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 been 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.

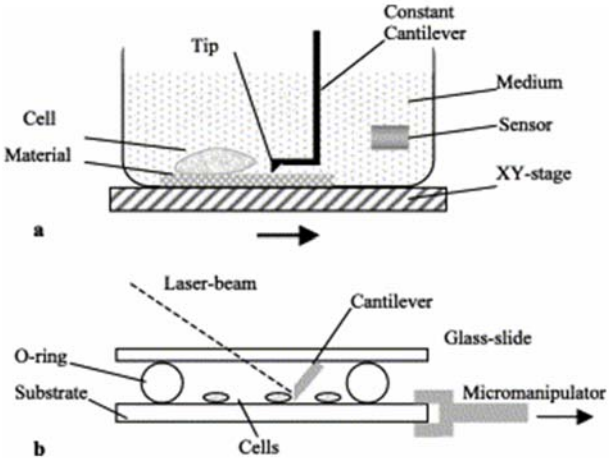

Figure 21. (a) A simplified schematic of the principle of measurement of a cell detachment with the use of shear force (Yamamoto *et al.*, 1998). (b) A schematic experimental set-up of a manipulation force microscope, which employs an inclined microcantilever and a laser beam deflection to measure the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

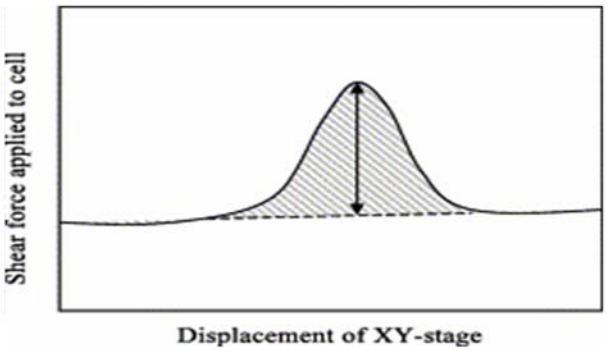

Figure 22. A schematic force–displacement curve. The cell detachment shear force is defined as the maximum force (Yamamoto *et al.*, 1998).

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 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 bacteriaemia. 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 bacteriaemia, 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.

References

Alfonso JL, Goldmann WH (2003) Feeling the forces: atomic force microscopy in cell biology. Life Sci **72:** 2553-2560.

An YH, Friedman RJ (1997) Laboratory methods for studies of bacterial adhesion. J Microbiol Meth **30:** 141-152.

An YH, Friedman RJ (1998) Concise review of mechanisms of bacterial adhesion to biomaterial surfaces. J Biomed Mater Res (Appl Biomater) **43:** 338-348.

Arciola CR, Campoccia D, Montanaro L (2002) Effects of antibiotic resistance of *Staphylococcus epidermidis* following adhesion to polymethylmethacrylate and to silicone surfaces. Biomaterials **23:** 1495-1502.

Bakker DP, Huijs FM, DeVries J, Klijnstra JW, Busscher JH, van der Mei HC (2003) Bacterial deposition to fluoridated and non-fluoridated polyurethane coatings with different elastic modulus and surface tension in a parallel plate and a stagnation point flow chamber. Col Surf B: Biointerf **32:** 179-190.

Balazs DJ, Triandafillu K, Chevolot Y, Aronsson B–O, Harms H, Descouts P, Mathieu HJ (2003) Surface modification of PVC endotracheal tubes by oxygen glow discharge to reduce bacterial adhesion. Surf Interf Anal **35:** 301-309.

Baumgartner JN, Cooper SL (1998) Influence of thrombus components in mediating staphylococcus aureus adhesion to polyurethane surfaces. J Biomed Mater Res **40:** 660-670.

Baveja JK, Willcox MDP, Hume EBH, Kumar N, Odell R, Poole-Werner LA (2004) Furanones as potential anti-bacterial coatings on biomaterials. Biomaterials **25:** 5003-5012.

Beech IB, Smith JR, Steele AA, Penegar I, Campbell SA (2002) The use of atomic force microscopy for studying interactions of bacterial biofilms with surfaces. Coll Surf B: Biointerf **23:** 231-247.

Bolshakova AV, Kiselyova OI, Filonov AS, Frolova OY, Lyubchenko YL, Yaminsky IV (2001) Comperative studies of bacteria with an atomic force microscopy operating in different modes. Ultramicrosc **86:** 121-128.

