Bacterial interactions with nanostructured surfaces

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CHAPTER 1

General Introduction: Current Developments in Bacterial Interactions with Nanostructured Surfaces and Aim of this Thesis

The formation of biofilm on material surfaces due to bacterial adhesion is a serious problem for both the health and economic field.[1–3] In marine environments, so-called macro-fouling is constituted by adhesion of organisms like tubeworms, mussels, barnacles which can be products of larvae settlement. Macro-fouling is preceded by adhesion of microorganisms, forming a biofilm as a substratum for more macroscopic organisms, including diatoms to adhere and growth.[4] Marine fouling on ship hulls causes tremendous increases in drag and associated fuel consumption. Hence economic losses due to the settlement of organisms on not only ship hulls (i.e. $56M per year for DDG-51 class naval ship),[5] but also power plant cooling systems, aquaculture systems, fishing nets, pipelines, submerged structures, oceanographic research instrumentation is enormous.[6]

In food industry, steel, aluminum and titanium are metals widely used which can be affected by the adhesion and colonization of bacteria.[7] Metal surfaces can corrode by way of microbially induced corrosion caused by biofilm formation with sulfide-producing bacteria.[8,9] With a decrease in efficiency and thus an increase in operating costs, biofilm formation is in part of a large factor in industrial process control. For instance in dairy industry, biofilm formation by thermophilic organisms causes reduced heat exchange in pasteurization machines.[10,11]

In the human body, microbial adhesion and growth can also cause serious health hazards by causing difficult to treat infections, especially on contaminating biomaterial implants and devices. Among many successful artificial organs and prostheses, dental implants and joint arthroplasties have become the most popular clinical applications. However, aside from the success rate of these surgeries, the aging of the baby boomer generation and the outbreak of obesity have made the use of biomaterials implants and devices indispensable in modern medicine. Total hip and knee arthroplasties for instance, are projected to grow at an increasingly high rate over the next few decades. At the same time, as a general drawback of biomaterial implants and devices, orthopedic joint infection is a major hazard in orthopedic surgeries. During the first two years following the implantation of a total knee arthroplasty, infection was the second main reason for failure, presumed aseptic biomechanical loosening being the number one reason.[12,13] Since the frequency of these procedures is increasing, revisions of total hip and total knee arthroplasties are estimated to increase at rates as high as 137% and 601%, respectively between 2005 and 2030[14] at almost double costs compared to primary arthroplasties.[15]

In medical applications, mechanical removal of biofilms is considered a last resort solution, as compared to applications in industry, which can more easily handle such an expensive yet effective approach. Extensive debridement and high risk revision surgery are used to detach and mechanically remove biofilm from microbially colonized biomaterial implants and devices, at the risk of further complications by infection. Some methods of treatment and prevention include antibiotic therapy, but with the increasing number of resistant strains and the desensitizing properties of the biofilm mode of growth, antibiotics are rendered highly ineffective now more than ever before.[16–18]

The ability of bacteria to adhere to a biomaterial surface comes through reciprocal action between cell surface structures and particular molecular groups of the biomaterial.[19–21] Accordingly, different approaches have been developed to prepare infection-resistant biomaterials.[22–25] Cationic coatings with alkylated quaternary ammonium groups can kill bacteria upon contact and constitute one way to prevent growth.[26] Polymers can also work as a reservoir for antibiotics housing.[27] However, with time antimicrobial efficacy of such release coatings decreases, eventually dropping below the minimum inhibitory concentration. This implies that when infection occurs, the coating may have become ineffective.

A different approach explored in the prevention of bacterial adhesion and biofilm formation that yet has to find its way to clinical use is to mechanically or chemically engineer specific surface properties that directly repel bacteria,[28,29] such as through engineered roughness or hydrophobicity.[30] The nature of hydrophobic and hydration forces plays a key role on the mediation of a solute (e.g. protein) adsorption and cell adhesion for biological systems.[31,32] As all surface modification approaches, it should be taken into account that adsorption of proteins and other macromolecules ("conditioning film formation") generally precedes adhesion of infectious organisms which may affect the efficacy of the surface modifications applied.

