Summary

Worldwide 85 million people use contact lenses (CL's) as an alternative to spectacles. However, this large population is at risk and makes the use of CL's an important health concern, as CL wear can induce microbial keratitis, which may result in permanent visual loss. Bacteria adhere to the lens surface and form an infectious biofilm, depending on the physico-chemical surface properties determined by the bacterial cell, the lens and the suspending liquid. Different episodes of usage cycle in wearing the contact lens affect the physico-chemical properties of the lens surface and therefore influence the adhesion of bacteria, as outlined in **Chapter 1**.

Similarly, as presented in **Chapter 2,** bacterial cell surfaces play a crucial role in their adhesion to surfaces and can be altered during lens care. Physico-chemical cell surface properties of *P. aeruginosa,* isolated from a case of contact lens associated keratitis, have been determined for mid-exponential and early-stationary phase cells and for cells after exposure to a lens care solution or after mechanical damage by sonication. Exposure to a lens care solution and mechanical cell surface damage reduced the cell surface hydrophobicity and water contact angles decreased from 129 to 96 and 83 degrees, respectively. Zeta potentials in saline (-9 mV) were hardly affected after mechanical damage, but tri-modal zeta potential distributions, with sub-population zeta potentials at -11, -28 and -41 mV, were observed after exposure of bacteria to a lens care solution. X-ray photoelectron spectroscopy indicated changes in the amounts of oxygen-, nitrogen- and phosphorus-rich cell surface components. Mid-exponential phase cells had more nitrogen-rich cell surface components than early-stationary phase cells, but water contact angles and zeta potentials were not very different. In addition, mid-exponential phase cells adhered

better than early-stationary phase cells to hydrophobic and hydrophilic substrata in a parallel plate flow chamber. The capacity of *P. aeruginosa* to adhere was decreased after inflicting cell surface damage. Exposure to a lens care solution yielded a larger reduction in adhesion capacity than sonication, likely because sonication left most of the cells in a viable state, in contrast to exposure to a lens care solution.

In **Chapter 3,** elemental surface compositions of contact lenses were measured after exposure to different lens care solutions (LCS) using X-ray photoelectron spectroscopy and related to adhesion and detachment of *P. aeruginosa*. Etafilcon A and polymacon contact lenses, prior to and after exposure to LCS were fixed on the bottom plate of a parallel plate flow chamber after which *P. aeruginosa* #3 was allowed to adhere during a 2 h time period. After adhesion, bacterial detachment was stimulated by perfusing the chamber with a LCS or by passing an air-bubble through the chamber. After exposure to a LCS the adhesion of *P. aeruginosa* #3 could either be enhanced or decreased, depending on the contact lens and LCS involved. Initial deposition rates of *P. aeruginosa* #3 could not be related with changes in elemental surface composition of the contact lenses, but decreased with an increasing ratio of oxygen involved in O=C bonds relative to oxygen in O-C bonds. *P. aeruginosa* #3 adhered tenaciously to both contact lens surfaces and the passage of an air bubble through the flow chamber detached only up to 9% of the adhering bacteria. Alternatively, the LCS most effective in decreasing bacterial adhesion after exposure (LCS A), was least effective in detaching adhering *P. aeruginosa* #3 (8% to 15%), while the other LCS detached up to 42% of adhering bacteria.

In **Chapter 4**, the adhesion of two physico-chemically characterized bacterial strains to a surface hydrophilic (CL A, water contact angle 57 degrees) and

hydrophobic (CL B, water contact angle 106 degrees) hydrogel contact lens with and without an adsorbed tear film in a parallel plate flow chamber were determined. Hydrophobicity (by water contact angles), charge (by particulate microelectrophoresis) and elemental composition (by X-ray photoelectron spectroscopy) of the surfaces of seven bacterial strains were characterized, after which two strains were selected for further studies. On CL surfaces, hydrophobicity, elemental composition, and mean surface roughness (by atomic force microscopy) were determined, as well as the protein composition of tear films adsorbed on these lenses (by SDS-PAGE). Bacterial cell surfaces were relatively uncharged and water contact angles on lawns of different strains ranged from hydrophobic to hydrophilic. After adsorption of tear film components, N/C elemental surface concentration ratios increased on CL A and CL B and differences in water contact angles between both lenses reduced to range from 48 (CL A) to 69 degrees (CL B). However, different protein compositions were inferred. The surface roughness of CL A increased from 4 nm to 13 nm, while it remained 16 nm for CL B. Adhesion of hydrophobic *P. aeruginosa* #3 was more extensive than of hydrophilic *Staphylococcus aureus* 799, with no differences between both lenses. The hydrophobicity of *P. aeruginosa* #3 after cell surface damage decreased and its adhesion was reduced on CL A and strongly reduced on CL B. In addition, passage of an air-liquid interface yielded more detachment of *S. aureus* 799 than of *P. aeruginosa* #3 from the CL surfaces. In conclusion, the hydrophobicity of CL surfaces dictates the composition of the adsorbed tear film and therewith plays an important role in bacterial adhesion to lenses.

