enzyme immunoassay	10^4–10^6 CFU/ml	poultry environmental samples	48 h	(Leon-Velarde et al. 2009)
S. Typhimurium (ATCC 53648)	anti-*S.* Typhimurium mAb; physical absorption	sandwich ELISA; electrochemical detection (chronoamperometry)	5×10^3 cells/ml	spiked chicken meat	n.a.	(Salam and Tothill 2009)
	anti-*S.* Typhimurium mAb; covalent linking		20 CFU/ml			
S. Typhimurium LT 2	anti-*Salmonella* magnetic beads; anti-*Salmonella*-HRP (rabbit pAb)	electrochemical magneto-immunosensing	5×10^3 CFU/ml	pure culture	50 min	(Liebana et al. 2009)
			7.5×10^3 CFU/ml	milk diluted 1/10 in LB		
			0.108 × CFU/ml	spiked milk, pre-enriched		
S. Typhymurium	mAb	ellipsometry	10^3 CFU/ml.	pure culture	n.a.	(Bae et al. 2005)
S. Typhimurium	goat IgG against *Salmonella* CSA-1	fluorescence resonance energy transfer based method	10^3 cells/ml.	pure culture	n.a.	(Ko and Grant 2006)
			10^5 CFU/g	spiked pork	5 min	
S. Typhimurium (ATCC 14028)	AP labeled anti-*Salmonella*, Ab-coated magnetic microbeads	immunomagnetic separation and immuno-optical absorption	2.2×10^4 CFU/ml	chicken carcass rinse	2 h	(Liu et al. 2001)
			2×10^4 CFU/ml	pure culture		
S. enteritidis isolated from food samples	HRP-labeled pAb	microcantilever	10^5 CFU/ml	pure culture	40 min	(Ricciardi et al. 2010)
S. Typhimurium	biotinylated anti-*Salmonella* CSA-1	surface plasmon resonance	1×10^6 CFU/ml	spiked chicken carcass rinse	n.a.	(Lan et al. 2008)
S. enteritidis	mAb MO9; anti-S. enteridis	piezoelectric immunosensor	1×10^5 cells/ml	pure culture	35 min	(Si et al. 2001)
S. Typhimurium (ATCC 14028)	anti-*Salmonella* CSA-1	quartz crystal microbalance	10^5 and 10^7 cells/ml	pure culture	hours	(Su and Li 2005)

	Sensing element	Method	Detection limit	Medium	Assay time	Ref.
S. Typhymurium	pAb, polyvalent somatic O antibody	quartz crystal acoustic wave device	10^2 to 10^{10} cells/ml	pure culture	3 h 40 min	(Olsen et al. 2003)
S. paratyphi A S. enteriditis S. Typhimurium	mAb MO2, MO4 and MO9	quartz crystal microbalance	6×10^4 cells/ml 6×10^4 cells/ml 8×10^4 cells/ml	pure culture	40 min	(Wong et al. 2002)
S. Typhi (strain SKST), a clinical isolate	pAb flagellar and mAb	sandwich ELISA	10^4-10^5 CFU/ml	pure culture	6 h	(Kumar et al. 2008)
			10^2 CFU/ml	spiked milk, vegetable /meat/ chicken rinse		
S. Typhimurium (KCCM 11806)	mAb against S. Typhimurium	surface plasmon resonance	10^2 CFU/ml	pure culture	n.a.	(Oh et al. 2004)
S. Typhimurium	rabbit pAb against *Salmonella*	Faradic impedimetric immunosensor	10^5 CFU/mL	spiked milk	2 h	(Mantzila et al. 2008)
S. Typhimurium	mAb against *S.* Typhimurium	microarray, fluorescence staining	n.a.	pure culture	n.a.	(Choi et al. 2008)
>*20 Salmonella* serovars	35 antisera against O- and H- antigens	microarray, fluorescence staining	n.a.	pure culture	n.a.	(Cai et al. 2005)
S. Typhimurium (ATCC 14028), heat killed	anti-*Salmonella* goat pAb (capture Ab); pAb HRP-anti–*Salmonella* (detection Ab)	microarray, microfluidics, chemiluminescence	2×10^7 cells/ml	pure culture	18 min	(Karsunke et al. 2009)
			3×10^6 cells/ml		13 min	(Wolter et al. 2008)
S. Typhimurium (ATCC 14028), heat killed	Antibody-labeled microspheres	flow cytometry	2.5-500	pure culture	180 min	(Dunbar et al. 2003)
S. Typhimurium (ATCC 14028 and wild type)	anti-*S.* Typhimurium mouse mAb, rabbit pAb	multiplexed assay, chemiluminescence	10^4 - 10^5 cells/ml	spiked human fecal and beef samples	60 min	(Magliulo et al. 2007)
S. Typhimurium, heat killed	rabbit pAb anti-*Salmonella* sp. (capture Ab), anti-S. Typhimurium LPS, mAb (detection Ab)	flow-through fluorescence assay	8×10^4	spiked food and fecal samples	15 min	(Taitt et al. 2004)
			8×10^3		60 min	

pAb: poyclonal antibody; mAb: monoclonal antibody; n.a.: not available; CSA: common structural antigens; HRP: horseradish peroxidase; AP: alkaline phosphatase.