Bos R, van der Mei HC, Gold J, Busscher HJ (2000) Retention of bacteria on a substratum surface with micro-patterned hydrophobicity. FEMS Microbiol Let **189:** 311-315.

Bowen R, Hilal N, Lovitt RW, Wright CJ (1998) Direct measurements of a force of adhesion of a single biological cell using an atomic force microscope. Col Surf A: Physicochem Eng Asp **136:** 231-234.

Boyd RD, Verran J, Jones MV, Bhakoo M (2002) Use of Atomic Force Microscope to determine the effect of substratum surface topography on bacterial adhesion. Langmuir **18:** 2343-2346.

Bruinsma GM, van der Mei HC, Busscher HJ (2001) Bacterial adhesion to surface hydrophilic and hydrophobic contact lenses. Biomaterials **22:** 3217-3224.

Buczynski BW, Kory MM, Steiner RP, Kittinger TA, Ramsier RD (2003) Bacterial adhesion to zirconium surfaces. Col Surf B: Biointerf **30:** 167-175.

Bunt CR, Jones DS, Tucker IG, (1993) The effects of pH, ionic strength and organic phase on the bacterial adhesion to hydrocarbons (BATH) test. Intern J Pharm **99:** 93-98.

Bunt CR, Jones DS, Tucker IG, (1995) The effects of pH, ionic strength and polyvalent ions on the cell surface hydrophobicity of *Escherichia coli* evaluated by the BATH and HIC methods. Intern J Pharm **113:** 257-261.

Chang HT, Rittmann BE, Amar D, Heim R, Ehrlinger O, Lesty Y (1991) Biofilm detachment mechanisms in a liquid fluidised bed. Biotechnol Bioeng **38:** 499-506.

Chang CC, Merritt K (1992) Microbial adherence on poly(methyl methacrylate) (PMMA) surfaces. J Biomed Mater Res **26:** 197-207.

Christensen GD, Baldassarri L, Simpson WA (1994) Colonization of medical devices by coagulase-negative staphylococci". In: Infections Associated with Indwelling Medical Devices, 2nd edition. Bisno AL, Waldvogel FA (eds). American Society for Microbiology, Washington, DC. Chapter 5,

Cordero J, Munuera L, Folgueira MD (1996) The influence of the chemical composition and surface of the implant on infection. Injury **27:** S-C34-37.

Costerton JW (1999) Introduction to biofilm. Intern J Antimicrob Agents **11:** 217-221.

C osterton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science **284:** 1318-1322.

Davenas J, Thevenard P, Philippe F, Arnaud MN, (2002) Surface implantation treatments to prevent infection complications in short term devices. Biomol Eng **19:** 263-268.

DeJong P TeGiffel MC, Kiezebrink EA, (2002) Prediction of the adherence, growth and release of microorganisms in production chains. Intern J Food Microbiol **74:** 13-25.

Dickinson RB, Cooper SL (1995) Analysis of shear-dependent bacterial adhesion kinetics to biomaterial surfaces. Bioeng Food Nat Prod **41:** 2160-2174.

Dickinson RB, Nagel JA, McDevitt D, Foster TJ, Proctor RA, Cooper SL (1995) Quantitative comparison of clumping factor- and coagulase mediated *Staphylococcus aureus* adhesion to surface-bound fibrinogen under flow. Infect Immun **63:** 3143-3150.

Dickinson RB, Nagel JA, Proctor RA, Cooper SL (1997) Quantitative comparison of shear-dependent *Staphylococcus aureus* adhesion to three polyurethane ionomer analogs with distinct surface properties. J Biomed Mater Res **36:** 152-162.

Diekema DJ, Pfaller MA, Schmitz FJ (2001) Survey of infections due to staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and the Pacific region for the Sentry antimicrobial surveillance program, 1997-1999. Clin Infect Dis **32:** 114-132.

Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microb Reviews **15:** 167-193.

Duddridge JE, Kent CA, Laws JF (1982) Effect of surface shear stress on the attachment of *Pseudomonas fluorescens* to stainless steel under defined flow conditions. Biotechnol Bioeng **XXIV:** 153-164.

Dufrene YF (2001) Application of Atomic Force Microscopy to microbial surfaces: from reconstituted cell surface layers to living cells. Micron **32:** 153-165.