Chapter 5 describes how wear affects the surface properties of lenses and the adhesion of *P. aeruginosa.* Ten new CL wearers used ionic, Etafilcon A lenses with

58 % water on both eyes for approximately 10 h each day during 10 and 50 days. All lenses were treated daily with an appropriate lens care solution. After wearing the CL for 10 days (first pair of lenses) and 50 days (second pair, representing overwear), hydrophobicity by water contact angles, surface roughness by atomic force microscope, elemental surface composition by X-ray photoelectron spectroscopy (XPS), and adsorbed proteins by SDS-PAGE were determined on one lens. The lens from the contra-lateral eye was placed in a parallel plate flow chamber for bacterial adhesion after each time interval. Water contact angles on lenses changed from 45 ± 10 degrees on unused lenses to 61 ± 25 degrees after 10 days of wear and changed significantly ($p<0.05$) to 27 ± 14 degrees after 50 days of wear. Surface roughness increased significantly ($p<0.05$) from 4 ± 2 nm (unused) to 10 ± 7 nm after 50 days of wear. These changes were accompanied by adsorption of proteinaceous material as evidenced by XPS and SDS-PAGE, demonstrating adsorption of lysozyme, tear lipocalin and a 30 kD protein. Initial bacterial adhesion to worn CL's was lower than to unworn CL's, while furthermore detachment of adhering bacteria from worn lenses was easier than from unworn lenses. The changes observed in physico-chemical surface properties of the lenses after wearing the CL for 50 days were accompanied by complaints about discomfort by 6 out of the 10 new CL wearers. Multiple regression analysis revealed that the most predictive variables for an effect on initial deposition after 10 days of wear were hydrophobicity, roughness, the presence of nitrogen-rich material, including the presence of a 30 kD protein, and the presence of oxygen-rich material, i.e. the type of oxygen adsorbed (O=C or O-C). After 50 days of wear, roughness and the presence of tear lipocalin were most predictive. This study demonstrates that the physico-chemical surface properties change after wear and

overwear, while overwear of the lenses decreases initial adhesion of *P. aeruginosa* #3 under the present experimental conditions.

In a similar study (**Chapter 6**), surface properties of RGP lenses prior to and after wear that are influential on adhesion of *P. aeruginosa* were determined. After 10 and 50 days of wear and after end-stage use, lenses were collected for determination of physico-chemical surface properties and bacterial adhesion in a parallel plate flow chamber. Water contact angles on unused RGP lenses amounted 47 ± 13 degrees and were affected by wear. In addition, %O at the lens surfaces, as determined by XPS increased after use for 10 and 50 days, but decreased after end-stage wear. The %N hardly increased after wear and, in line, SDS-PAGE did not indicate adsorbed proteins. The surface roughness of the lenses, as measured by AFM amounted 9 nm after 10 and 50 days of use, but end-stage lenses were significantly rougher (48 ± 23 nm). Moreover, initial deposition of *P. aeruginosa* #3 increased with increasing roughness for end-stage lenses. Multiple regression analysis, however, revealed that both physical and chemical surface properties were predictive for initial bacterial deposition to lens surfaces. After 10 days of wear, bacterial deposition was governed by the water contact angle, surface roughness, %O, %N, and %Si, while after 50 days of wear the surface roughness, %N, and %Si were found predictive for bacterial deposition. Initial bacterial deposition to end-stage lenses was solely dependent on the surface roughness. Summarizing, physico-chemical surface properties of RGP lenses change slightly during the first 10 to 50 days of wear, but end-stage lenses all had increased surface roughness, concurrent with increased bacterial adhesion.

In the general discussion of this thesis (**Chapter 7**), it is pointed out that the methods used to determine physico-chemical properties of the lens surfaces are all

adequate and yield a good characterization of the surfaces, but that the XPS and SDS-PAGE technique to identify adsorbed proteins and other macromolecular components on the lens surfaces might be extended with, for instance MALDI-MS. From a mechanistic point of view, it is emphasized that the clinical study in combination with laboratory studies on bacterial adhesion is unique, as it allows to study multiple surface properties in relation with bacterial adhesion, instead of focussing on single factors as done frequently in other studies.

From a clinical point of view, the studies carried out on soft hydrogel lenses are important as they show, within the limitation of the strain used, that wear nor overwear increases adhesion of *P. aeruginosa,* suggesting that overwear of these lenses does not increase the risk of inducing keratitis. This is opposite to the situation with RGP lenses. End-stage RGP lenses has been found to be significantly rougher than unused lenses with a concurrent increase in bacterial adhesion. Therewith, this study underlines the need to develop wearing schedules for RGP lenses, similar as existing for soft hydrogel lenses.