Biomaterials 31 (2010) 5510–5519

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Clinical observations of biofouling on PEO coated silicone hydrogel contact lenses

Helmut Thissen [a,b,*], Thomas Gengenbach [a], Renee du Toit [b], Deborah F. Sweeney [b], Peter Kingshott [b,c], Hans J. Griesser [b,d], Laurence Meagher [a,b]

[a] CSIRO Molecular and Health Technologies, Bayview Avenue, Clayton 3168 VIC, Australia
[b] CRC for Eye Research and Technology (now Vision CRC), Rupert Myers Building, Sydney 2052 NSW, Australia
[c] Interdisciplinary Nanoscience Center, Aarhus University, Ny Munkegade, Building 521, 8000 Aarhus C, Denmark
[d] Ian Wark Research Institute, University of South Australia, Mawson Lakes 5095 SA, Australia

ARTICLE INFO

Article history:
Received 1 December 2009
Accepted 17 March 2010
Available online 24 April 2010

Keywords:
Contact lens
Surface modification
In vivo test
XPS
AFM

ABSTRACT

Silicone hydrogel contact lenses, which have been a major advance in the field of vision correction, require surface modification or coatings for comfort and biocompatibility. While current coatings show adequate clinical performance, advanced coatings may improve the biocompatibility of contact lenses further by reducing biofouling and related adverse clinical events. Here, we have produced coatings on Lotrafilcon A contact lenses by deposition of a thin film of allylamine plasma polymer (ALAPP) as a reactive interlayer for the high density grafting of poly(ethylene oxide) dialdehyde ($PEO(ALD)_2$), which had previously shown complete resistance to protein adsorption *in vitro*. The performance of these contact lenses was evaluated in a controlled clinical study over 6 h using Focus® Night and Day™ (also known as Air Optix® Night & Day®) contact lenses as control lenses. Surface modified lenses were characterised by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) before and after wear. Clinical data showed a high level of biocompatibility of the PEO coated lenses equivalent to control lenses. Surface analysis of worn contact lenses demonstrated that the high density PEO coating is effective in reducing biofouling *in vivo* compared to control lenses, however small amounts of protein deposits were still detected on all worn contact lenses. This study highlights that elimination of biofouling *in vivo* can be much more demanding than *in vitro* and discusses issues that are important for the analysis of worn contact lenses as well as the design of improved contact lenses.

1. Introduction

The development of highly oxygen permeable silicone hydrogel contact lens materials has been a major advance in the field of vision correction [1]. Contact lenses made from these materials satisfy the metabolic needs of the cornea, maintain its physiological health, and can be worn continuously for up to 30 days. However, these materials require coatings for improved comfort and biocompatibility. While currently used coatings have demonstrated adequate clinical performance, advanced coatings may improve the biocompatibility further. In particular, it is known that contact lenses rapidly accumulate proteins and other tear film components when inserted into the eye and it is assumed that bacteria, which can potentially cause adverse clinical events, can adhere to these adsorbed biomolecules [2]. Although small in numbers, adverse clinical events related to bacterial attachment and colonisation on contact lenses can be 'sight threatening' (eg. microbial keratitis (MK)) or 'significant' (eg. contact lens induced acute red eye (CLARE), contact lens induced peripheral ulcers (CLPU) and infiltrative keratitis (IK)). Bacterial attachment and colonisation on contact lenses may also be related to 'non-significant' adverse events such as asymptomatic infiltrative keratitis (AIK) and asymptomatic infiltrates (AI). These adverse clinical events remain a challenge for contact lens manufacturers [3] and therefore low-biofouling characteristics are believed to be an important requirement for advanced contact lens coatings.

The reduction of biofouling on contact lenses is related to the understanding of the composition and regulation of the preocular tear film, which plays a critical role in maintaining corneal and conjunctival integrity by protecting against microbial challenge and preserving visual acuity. Furthermore, the reduction of biofouling is related to the understanding of interactions between tear film components and contact lens surfaces. In this respect, several studies have correlated deposits on the contact lens surface originating from the tear film with clinical characteristics of the lens [1,4,5].

The phenomenon of biofouling has attracted much attention due to its relevance in a broad variety of applications, including

* Corresponding author. CSIRO Molecular and Health Technologies, Bayview Avenue, Clayton 3168 VIC, Australia. Tel.: +61 3 9545 2191; fax: +61 3 9545 2446.
E-mail address: helmut.thissen@csiro.au (H. Thissen).