Among the engineered surfaces, nanostructured surfaces are new and their possible merits as infection-resistant implant surfaces, or for that matter anti-adhesive surfaces in general, has never been truly explored.[33] Surface roughness and hydrophobicity on a microscale are known to alter surface hydrophilicity and hydrophobicity to more extreme values with a possible impact on bacterial adhesion and growth on biomaterials both in vitro[34] and in vivo.[35] Adhesion of staphylococci was notably reduced on pillar-patterned poly(ethylene glycol) hydrogels when the spacing between the structures was 1.5 μm or less. This suggests the critical length scale of surface features for more effective prevention of bacterial adhesion should be nanoscale (i.e. smaller than the size of a bacterium).[36] The importance of the effects of nanoscale features have also been reported recently.[37,38] The smaller contact area between bacteria and the surface and higher hydrophilicity caused by the nanostructures resulted in reduced adhesion and biofilm formation on the nanostructured gold surfaces.[39] Titanium dioxide (TiO_2) nanotube surfaces have also been shown to reduce bacterial adhesion, growth and viability.[40,41] Gentamicin-loaded nanotubes have been used to decrease bacterial growth.[42] Nanopillared structures were able to effectively kill bacteria due to the mechanical rupture of the bacterial cell membrane by the pillars in Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus 65.8^T, and spores of Bacillus subtilis NCIMB 3610^T.[43] Another study has found that when air is entrapped on a nanostructured alumina (Al_2O_3) surface, a superhydrophobic surface develops that reduces initial adhesion of Escherichia coli K-12 and Staphylococcus aureus ATCC 12600.[44] These studies demonstrate that substratum nanostructures can significantly modulate bacterial adhesion and growth, while triggering bacterial cell death. This is similar to earlier work that nanostructured Teflon surfaces become superhydrophobic[45] with merits on the biocompatibility of Teflon applications in the human

body,[46,47] although at the time the word NANO yet had to be introduced. The fact that nanostructured surfaces still have to find their way as an anti-adhesive biomaterial, probably has to do with the fact that bacterial adhesion and viability is multifactorial depending on bacterial size, physiology, and topographical dimensions which can be conflicting even when the same materials with the same bacterial species are studied.[30]

Also, it would be a logical scheme to explore the enlarged surface area of coatings on top of a nanostructured surface for housing antibiotics, which would yield a unique possibility to create higher local concentrations than can be achieved using smooth surfaces,[30] while the minimal contact between bacteria and a nanostructured surface may leave bacteria unresponsive to their adhering state in their antibiotic susceptible, planktonic regimes.[48]

Numerous methods exist to create nanostructured surfaces that can roughly be divided into structures with a random or periodic roughness[49] and include, simple electrochemical etching process, and lithographically fabricated nanostructures, including pillars and pores, of differing shapes and dimensions.[50,51] Although electrochemical methods are more engaging with regards to hard-metal surface processing, including but not limited to Ti, there is also the lithographic approach, which on the other hand, can be more costly but yields results that are more exact and precise.[52] Due to curved shape of implants and their relatively large surfaces, electrochemical techniques have a major advantage to fabricate nanostructures on them. Nonetheless, the anodization process contains particular deficiencies such as constraints on controlling the pattern or structure dimensions, and homogeneity which can be solved by enforcing a two or three step anodization.[53,54] Periodic nanostructured surfaces are easier to characterize than random rough surfaces although more tedious to prepare and therefore randomly rough surfaces are looked at mostly for applications. Periodically rough surfaces on the other hand, are more ideal to study mechanisms of bacterial interaction with nanostructured surfaces.

AIM OF THE THESIS

The aim of the thesis to extend our understanding of bacterial interactions with nanostructured surfaces and explore the use of nanostructured surfaces coated with antibiotics. To this end, we developed a simple 3D anodization technique to nanostructure metal surfaces and used an interference lithography to produce highly precise Si surfaces on which a variety of experiments will be carried out to answer the above aim.

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