Bacterial interactions with nanostructured surfaces

Hizal, Ferdi

CHAPTER 6

General Discussion

Metals are widely used in water-distribution systems, filtration processes, cooling facilities and power plants and in the biomedical field. However, wherever there is an aqueous environment, a surface support and available nutrients regardless how limited in the amount, a biofilm will eventually form. This thesis proudly revealed two main outcomes that are of relevance in applications where biofilm formation is considered troublesome;

1- a way to produce large scale micro-nanoscaled surfaces with an impact on bacterial adhesion, detachment and transmission.

2- a new phenomenon named "pressure-induced EPS" production and associated cell death which should be taken into account for future studies on "nanostructured" surface designs for reducing biofilm formation.

Due to their high area to volume ratio and unique chemical and physical properties, nanoscaled surfaces have consistently been called as being promising to control biofilm formation in widely varying applications, notably including biofilm-associated infections related to biomaterials implants and devices.[1] This "control" is often attributed to be either with respect to bacterial adhesion or detachment, or enhanced housing capacities of antibiotics. Nanostructured surfaces can be fabricated with a random[2–4] or a periodic[5–7] nanostructure. Randomly structured surfaces are usually cheap, easy to fabricate and sturdy compared to periodically structured surfaces, which are ideal to study biological interaction mechanisms, such as bacterial adhesion and biofilm formation.

Throughout this thesis, we have explored a number of different advanced nanostructured surfaces, including nanoporous and nanopillared aluminum (Chapter 2), titanium (Chapter 5), and high-precision nanopillared silicon surfaces (Chapters 3 and 4). Common to all nanostructured surfaces included in this thesis, are reduced bacterial adhesion forces as compared with bacterial adhesion forces on the smooth counterparts of the same materials. The mere presence of an adhesion force, regardless how small, was sufficient to cause bacteria to adhere to nanostructured surfaces under convective-diffusional mass transport at shear rates as low as 6 s^{-1} (Chapters 3 and 5). However, convective-diffusion at a much higher (37 s^{-1}) shear rate (Chapter 2) did show reduced bacterial adhesion to nanostructured surfaces, indicating that environmental factors such as the presence of high shear have a major impact on the usefulness of nanostructured surfaces in controlling bacterial adhesion.[8]

Though reduced bacterial adhesion forces on nanostructured surfaces do not necessarily affect adhesion, detachment may well be higher as seen in Chapter 3 where the fluid shear rate was increased from 6 to 60 s^{-1} to trigger bacterial detachment. Bacteria adhering with the lowest adhesion forces detached the most compared to strongly adhering ones (e.g. those adhering on nanopillared *versus* smooth surfaces). Similarly, during transmission (Chapter 3) from one surface to another, an interplay of adhesion and detachment, adhesion force itself determines the detachment rate from the donor under a vertical pulling force separating donor and receiver surfaces, as well as adhesion to the receiver. The adhesion force on the receiver surface should overcome the adhesion force on the donor surface to firstly detach bacteria. Bacteria tend to leave the surface with low adhesion force and adhere to the

one with a higher adhesion force during transmission. Over and above, transmission from a mature biofilm (as opposed to a (sub-)monolayer of adhering bacteria) to a clean surface involves an additional force; the cohesion force between bacteria in the biofilm. Both (sub-)monolayer bacterial and biofilm transmission cases have their own potential of occurrence, depending on many factors including but not limited to environmental shear, hygienic conditions, and nutrients availability. However, transmission of bacteria in contaminating (sub-)monolayers is more likely to occur than biofilm transmission, thinking for instance about contamination of hands from sneezes or aerosols to environmental surfaces and transmission between hands and door knobs, cart handles, surgical tools.[9–11] Biofilm transmission is rare and occurs for instance from difficult to clean endoscopes or from contact lens cases to lenses.[12,13]

The data obtained on bacterial adhesion numbers before or during adhesion and detachment numbers have been tested for statistical significance throughout all chapters and have often been found significant. Yet differences were quite often, though not always (Chapter 2) small and it is hard if not impossible to estimate whether or not these relatively small differences have any practical or clinical significance. The entirely new and unpredicted observation of EPS production induced by high local pressures exerted on bacteria adhering to nanostructured surfaces may be of greater practical and clinical significance than differences in bacterial numbers. Pressure-induced EPS production is new and provides bacteria adhering on nanostructured surfaces under high local pressure with an additional amount of protective matrix, which may make them more difficult to control by antibiotics. How this would balance with the small reductions in adhesion numbers, increased detachment or reduced transmission remains currently to be determined. In favor of nanostructured surfaces, we have also observed increased cell death as induced by high local pressures on bacteria adhering to nanostructured surfaces. Theoretically, pressure-induced EPS production may open efflux channels in an uncontrolled way capitulating the membrane barrier and causing spill of intracellular material yielding cell death.

