

detachment forces can be measured. For this reason, two variations of the AFM technique as force sensing have been used. The first one by Yamamoto *et al.* (1998) utilizes a microcantilever to measure the detachment force of a cell that has adhered to a material (Fig. 21a). As the cell adheres to a material in a medium, the XY-stage of the microscope is moved at a constant velocity. When the tip of the microcantilever touches the cell, a lateral load is applied to it and the cantilever is deflected corresponding to the deformation of the cell and the required shear force to detach the cell from the material.

The deflection of the cantilever is measured and the shear force applied to the cell, F , is calculated by Equation. (4):

$$F = k * \delta l \quad (4)$$

Where k is the force spring constant of the cantilever and δl is the deflection of the cantilever. The shear force applied to the cell is recorded as a function of the displacement of the XY-stage and thus graphs like that in Figure 22 are generated. The required shear force to detach the cell is equal to the maximum force that appears in the force-displacement curves. The integrated area underneath the curve is supposed to be the total energy necessary to detach the cell from the materials. Experiments using this technique have been done with murine fibroblasts and shear forces of detachment in the range from 300 to 500 nN have been reported.

The second variation (Sagvolden *et al.*, 1999) involves the use of an inclined atomic microscope cantilever and the laser beam deflection to measure the force. The set up is shown schematically in Figure 21b. The substrate moves at a constant velocity so that the cell is displaced and when it touches the tip of the cantilever the force on the cell increases. Gradually the cell is released from the surface and finally it is moved freely as the last bond is broken. The required force is recorded by the cantilever deflection with the help of a laser beam and a CCD array. During the experiment the typical force-displacement curve is recorded and the detachment force and energy is calculated. Experiments with this technique have been done with silica microspheres coated with glutaraldehyde and with cervical carcinoma cells cultured in hydrophobic or hydrophilic polystyrene substrates. Typical values of the detachment force have been measured in the range from 20 to 200 nN.

Discussion

All the above techniques provide us with an impressive array of tools for investigating bacteria-material interactions *in vitro*. Each one has certain advantages and disadvantages with respect to the others based on the sophistication of the equipment, the cost, the calibration of the force transducers, especially in the lower range, the optical observation, the non-disturbance of the bacteria under investigation etc.

In global tests, (static assays, flow chambers, rotating disc) where populations of bacteria are involved, a formidable problem is that of the existence of subpopulations of bacteria with stochastic adhesive

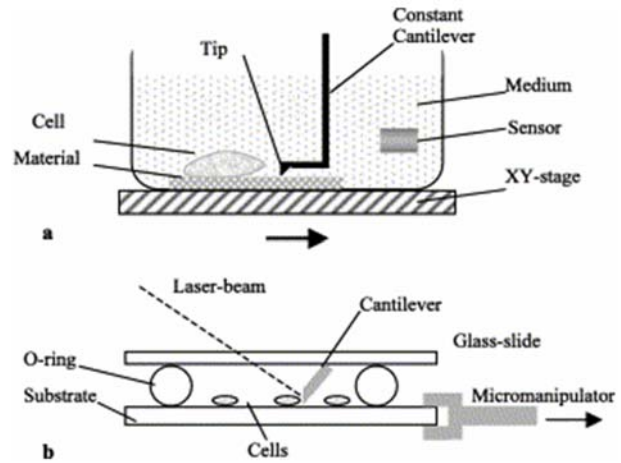


Figure 21. (a) A simplified schematic of the principle of measurement of a cell detachment with the use of shear force (Yamamoto *et al.*, 1998). (b) A schematic experimental set-up of a manipulation force microscope, which employs an inclined microcantilever and a laser beam deflection to measure the force (Sagvolden *et al.*, 1999).