Table 1. Antibody-based detection of *Salmonella*

an expected result, considering the immobilization efficiency is affected by multiple factors, including the substrate surface chemistry; the purity, clonal type and affinity of the antibody; the type and expression level of the bacterial antigen; the incubation duration; and the medium. Since microarrays employ multiple antibodies it is important to evaluate their cross-reactivities to avoid false positive results. One example is the work by Rivas et al. evaluating the binding capacities and cross-reactivities of 200 different antibodies for the detection of environmental toxins (Rivas et al. 2008). Our work focuses on determining how these factors affect the efficiency of capturing pathogenic bacteria. When coupled with microfluidics techniques, antibody-based detection can lead to miniaturized and automated detectors, which are in great demand for field applications (Karsunke et al. 2009; Wolter et al. 2008). Our work on using immunoimmobilization for biosensor applications has just been submitted for publication. Besides the direct capturing of bacterial cells, microarrays prepared using antibodies, proteins or carbohydrates have been used to detect pathogenic bacteria including *Salmonella*. These microarrays do not target the bacterial cell, and further reviews can be found in Bacarese-Hamilton's work (Bacarese-Hamilton et al. 2002).

5. Summary and outlook

We have presented the immobilization and manipulation of *Salmonella* cells on flat substrates in order to conduct tests on individual bacteria and to develop a biosensor technology based on capturing living organisms. Such immobilization is achieved using antibodies covalently tethered to flat substrates such as silicon wafers in order to capture living bacteria in their physiological environment**.** The immobilization process takes advantage of the specific interaction between an antibody and the corresponding antigen on a bacterial surface. Thus, the method offers a high immobilization efficiency with a rate of initial attachment of over 100 microorganisms per minute per 100×100 μm^2 area for a bulk concentration of microorganisms of ~2×10^6 CFU/ml. Because the surfaces to which the targeted organisms are tethered are highly polished and flat, the detection of these organisms is highly efficient: a single bacterium in a field with an area of 200×200 μm^2 can easily be imaged under optical microscope. The key to the success of immunoimmobilization is the combination of proper selection of antibody-antigen pairs and optimization of the surface chemistry for antibody linkage. If the surface chemistry is designed carefully, living bacterial cells can be immobilized on a variety of substrates, including silicon wafer, glass, gold and steel. Our work of the last 5-6 years on *Salmonella* and *E. coli* suggests that the most efficient and reliable immunoimmobilization involves a limited number of specific surface antigens such as pili, flagella or O-antigens and the corresponding antibodies. Bacterial cells immobilized in this way are linked robustly enough to be tethered to their locations but still maintain their viability and functionality without any noticeable hindrance. The technique provides a promising platform for the *in situ* investigation of individual or small groups of localized bacterial cells in their natural physiological environments, which offers substantial promise for the future. For example, our work has proven that multiple puncturings of the cell wall of a bacterium by means of an AFM tip does not kill the organism. Until this technique was published, many prominent microbiologists had believed that the puncturing process would undoubtedly kill the organism. This new phenomenon opens up the possibility of introducing macromolecules or nanoparticles into the cytoplasm of an individual living bacterium and observing the response of the bacterium to the intrusion. This possibility is a fertile ground for new science, and only time will show how fruitful.

The high efficiency and specificity of immunoimmobilization can also be utilized in biosensor technology for the rapid detection and identification of pathogenic species in field applications and the sorting of specific species from mixed consortia. To sum up, immunoimmobilization as described in this chapter has great potential both in fundamental and in practical applications.

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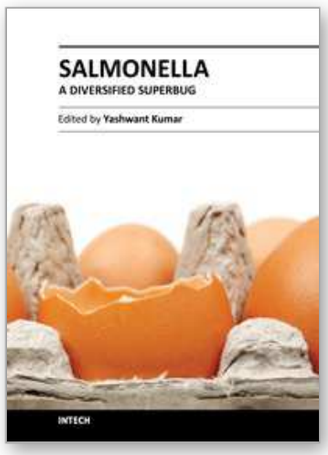

Salmonella - A Diversified Superbug
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Salmonella is an extremely diversified genus, infecting a range of hosts, and comprised of two species: enterica and bongori. This group is made up of 2579 serovars, making it versatile and fascinating for researchers drawing their attention towards different properties of this microorganism. Salmonella related diseases are a major problem in developed and developing countries resulting in economic losses, as well as problems of zoonoses and food borne illness. Moreover, the emergence of an ever increasing problem of antimicrobial resistance in salmonella makes it prudent to unveil different mechanisms involved. This book is the outcome of a collaboration between various researchers from all over the world. The recent advancements in the field of salmonella research are compiled and presented.

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