Dufrene YF (2002) Atomic Force Microscopy: a powerful tool in Microbiology. J Bacteriol **184:** 5205-5213.

Dufrene YF (2003) Recent progress in the application of atomic force microscopy imaging and force spectroscopy to microbiology. Cur Opin In Microbiol **6:** 317-323.

Duguid IG, Evans E, Brown MR, Gilbert P (1992) Effect of biofilm culture upon the susceptibility of *Staphylococcus epidermidis* to tobramycin. J Antimicrob Chemother **30:** 803-810.

Edwards KJ, Rutenberg AD (2001) Microbial response to surface microtopography: the role of metabolism in localized mineral dissolution. Chem Geol **180:** 19-32.

Fallgren C, Ljungh A, Shenkman B, Varon D, Savion N (2002) Venous shear stress enhances platelet mediates *staphylococcal* adhesion to artificial and damaged biological surfaces. Biomaterials **23:** 4581-4589.

Fang HHP, Chan K-Y, Xu L-C (2000) Quantification of bacterial adhesion forces using atomic force microscopy. J Microbiol Meth **40:** 89-97.

Flemming RG, Capelli CC, Cooper SL, Proctor RA (2000) Bacterial colonization of functionalized polyurethanes. Biomaterials **21:** 273-281.

Fletcher M, Marshall KC (1982) Bubble contact angle method for evaluating substratum interfacial characteristics and its relevance to bacterial attachment. Appl Environ Microbiol **44:**184–192.

Francois P, Vaudaux P, Nurdin N, Mathieu HJ, Descouts P, Lew DP (1996) Physical and biological effects of a surface coating procedure on polyurethane catheters. Biomaterials **17:** 667-678.

Garcia-Saenz MC, Arias-Puente A, Fresnadillo-Martinez MJ, Matilla-Rodriguez A (2000) *In vitro* adhesion of *Staphylococcus epidermidis* to intraocular lenses. J Cataract Refract Surg **26:** 1673-1679.

Geesey GG (2001) Bacterial behaviour at surfaces. Cur Opin In Microbiol **4:** 296-300.

Gottenbos B, van der Mei HC, Busscher HJ (2000) Initial adhesion and surface growth of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* on biomedical polymers. J Biomed Mater Res **50:** 208-214.

Gottenbos B, Busscher HJ, Van Der Mei HC, Nieuwenhuis P (2002) Pathogenesis and prevention of biomaterial centered infections. J Mat Sci: Mat In Med **13:** 717-722.

Gotz F (2002) *Staphylococcus* and biofilms. Mol Microb **43:** 1367-1378.

Gray ED, Peters G, Verstegen M, Regelmann WE (1984) Effect of extracellular slime substance from *Staphylococcus epidermidis* adhesion on human cellular immune response. Lancet **18:** 365-367.

Harris LG, Tosatti S, Wieland M, Textor M, Richards RG (2004) *Staphylococcus aureus* adhesion to titanium oxide surfaces coated with non-functionalized and peptide-functionalized poly (L-lysine)-grafted-poly(ethylene glycol) copolymers. Biomaterials **25:** 4135-4148.

Hauert R (2003) A review of modified DLC coatings for biological applications. Diam Rel Mater **12:** 583-589.

Heilmann C, Schweitzer O, Gerke C, Vanittanakom N, Mack D, Gotz F (1996) Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. Mol Microbiol **20:** 1083-1091.

Henriques M, Azeredo J, Oliveira R (2004) Adhesion of Candida albicans and Candida dubliniensis to acrylic and hydroxyapatite. Col Surf B Biointerf **33:** 235-241.

Hermansson M (1999) The DLVO theory in microbial adhesion. Coll Surf B: Biointerf **14:** 105-119.

Herrmann M, Vaudaux PE, Pittit D (1988) Fibronectin, fibrinogen, and laminin act as mediators of adherence of clinical staphylococci isolates to foreign material. J Infect Dis **158:** 693–701.

Hubble J, Ming F, Eisenthal R, Whish W (1996) Progressive detachment of cells from surfaces: a consequence of heterogeneous ligand populations or a multi-site binding equilibrium? J Theor Biol **182:** 169-171.

Hubble J (2003) Monte Carlo simulation of biospecific interactions between cells and surfaces. Chem Eng Sci **58:** 4465-4474.

Huebner J, Goldmann DA (1999) Coagulase-negative staphylococci: role as pathogens. Ann Rev Med **50:** 223-226.