0142-9612/$ – see front matter
doi:10.1016/j.biomaterials.2010.03.040

particularly biomedical applications. Many undesirable biological responses such as platelet adhesion and activation and bacterial attachment and colonisation have been found to be related to biofouling, which takes place immediately after insertion of a material surface into a biological medium or environment [6]. On contact lenses in particular, it has been demonstrated that biofouling is related to the main tear film components including proteins, lipids and mucins [7,8].

A variety of strategies have been used to create low-fouling surfaces for biomedical applications, including surface coatings comprising neutral hydrophilic polymers such as polyacrylamide and poly(ethylene oxide) (PEO) (synonymous with poly(ethylene glycol) (PEG)) [9], polymers based on phospholipids [10], naturally occurring polymers such as dextran [11] and pullulan [12] and switchable polymers based on *N*-isopropylacrylamide [13,14]. However coatings based on PEO have received by far the most attention [15].

Again a variety of methods have been used to produce PEO based coatings with the aim of reducing biofouling, including the assembly of oligo(ethylene oxide) self-assembled monolayers [16], 'grafting to' reactions of pre-formed polymers, 'grafting from' polymerisation techniques [17,18] and crosslinking methods [19].

In the case of 'grafting to' approaches, coatings produced under 'cloud point' conditions have been shown to yield superior results [20,21,22]. Under these conditions, marginal solvation of the PEO results in reduced chain repulsion during surface immobilisation, and hence in an increased surface density of the grafted polymer chains. The effectiveness of high density PEO surfaces prepared by this method has previously been demonstrated *in vitro* in regard to reducing or preventing protein adsorption [20], cell attachment [23,24] and tissue migration [25] as well as bacterial attachment [26]. While the effectiveness of PEO coatings for the reduction or prevention of biofouling has been established in many *in vitro* studies, *in vivo* studies have been limited [27].

In this study, we have surface modified silicone hydrogel contact lenses with the aim to reduce biofouling *in vivo* while at the same time maintaining the excellent clinical performance and biocompatibility of currently commercially available silicone hydrogel lenses. Untreated Lotrafilcon A contact lenses (supplied by CIBA Vision, Atlanta GA, USA) were used as the substrate material. An allylamine plasma polymer (ALAPP) was deposited on dry contact lenses to produce a thin, pinhole-free coating with excellent adhesion. In addition to representing a robust and well established coating procedure, plasma polymerisation also provided amino functional groups for the subsequent covalent attachment of poly(ethylene oxide) dialdehyde ($PEO(ALD)_2$), which was carried out under 'cloud point' conditions to achieve high grafting density. The biocompatibility and clinical performance of ALAPP–$PEO(ALD)_2$ coated Lotrafilcon A contact lenses were evaluated during a controlled 6 h clinical study. In addition, the surface of lenses was evaluated before and after wear using X-ray photoelectron spectroscopy (XPS) and before wear using atomic force microscopy (AFM).

2. Materials and methods

2.1. Substrate materials

For all surface modification experiments, uncoated Lotrafilcon A contact lenses (supplied by CIBA Vision, Atlanta GA, USA) were used. In addition, polished silicon wafers [Si] (M.M.R.C. Pty Ltd, Mt. Waverley, VIC, Australia) with a size of 1.0×1.0 cm^2 and a nominal oxide layer thickness of 3 nm were used as a non-compliant surface with which to calibrate the AFM photodetector.

2.2. Plasma polymerisation

Plasma polymerisation was carried out in a custom-built reactor as described elsewhere [28]. Briefly, the cylindrical reactor chamber was defined by a height of 350 mm and a diameter of 170 mm. Samples were placed on a metal mesh, positioned 15 mm above the lower, circular electrode with a diameter of 95 mm. The distance between the lower and the upper (U-shaped) electrode was 125 mm. Allylamine (Aldrich, 98% purity) was used as the monomer. The parameters chosen for plasma polymer deposition were a frequency of 200 kHz, a power of 20 W and an initial monomer pressure of 0.200 mbar. The treatment time was 25 s.

