THE MECHANICS OF *STAPHYLOCOCCUS EPIDERMIDIS* ADHESION TO VARIOUS CHEMICALLY FUNCTIONALIZED SURFACES

Maria G. Katsikogianni and Yannis F. Missirlis
Laboratory of Biomechanics and Biomedical Engineering, Department of Mechanical Engineering, University of Patras, Rion, Patras, Greece

INTRODUCTION

Infection is by far one of the major complications which impede the long-term use of artificial organs and medical devices[1]. The critical step in the development of infections is bacterial adhesion to the biomaterial surface, which is mediated by interactions between the material and the bacterial surfaces. The effect of specific chemical functionalities on the initial adhesion of bacteria under flow was investigated using well-characterized chemically functionalized surfaces prepared by self-assembly of silane monolayers on glass and further modification of them.

MATERIALS AND METHODS

Bacteria: The reference ATCC 35984 slime producing strain of *S. epidermidis* was used. Bacteria were suspended in 100 mM PBS pH 7.4, at a concentration of $3x10^8$ bacterial cells/ml.

Substrates: amino (NH_2)-terminated self-assembled monolayers (SAMs) on glass surfaces by chemical vapor deposition of the silane[2], positively charged NH_2-terminated surfaces by adsorption of poly-L-lysine hydrobromide 0.01% wt/vol to glass[3], carboxyl (COOH)-terminated surfaces by carboxylation of NH_2-terminated with 0.1 M succinic anhydride in dimethylformamide[4] and 0.1 M EDC(1-Ethyl-3-(3-dimethylaminopropyl)-carbodimide) pH 5.5 – 0.04 M sulfo-NHS (N-Hydroxysulfosuccinimide) pH 7.5 treated COOH-terminated surfaces[5] were used as substrates. Hydrolyzed and oxidized glass, in NaOH 5M and H_2SO_4/H_20_2 (3/1), was used as control.

Parallel Flow Chamber: Bacterial adhesion to the substrates was examined under two shear rates, 50 and 2000 s^{-1}, after two hours.

Techniques: The number and pattern of the adherent bacteria was evaluated by Scanning Confocal Laser Microscope; the adherent bacteria to the various substrates were fixed in paraformaldehyde, fluorescently labeled with the DNA-binding stain SYTO 9 and examined. Surface topography and roughness were examined by Atomic Force Microscope in contact mode, surface wettability and energy were evaluated by Contact angle measurements of three probe liquids; water, methylene iodide and glycerol.

RESULTS AND DISCUSSION

NH_2-terminated surfaces appear moderately hydrophobic (θwater ~50 deg). Positively charged NH_2-terminated and negatively charged COOH-terminated surfaces present similar contact angles (θwater ~26 and 28 deg respectively), the sulfo-NHS ester-terminated surfaces appear relatively hydrophobic (θwater ~70 deg) with low surface energy, whereas the glass presents an extremely hydrophilic surface (θwater ~10 deg) and high surface energy. The AFM images showed that all the various substrate have similar topography and roughness values. The confocal microscopy observations revealed that, under low shear rate conditions, the sulfo-NHS ester-terminated surface yields the highest number of adherent bacteria, followed by the positively charged and not charged NH_2-terminated surfaces. The negatively charged COOH-terminated surface and especially the hydrolyzed-oxidized glass retain the lowest number of adherent bacteria. These results were in qualitative agreement with the predictions of the Extended (X) DLVO Theory[6]. Increase in shear from 50 to 2000 s^{-1} significantly decreased bacterial adhesion to all the substrates. The highest decrease appeared to the glass followed by the COOH- and the sulfo-NHS ester-terminated substrates. This means that the bacterial NH_2 groups could not form covalent bonds with the sulfo-NHS ester-terminated surface under high hydrodynamic forces. The lowest decrease in bacterial adhesion with the increase in shear rate appeared to both the positively charged and not charged NH_2-terminated surfaces, revealing macromolecular interactions between the bacterial surfaces and these substrates[7].

CONCLUDING REMARKS

The XDLVO Theory, in which the van der Waals, the electrostatic and the acid-base interactions are included, qualitatively predicts the increase in adhesion of a hydrophilic and highly negatively charged bacterium with the decrease in substratum surface energy and charge, or to positively charged substrates, under low shear rate. Increased shear rate, and therefore increased hydrodynamic forces, significantly decrease bacterial adhesion to all the substrates, but in a way that is not predicted by the XDLVO Theory. Therefore, apart from the physicochemical interactions that this Theory accounts for and the hydrodynamic forces, bacterial polymeric structures that contribute in bacteria-material macromolecular interactions must also be taken into consideration.

REFERENCES

1. Vuong C., Otto M. Microbes Infect 2002;4(4):481.
2. Sugimura H., Hozumi A., Kameyana T., Takai O. Surf Interf Analysis 2002;34:550.
3. Vadillo-Rodriguez V., Busscher H.J., Norde W., de Vries J., Dijkstra R.J.B., Stokross I., van der Mei H.C. Appl Enironm Microbiol 2004;70:5441.
4. An Y., Chen M., Xue Q., Liu W. J Colloid Interf Sci 2007;311:507.
5. Grabarek Z., Gergely J. Anal Biochem 1990;**185:**131.
6. van Oss C.J., Good R.J., Chaudhury M.K. J Colloid Interface Sci 1986;111:378.
7. Ma H., Dickinson R.B. J Theoretical Biol 2004;226:237.