Isberg RR, Barnes P (2002) Dancing with the host: flow-dependent bacterial adhesion. Cell **110:** 1-4

James NR, Jayakrishnan A (2003) Surface thiocyanation of plasticized poly(vinyl chloride) and its effect on bacterial adhesion. Biomaterials **24:** 2205-2212.

Jansen B, Hartmann C, Schumacher-Perdreau F, Peters G (1991) Late onset endophthalmitis associated with intraocular lens: a case of molecularly proved *S. epidermidis* aetiology. Br J Ophthalml **75:** 440-441.

Jenal U (2004) Cyclic di-guanosine-monophosphate comes of age: a novel secondary messenger involved in modulating cell surface structures in bacteria? Cur Opin Microbiol **7:** 185-191.

Jucker BA, Zehnder AJB, Harms H (1998) Quantification of polymer interactions in bacterial adhesion. Environ Sci Technol **32:** 2909-2915.

Karamanos NK, Panagiotopoulou HS, Syrokou A, Frangides C, Hjerpe A, Dimitrakopoulos G, Anastassiou ED (1995) Identity of macromolecules present in the extracellular slime layer of *Staphylococcus epidermidis*. Biochimie **77:** 217-224.

Kiremitci-Gumustedereliogl u M, Pesmen A (1996) Microbial adhesion to ionogenic PHEMA, PU and PP implants. Biomaterials **17:** 443-449.

Kirov SM (2003) Bacteria that express lateral fragella enable dissection of the multifunctional roles of fragella in pathogenesis. FEMS Microbiol Let **224:** 151-159.

Klapper I, Rupp CJ, Cargo R, Purvedorj B, Stoodley P (2002) Viscoelastic fluid description of bacterial biofilm material properties. Biotech Bioeng **80:** 289-296.

Kohnen W, Kolbenschlag C, Teske-Keiser S, Jansen B (2003) Development of a long-lasting ventricular catheter impregnated with a combination of antibiotics. Biomaterials **24:** 4865-4869.

Krimer V, Merkert H, Von Eiff C, Frosch M, Eulert J, Lohr JF, Hacker J, Ziebuhr W (1999) Detection of *Staphylococcus aureus* and *Staphylococcus epidermidis* in clinical samples by 16S rRNA-directed in situ hybridisation. J Clin Microbiol **37:** 2667-2673.

Kuusela P, Vartio T, Vuento M, Myhre EB (1985) Attachment of staphylococci and streptococci on fibronectin, fibronectin fragments, and fibrinogen bound to a solid phase. Infect Immunol **50:** 77-85.

Lejeune P (2003) Contamination of abiotic surfaces: what a colonizing bacterium sees and how to blur it. Trend in Microbiol **11:** 179-184.

Liu Y, Tay J-H (2001) Metabolic response of biofilm to shear stress in fixed-film culture. J Appl Microbiol **90:** 337-342.

Liu Y, Tay J-H (2002) The essential role of hydrodynamic shear force in the formation of biofilm and granular sludge. Water Res **36:** 1653-1665.

Lower SK, Tadanier CJ, Hochella MF (2000) Measuring interfacial and adhesion forces between bacteria and mineral surfaces with biological force microscopy Geochim Cosmochim Acta **64:** 3133-3139.

Mack D (1999) Molecular mechanisms of *Staphylococcus epidermidis* biofilm formation. J Hosp Inf **43:** S113-S125.

Mah T-FC, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends in Microbiol **9:** 34-39.

Maki DG, Stolz SM, Wheeler S, Mermel LA (1997) Prevention of central venous catheter-related bloodstream infection by use of an antiseptic-impregnated catheter: a randomised, controlled trial. Ann Intern Med **127:** 257-266.

Mascari L, Ymele-Leki P, Eggleton CD, Speziale P, Ross JM (2003) Fluid shear contributions to bacteria cell detachment initiated by a monoclonal antibody. Biotechnol Bioeng **83:** 65-74.

Mayer C, Moritz R, Kirschner C, Borchard W, Maibaum R, Wingender J, Flemming H-C (1999) The role of intermolecular interactions: studies on model systems for bacterial biofilms. Intern J Biol Macromol **26:** 3-16.