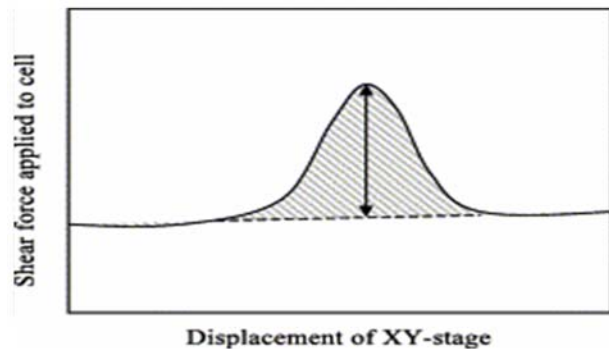


Figure 22. A schematic force-displacement curve. The cell detachment shear force is defined as the maximum force (Yamamoto *et al.*, 1998).

expression and of the uncertainty of the various degrees of adhesion of individual bacteria. For this reason, lately, probabilistic approaches attempt to characterize more accurately the attachment/detachment process. On the other hand, in single cell manipulation experiments, Monte Carlo simulations have been applied to understand the stochastic kinetics of the receptor-ligand bonds.

In all these techniques, the assumptions of the underlying mechanisms of the bacteria approaching the surface, the kinetics of receptor expression, the generation of focal adhesion points, the hypothesis regarding bond formation and breakage, and the number of specific receptors for the corresponding ligands add to the approximate nature of such investigations. In addition, the lack of a standard experimental procedure does not help in the impartial comparison of the techniques.

Measuring interaction forces using the AFM has the advantage of using a reliable device, however, the possible destructive deformation of bacteria due to the geometry of the tip is of concern and maybe a source for the observed variation of results. Moreover, the need to use a

physicochemical treatment in order to firmly anchor the cells to the probe may alter the cell surface properties, leading to false results.

Therefore, since the molecular and physical interactions that govern bacterial adhesion to biomaterials have not been understood in detail all the available preventive measures that decrease the rate of bacterial infections should be taken. These preventive strategies could be: experienced therapy teams to insert and maintain indwelling devices, maximum sterile barriers, such as sterile gloves, masks, gowns, caps, large drapes and careful handwashing. Use of these precautions has been linked to a four-fold decrease in the rate of bacteraemia. Moreover, cutaneous antimicrobials and antiseptics, ionic silver cuffs, combination of antibiotics with heparin, antiseptic hubs and antimicrobial coatings of biomaterial surfaces have shown good results against microbial colonization and produced bacteraemia, especially when the right antibiotics are chosen against each type of bacteria.

Concluding Remarks

A large amount of research work has been done and great achievements have been made in understanding the mechanisms of bacterial adhesion and prosthetic infection. However, since bacterial adhesion is a very complicated process affected by many factors, such as bacterial-material properties, environment, and, furthermore the experimental evaluation of the relative contributions of these factors is extremely difficult, more investigations are still needed to advance our understanding of the mechanisms of bacterial adhesion and prosthetic infection, and to attain appropriate methods to prevent them from happening. Most of the studies so far have utilized: different materials (glass, metals, polymers), different bacterial strains-species and concentrations, different experimental procedures (static, flow, AFM, time, environment). Polymer systems used in biointeraction studies do not allow for systematic-controlled variations in material surface properties. Surface chemical modification often leads to surface heterogeneity and increased roughness, trace impurities, in many polymers used, result in uncertainties. Therefore, a rigorous study of the effects of surface chemistry/topography on bacterial adhesion and protein adsorption requires a model system that allows precise control of the type and the configuration of functional groups at the substratum surface under dynamic conditions. All the techniques mentioned here, although they cannot be used routinely in the clinical field because of the cost, the complexity of the set up and the time they need in order to give results, they are necessary in the research field of quantitative definition of bacteria-material interactions.

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Discussion with Reviewers

L. Harris: In the section entitled “Serum or Tissue Proteins”, the author mentions vWF factor. Can the author comment in more detail on the fact *S. aureus* in particular has an adhesin that recognises vWF factor?