2.3. PEO grafting

Grafting of poly(ethylene oxide) dialdehyde ($PEO(ALD)_2$) with a molecular weight of 3400 (Shearwater Polymers, Huntsville AL, USA) on the freshly deposited amino functionalised ALAPP surface of the Lotrafilcon A contact lenses was carried out as reported previously [20]. Briefly, covalent immobilisation of the graft polymer was achieved by reductive amination using $NaCNBH_3$ (Sigma, 90% purity) as the reducing agent. The reaction was carried out for 16 h under 'cloud point' conditions at 60 °C in 0.1 M sodium phosphate buffer at pH 7, containing 11% (w/v) K_2SO_4 (BDH Chemicals, Kilsyth VIC, Australia). Subsequently lenses were washed extensively with MilliQ™ and assessed for clinical quality using dark field optical microscopy. Flawless lenses were graded as clinical quality, while lenses with minor imperfections were used in characterisation studies etc. Finally lenses were transferred into a physiological PBS buffer solution and autoclaved.

2.4. XPS analysis

X-ray photoelectron spectroscopy (XPS) analysis of surface modified samples was performed on an AXIS HSi spectrometer (Kratos Analytical Ltd., Manchester, UK), equipped with a monochromatised AlK_α source. The pressure during analysis was typically 5×10^{-8} mbar. The elemental composition of samples was obtained from survey spectra, collected at a pass energy of 320 eV. High resolution spectra were recorded at a pass energy of 40 eV. In order to quantify contributions to the C1s peaks from chemically different species, curve fitting of the corresponding high resolution spectra was performed using the Vision 1.5 instrument software. The curve fit protocol employed Gaussian/Lorentzian product functions (20–30% Lorentzian, 70–80% Gaussian), each defined by position, width and height, and used a Simplex algorithm for the actual minimisation. In the case of C1s spectra, all peak components were constrained to have the same, but variable, width. No other constraints were applied. Binding energies were referenced to the aliphatic hydrocarbon peak at 285.0 eV. XPS reference spectra were recorded from the lyophilised powders of lysozyme (from chicken egg white, Sigma L-6876) and gastric mucin (from hog stomach, TCI MO 470) and on the phospholipid 1,2-distearoyl-sn-glycero-3-phosphocholine (Northern Lipids, Inc. PCS-030).

XPS results on worn contact lenses were obtained from lenses which were removed from the eye after 6 h wear under sterile conditions. After removal, the lenses were placed in a sterile saline solution and stored at 4 °C. Before XPS analysis, the lenses were washed extensively with MilliQ™ water and air dried in a laminar flow cabinet.

2.5. AFM analysis

The interaction forces between a silica particle and either ALAPP coated contact lenses or contact lenses with covalently grafted $PEO(ALD)_2$ layers were measured with a Nanoscope III Multimode Atomic Force Microscope (Digital Instruments, Inc., Santa Barbara, CA, USA) using the colloid probe method developed by Ducker et al. [29]. In this method, a spherical colloidal particle (pure silica, 4–5 μm in diameter, Bangs Laboratories, Fishers, IN, USA) was attached to the AFM cantilever spring via an epoxy adhesive (Epon 1004, Resolution Performance Products, Houston, TX, USA), providing a surface of known geometry.

Calibration of the AFM cantilever spring constant was carried out using the resonance method of Cleveland et al. [30] (error approx. 10%) and the average measured values were used to scale the force data. The cantilevers used were gold coated, triangular Si_3N_4 cantilevers (Veeco, Model NP) with a spring constant of 0.076 Nm^{-1}. Conversion of the cantilever deflection curves to plots of the force/radius as a function of separation distance and indentation versus applied load were carried out using a custom designed computer program. In the analysis and scaling of the force profiles, the average compliance slope calculated from 20 to 30 force curves obtained on clean silicon wafers was used to calibrate the AFM photodetector sensitivity. This process allowed the reduction in cantilever deflection, which occurs when carrying out measurements on deformable surfaces such as contact lenses, to be corrected for. The same optical alignment set up was used for the clean silicon wafers and the lenses. For polymer systems such as that studied here (i.e. the PEO $(ALD)_2$ covalently grafted layer), there will always be a finite thickness of compressed polymer between the underlying surface and the silica colloid sphere at large loads. However, in most systems the error is small, especially when the non-compressed thickness of the layer is large. The fittings and tubing (Teflon® or KelF™) used for injecting solutions, the AFM fluid cell, O-ring and syringes were cleaned by soaking in a surfactant solution (1% v/v, RBS-35, Pierce, Rockford, IL, USA) overnight followed by thorough rinsing with MilliQ™ water and soaking in AR grade ethanol overnight. These components were finally rinsed with AR grade ethanol and blown dry using a high velocity stream of high purity, compressed nitrogen. Solutions were