McWhirter MJ, McQuillan AJ, Bremer PJ (2002) Influence of ionic strength and pH on the first 60 min of Pseudomonas aeruginosa attachment to ZeSe and to TiO_2 monitored by ATR-IR spectroscopy. Col Surf B: Biointerf **84:** 17-25.

Medilanski E, Kaufmann K, Wick L, Wanner O, Harms H (2002) Influence of surface topography of stainless steel on bacterial adhesion. Biofoul **18:** 193-203.

Merriam CV, Fernandez HT, Citror DM, Tyrrell KL, Warren YA, Goldstein EJC, (2003) Bacteriology of human bite wound infections". Anaerobe **9:** 83-86.

Miele PS, Kogulan PK, Levy CS (2001) Seven cases of surgical native valve endocarditis caused by coagulase-negative staphylococci: an underappreciated disease. Am Heart J **142:** 571-576.

Ming F, Whish W, Hubble J, Eisenthal R (1998) Estimation of parameters for cell-surface interactions: maximum binding force and detachment constant. Enzyme Microbiol Technol **22:** 94-99.

Miorner H, Myhre E, Bjorck L, Kronvall G (1980) Effect of specific binding of human albumin, fibrinogen, and immunoglobulin G on surface characteristics of bacterial strains as revealed by partition experiments in polymer phase system. Infect Immunol **29:** 879–885.

Missirlis YF, Spiliotis AD (2002) Assessment of techniques used in calculating cell-materials interactions. Biomolec Engin **19:** 287-294.

Mohamed N, Rainier TR, Ross JM (2000) Novel experimental study of receptor-mediated bacterial adhesion under the influence of fluid shear. Biotechnol Bioeng **68:** 628-636.

Monzón M, Concepción Oteiza C, Leiva J, Lamata M, Amorena B (2002) Biofilm testing of *Staphylococcus epidermidis* clinical isolates: low performance of vancomycin in relation to other antibiotics. Diagn Microbiol Infect Dis **44:** 319-324.

Morra M, Cassinelli C (1996) *Staphylococcus epidermidis* adhesion to films deposited from hydroxyethylmethacrylate plasma. J Biomed Mater Res 31: 149-155.

Morra M, Cassinelli C (1997) Bacterial adhesion to polymer surfaces: A critical review of surface thermodynamic approaches. J Biomater Sci Polymer Edn **9:** 55-74.

Moter A, Gobel UB (2000) Fluorecence in situ hybridization (FISH) for direct visualization of microorganisms. J Microbiol Meth **41:** 85-112.

O'Gara JP, Humphreys H (2001) *Staphylococcus epidermidis* biofilms: importance and implications. J Med Microbiol **50:** 582-587.

O'Toole G, Kaplan HB, Kolter R (2000) Biofilm formation as microbial development. Ann Rev Microbiol **54:** 49-79.

Ong Y-L, Razatos A, Georgiou G, Sharma MM (1999) Adhesion forces between *E. coli* bacteria and biomaterial surfaces. Langmuir **15:** 2719-2725.

Pagano L, Tacconelli E, Tumbarello M, (1997) Bactaraemia in patients with hematological malignancies. Analysis of risk factors, etiological agents and prognostic indicators. Haematologica **82:** 415-419.

Paragioudaki M, Stamouli V, Kolonitsiou F, Anastassiou ED, Dimitrakopoulos G, Spiliopoulou I (2004) Intravenous catheter infections associated with bacteraemia: a 2-year study in a University Hospital. Eur Soc Clin Microbiol Infect Dis **10:** 431-435.

Park KD, Kim YS, Han DK, Kim YH, Lee EHB, Suh H, Choi KS (1998) Bacterial adhesion on PEG modified polyurethane surfaces. Biomaterials **19:** 851-859.

Paulsson M, Gouda I, Larm O, Ljungh A (1994) Adherence of coagulase-negative staphylococci to heparin and other glucosaminoglycans immobilized on polymer surfaces. J Biomed Mater Res **28:** 311-317.

Perdreau-Remington F, Stefanik D, Peters G (1996) A four-year prospective study on microbial ecology of explanted prosthetic hips in 52 patients with aseptic prosthetic joint loosening. Eur J Clin Microbiol Infect Dis **15:** 160-165.

Pessoa-Silva CL, Miyasaki CH, De Almeida MF, Kopelman BI, Raggio RL, Wey SB (2001) Neonatal late-onset bloodstream infection: attributable mortality, excess of length of stay and risk factors. Eur J Epidemiol **17:** 715-720.