Authors: *S. aureus* has the ability to interact with and bind to several different plasma and extracellular matrix proteins such as fibrinogen, collagen, vitronectin and laminin, via protein adhesins of MSCRAMM (microbial surface components recognizing adhesive matrix molecules) family, which, in most cases are covalently anchored to the cell wall peptidoglycan. The first molecularly characterized MSCRAMMs of *S. aureus* are fibronectin-binding protein A (FnBPA), a collagen-binding protein (Can) and a fibrinogen-binding protein, clumping factor A (ClfA) (Foster and Hook, 1998). In addition to blood and matrix proteins, *S. aureus* interacts with platelets (Fallgren *et al.*, 2002). Among the factors released by platelets is von Willebrand factor (vWf), a large multifunctional glycoprotein characterized by high molecular weight multimers. Concerning bacterial proteins binding to vWf, there are only a few reports. The binding of *S. aureus* to vWf was first reported in 1997 (Hermann *et al.*, 1997) and later it was shown that protein A mediates the adherence of *S. aureus* to vWf (Hartleib *et al.*, 2000). In addition, a secreted *S. aureus* protein (vWbp) that binds vWf has recently been identified (Bjerketorp *et al.*, 2002). Therefore vWf binds to and promotes the surface adhesion of *S. aureus*.

L. Harris: Have the flow chambers been used to evaluate the influence of bacterial adhesins and their effect on adhesion to different biomaterials?

Authors: Dickinson *et al.* (1995, 1997) used a radial flow chamber in order to evaluate receptor-mediated bacterial adhesion under the influence of fluid shear and they showed that bacteria-surface interactions are influenced by the presence of proteins on the substratum surface (Figs. 14 and 15). Mohamed *et al.* (2000) used a parallel plate flow chamber and they showed that in the case of higher number of receptors/cell, *S. aureus* adhesion to collagen coated coverslips increases between shear rates 50-300 s⁻¹ and then decreases for shear rates higher than 500 s⁻¹ (Fig. 5). However, it has not been shown directly whether and how functional properties of bacterial adhesins are directly modulated by shear. To our knowledge, a directly related study of the influence of bacterial adhesins on adhesion, under the influence of flow conditions, is that

of Thomas *et al.* (2002) which showed that *E. coli* (expressing lectin-like adhesin FimH) attachment to erythrocytes switched from loose to firm upon a 10-fold increase in shear stress, due to increased bond formation (kinetic effects) and adhesin's ability to act as a force sensor. However, direct adhesin-biomaterial surface evaluation using flow chambers has not been reported yet.

J Douglas: What progress has been made in preventing bacterial adhesion to biomaterials either by changing biomaterial surface chemistry or by incorporating antimicrobial agents?

Authors: Coatings and surface treatments have been extensively studied (see Material Surface Characteristics) and a particular interest was devoted to silver as it combines antimicrobial activity and low human toxicity. Both physicochemical methods and surface engineering techniques (surface implantation) have been used in order to produce new, antibacterial surface properties. *In vitro* experimental results have shown that increased material hydrophilicity, antimicrobial coatings of biomaterial surfaces and especially ionic silver, and combination of antibiotics with heparin have good results against microbial colonization and bacteraemia. Clinical trials have shown that silver coated hemodialysis catheter offered a 42%, 65% and 66% reduction in bacterial positive cultures from skin, blood and catheter tip respectively (Bambauer *et al.*, 1998).

J Douglas: What are the problems associated with such strategies?

Authors: The main problems associated with changing biomaterial surface chemistry (surface energy) and incorporating antimicrobial agents are first of all the probable heterogeneity of the produced surface, especially when we have to deal with rough surfaces, and the probable

dissociation of the thin film antimicrobial coating, especially under high shear stresses. Moreover, surface treatments are not effective for long-term applications due to surface fouling and only surface bound antimicrobial technology offers advantages for long term applications. But even then, antimicrobial coatings should be checked for their bactericidal effects since immobilized ones are not as effective as soluble ones (James and Jayakrishnan, 2003) and atomic silver has not antibacterial effects in comparison to ionic silver (Davenas *et al.*, 2002).

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