The proof of the pudding is always in the eating, and for possible use in biomaterials implants and devices, animal studies are inevitable, if only to study the competition between tissue integration *versus* biofilm formation that determines the ultimate faith of a biomaterials implant or device *in vivo*. Furthermore, robustness tests (e.g. hammering, screwing, scratching etc. as occurring during practical use) of nanostructured surfaces are to be carried out in order to enable their use in real-life, including clinical applications.

REFERENCES

1. Ramasamy, M.; Lee, J. Recent Nanotechnology Approaches for Prevention and Treatment of Biofilm-Associated Infections on Medical Devices. *Biomed Res. Int.* 2016, *2016*, 1851242.
2. Wu, Y.; Zitelli, J. P.; TenHuisen, K. S.; Yu, X.; Libera, M. R. Differential Response of Staphylococci and Osteoblasts to Varying Titanium Surface Roughness. *Biomaterials* 2011, *32* (4), 951–960.
3. Nejadnik, M. R.; Van der Mei, H. C.; Norde, W.; Busscher, H. J. Bacterial Adhesion and Growth on a Polymer Brush-Coating. *Biomaterials* 2008, *29* (30), 4117–4121.
4. Raulio, M.; Järn, M.; Ahola, J.; Peltonen, J.; Rosenholm, J. B.; Tervakangas, S.; Kolehmainen, J.; Ruokolainen, T.; Narko, P.; Salkinoja-Salonen, M. Microbe Repelling Coated Stainless Steel Analysed by Field Emission Scanning Electron Microscopy and Physicochemical Methods. *J. Ind. Microbiol. Biotechnol.* 2008, *35*, 751–760.
5. Xu, L.-C.; Siedlecki, C. A. *Staphylococcus epidermidis* Adhesion on Hydrophobic and Hydrophilic Textured Biomaterial Surfaces. *Biomed. Mater.* 2014, *9* (3), 35003.
6. Ivanova, E. P.; Hasan, J.; Webb, H. K.; Gervinskas, G.; Juodkazis, S.; Truong, V. K.; Wu, A. H. F.; Lamb, R. N.; Baulin, V. A.; Watson, G. S.; Watson, J. A.; Mainwaring, D. E.; Crawford, R. J. Bactericidal Activity of Black Silicon. *Nat. Commun.* 2013, *4*, 2838.
7. Epstein, A. K.; Wong, T.-S.; Belisle, R. A.; Boggs, E. M.; Aizenberg, J. Liquid-Infused Structured Surfaces with Exceptional Anti-Biofouling Performance. *Proc. Natl. Acad. Sci. U. S. A.* 2012, *109* (33), 13182–13187.
8. Luong-Van, E.; Rodriguez, I.; Low, H. Y.; Elmouelhi, N.; Lowenhaupt, B.; Natarajan, S.; Lim, C. T.; Prajapati, R.; Vyakarnam, M.; Cooper, K. Review: Micro- and Nanostructured Surface Engineering for Biomedical Applications. *J. Mater. Res.* 2013, *28* (2), 165–174.
9. Bhalla, A.; Pultz, N. J.; Gries, D. M.; Ray, A. J.; Eckstein, E. C.; Aron, D. C.; Donskey, C. J. Acquisition of Nosocomial Pathogens on Hands after Contact with Environmental Surfaces near Hospitalized Patients. *Infect.Control Hosp.Epidemiol.* 2004, *25*, 164–167.
10. Hayden, M. K.; Blom, D. W.; Lyle, E. A.; Moore, C. G.; Weinstein, R. A.; Hayden, M. K.; Blom, D. W.; Lyle, E. A.; Moore, C. G.; Weinstein, R. A. Risk of Hand or Glove Contamination After Contact With Patients Colonized With Vancomycin-Resistant Enterococcus or the Colonized Patients' Environment. *Infect. Control Hosp. Epidemiol.* 2012, *29* (2), 149–154.
11. Morgan, D. J.; Liang, S. Y.; Smith, C. L.; Johnson, J. K.; Harris, A. D.; Furuno, J. P.; Thom, K. A.; Snyder, G. M.; Day, H. R.; Perencevich, E. N. Frequent Multidrug-Resistant *Acinetobacter baumannii* Contamination of Gloves, Gowns, and Hands of Healthcare Workers. *Infect. Control Hosp. Epidemiol.* 2010, *31* (7), 716–721.
12. Kovaleva, J.; Peters, F. T. M.; Van der Mei, H. C.; Degener, J. E. Transmission of Infection by Flexible Gastrointestinal Endoscopy and Bronchoscopy. *Clin. Microbiol. Rev.* 2013, *26* (2), 231–254.
13. Qu, W.; Busscher, H. J.; Hooymans, J. M. M.; Van der Mei, H. C. Surface Thermodynamics and Adhesion Forces Governing Bacterial Transmission in Contact Lens Related Microbial Keratitis. *J. Colloid Interface Sci.* 2011, *358* (2), 430–436.