Peters G, Locci R, Pulverer G (1982) Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. J Infect Dis **146:** 479-482.

Raad I (1998) Intravascular-catheter-related infections. Lancet **351:** 893-898.

Razatos A, Ong Y-L, Sharma MM, Georgiou G (1998) Evaluating the interaction of bacteria with biomaterials using atomic force microscopy. J Biomater Sci Polym Edn **9:** 1361-1373.

Rijnaarts HHM, Norbe W, Bouwer EJ, Lyklema J, Zehnder AJB (1995) Reversibility and mechanism of bacterial adhesion. Col Surf B: Bioint **4:** 5-22.

Robichon D, Girard J-C, Cenatiempo Y, Cavallier J-F (1999) Atomic force microscopy imaging of dried or living bacteria. C R Acad Sci Paris, Life Sci **322:** 687-693.

Rupp ME, Archer GD (1994) Coagulase-negative staphylococci: pathogenesis associated with medical progress. Clin Infect Dis **19:** 231-245.

Sagvolden G, Giaever I, Pettersen EO, Feder J (1999) Cell adhesion force microscopy Proc Natl Acad Sci USA **96:** 471-476.

Sanderson NM, Guo B, Jacob AE, Handley PS, Cunniffe JG, Jones MN (1996) The interaction of cationic liposomes with the skin-associated bacterium *Staphylococcus epidermidis*: effects of ionic strength and temperature. Biochim Bioph Acta (BBA) – Biomembr **1283:** 207-214.

Scheuerman TR, Camper AK, Hamilton MA (1998) Effects of substratum topography on bacterial adhesion. J Col Interf Sci **208:** 23-33.

Schierholz JM, Beuth J, Pulverer G (2000) Evidence for a self-fulfilling hypothesis: chlorhexidine dressing for reduction of microbial colonization of the skin with central venous catheters. J Hosp Infect **44:** 241-242.

Scierholz JM, Beuth J (2001) Implant infections: a haven of opportunistic bacteria. J Hosp Infect **49:** 87-93.

Shi L, Ardehali R, Caldwell KD, Valint P (2000) Mucin coating on polymeric material surfaces to suppress bacterial adhesion. Col Surf B: Biointerf **17:** 229-239.

Silver S (2003) Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiol Rev **27:** 341-353.

Souvenir D, Anderson DEJ, Palpant S, Mroch H, Askin S, Anderson J, Claridge J, Eiland J, Malone C, Garrison MW, Watson P, Cambell DM (1998) Blood cultures positive for coagulase-negative staphylococci: antisepsis, pseudobacteraemia and therapy of patients. J Clin Microbiol **36:** 1923-1926.

Speranza G, Gottardi G, Pederzolli C, Lunelli L, Canteri R, Pasquardini l, Carli E, Lui A, Mniglio D, Brugnara M, Anderle M (2004) Role of chemical interactions in bacterial adhesion to polymer surfaces". Biomaterials **25:** 2029-2037.

Stewart PS, Costerton JW (2001) Antibiotic resistance of bacteria in biofilms. Lancet **358:** 135-138.

Stoodley P, Lewandowski Z, Boyle JD, Lappin-Scott HM (1999) Structural deformation of bacterial biofilm caused by short-term fluctuations in fluid shear: an in situ investigation of biofilm rheology. Biotechnol Bioeng **65:** 83-92.

Tacconelli E, Tumbarello M, Pittiruti M, Leone F, Lucia MB, Cauda R, Ortona L (1997) Central venous catheter-related sepsis in a cohort of 366 hospitalised patients. Eur J Clin Microbiol Infect Dis **16:** 203-209.

Taylor RL, Verran J, Lees GC, Ward AJP (1998) The influence of substratum topography on bacterial adhesion to polymethyl methacrylate. J Mater Sci: Mater Med **9:** 17-22.

Tegoulia VA, Cooper SL (2002) *Staphylococcus aureus* adhesion to self-assembled monolayers: effect of surface chemistry and fibrinogen presence. Col Surf B: Biointerf **24:** 217-228.

Trautner BW, Darouicher. O (2004) Role of biofilm in catheter-associated urinary tract infection. Am J Inf Contr **32:** 177-183.

Vacheethasanee K, Marchant RE (2000) Surfactant polymers designed to suppress bacterial (*Staphylococcus epidermidis*) adhesion on biomaterials. J Biomed Mater Res **50:** 302-312.

Vacheethasanee K, Temenoff JS, Higashi JM, Gary A, Anderson JM, Bayston R, Marchant RE (1998) Bacterial surface properties of clinically isolated *staphylococcus epidermidis* strains determine adhesion on polyethylene. J Mater Res **42:** 425-432.

Van Loosdrecht MCM, Lyklema J, Norde W, Schraa G, Zehnder AJB (1987) The role of bacterial cell wall hydrophobicity in adhesion. Appl Environ Microbiol **53:** 1893–1897.

Vaudaux P, Yasuda H, Velazco MI, Huggler E, Ratti I (1990) Role of host and bacterial factors in modulating staphylococcal adhesion to implanted polymer surfaces. J Biomater Appl **5:** 134-153.

Vaudaux PE, Waldvogel FA, Morgenthaler JJ, Nydegger UE (1984) Adsorption of fibronectin onto polymethylmethacylate and promotion of *Staphylococcus aureus* adherence. Infect Immunol **45:** 768–774.

Vincent J-L (2003) Nosocomial infections in adult intensive-care units. Lancet **361:** 2068-2077.

Von Eiff C, Peters G, Heilmann C (2002) Pathogenesis of infections due to coagulase-negative staphylococci. Lancet Infect Dis **2:** 677-685.

Vuong C, Otto M (2002) *Staphylococcus epidermidis* infections. Microbes Infect **4:** 481-489.

Whitehead KA, Colligon JS, Verran J (2004) The production of surfaces of defined topography and chemistry for microbial retention studies, using ion beam sputtering technology. Intern Biodeter Biodegrad **54**: 143-151.

Willcox MDP, Harmis N, Cowell B., Williams T, Holden BA (2001) Bacterial interactions with contact lenses; effects of lens material, lens wear and microbial physiology. Biomaterials **22:** 3235-3247.

Willing GA, Ibrahim TH, Etzler FM, Neuman RD (2000) New approach to the study of particle-surface adhesion using atomic force microscopy. J Col Interf Sci **226:** 185-188.

Woodyard LL, Bowersock TL, Turek JJ, McCabe GP, DeFord J (1996) A comparison of the effects of several silver-treated intravenous catheters on the survival of staphylococci in suspension and their adhesion to the catheter surface. J Contr Rel **40:** 23-30.

Yamamoto A, Mishima S, Maruyama N, Sumita M (1998) A new technique for direct measurement of the shear force necessary to detach a cell from a material. Biomaterials **19:** 871-879.

Zlatanova J, Lindsay SM, Leuda SH (2000) Single molecule force spectroscopy in biology using the atomic force microscopy. Progr Bioph Molec Biol **74:** 37-61.

Web References

Fletcher M, Decho A (2001) Biofilms. Encyclopedia of life siences. Nature Publising Group/ www.els.net

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, clumbing 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 bacteriaemia. 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).

Additional References

Bambauer R, Mestres P, Schiel R, Schneidewind J M, Goudjinou R, Latza R, Innlger R, Bambauer S, Shishansi P (1998) Surface treated large-bore catheters with silver based coatings versus untreated catheters for extracorporeal detoxification methods. ASAIO J **44:** 303-308.

Bjerketorp J, Nilsson M, Ljungh A, Flock J-I, Jacobsson K, Frykberg L (2002) A novel von Willebrand factor binding protein expressed by Staphylococcus aureus. Microbiol **148:** 2037-2044.

Hartleib J, Kohler N, Dickinson R B, Chhatwal G S, Sixma J J, Hartford O M, Foster T J, Peters G, Kehrel B E, Herrmann M (2000) Protein A is the von Willebrand factor binding protein on *Staphylococcus aureus*. Blood **96:** 2149-2156.

Herrmann M, Hartleib J, Kehrel B, Montgomery R R, Sixma J J, Peters G (1997) Interaction of von Willebrand factor with *Staphylococcus aureus*. J Infect Dis **176:** 984–991.

Foster T J, Hook M (1998) Surface protein adhesins of *Staphylococcus aureus*. Trends Microbiol **484:** 484-488.

Thomas W E, Trintchina E, Forero M, Vogel V, Sokurenko E V (2002) Bacterial adhesion to target cells enhanced by shear force. Cell **109:** 913-923.