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HYDROGELS FROM POLYACRYLIC ACID FOR REDUCTION OF BIOADHESION ON SILICONE CONTACT LENSES

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Abstract: Contact lenses suffer from two limitations: low oxygen permeability and deposition of protein and lipids. In order to prevent bioadhesion, surface must be completely inert to all biological reactions. To achieve this, surface properties must be tailored. Also, to improve comfort, surface must be highly wettable and lubricous. In this paper the surface of silicone contact lenses was modified by plasma induced copolymerization of acrylic acid. A wettable surface was generated and in addition carboxyl groups that were created on the surface provided an ideal reactive platform for subsequent grafting of polyethylene glycol. Each surface modification step was analysed by XPS and contact angle measurements. Lysozyme adsorption on modified silicone contact lenses was analysed by surface-MALDI-ToF-MS and XPS. After incubation with lysozyme, surface-MALDI-TOF-MS and XPS analysis showed a reduction of adsorbed lysozyme on hydrogel modified contact lenses. Surface modification of silicone with PEG is a method for reduction of protein adsorption on contact lenses.

Keywords: hydrogels, PEG, protein adsorption, contact lenses.

1. INTRODUCTION

Formation of protein deposits on the lens from the tear fluid is one of the problems encountered with contact lens use. The deposit clouds the lens, causes the wearer discomfort, reduces oxygen permeability and may cause inflammatory conditions. The development of lens materials that exhibit minimal protein adsorption is therefore desirable. The rate and amount of adsorbed protein are dependent on physical-chemical properties of the polymeric surface such as wettability and surface charge density. Therefore, surface modification procedures are developed to create ultrathin protein-repellent nanostructured and biologically functionalized interfaces at the implant surfaces. Silicone is a material that is widely used in many applications, but as material for contact lenses it has limited application. The reason is high hydrophobic nature of silicone which leads to inadequate material properties and performance of contact lenses when in contact with tear film. When in contact with biological fluids, all hydrophobic surfaces tend to adsorb and deposit lipids and proteins on the surface,
affecting biocompatibility of the whole material [1]. For contact lenses it means that the wearing comfort is affected, it can lead to dryness of natural lens and reduce oxygen permeability. For optimization of biocompatibility of silicone contact lenses, a specific surface design is needed to prevent nonspecific protein adsorption and bacterial adhesion. The specific surface modification of silicone however requires the introduction of suitable functional groups. Surface modification is achieved without influencing bulk properties of the material.

Grafting of poly(ethylene glycol) (PEG) is a common strategy for reducing the non-specific adsorption of biomolecules on surfaces [2−3]. Several theories – both qualitative and quantitative – have been proposed to explain the effectiveness of PEG as a component of protein-resistant surfaces [4]. An essential finding of these theories is that the PEG layer might provide an interfacial barrier to prevent the protein from interacting with the underlying substrate. The protein rejecting capability of PEG coatings is expected to depend on a range of parameters, including PEG molecular weight, interfacial PEG chain density, polymer chain architecture, etc., the interfacial graft density proving to be the most crucial property of a PEG layer for minimizing protein adsorption [5−6].

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In this paper the functionalization of the silicone surface is achieved by plasma-induced graft-*co*-polymerisation of acrylic acid (AAc). Plasma-induced grafting of hydrogels like polyacrylic acid offers a possibility of providing permanent minimization of the surface energy of hydrophobic polymer surfaces [7]. Acrylic acid was chosen as hydrogel building monomer to generate a stable, hydrophilic surface with an additional possibility to use the carboxylic groups for further modifications. In this paper a hydrogel layer of polyethylenimine (PEI) is attached to the PAAc modified silicone surface. Additionally, methoxy-aldehyde polyethylene glycol (M-PEG-ald) is grafted on the surface to prevent nonspecific adsorption of proteins.

All modification steps were verified by means of X-ray photoelectron spectroscopy (XPS) and contact angle measurements. Protein adsorption studies were carried out by means of surface MALDI-ToF-MS. Modified PEG graft-modified silicone surface can be used as a material with a nonfouling surface while still keeping the excellent bulk properties of silicone.

2. MATERIALS AND METHODS

2.1. Materials

Referent material was Sylgard® 184, a two component silicone elastomeric kit obtained from Dow Corning. Acrylic acid was obtained from Fluka (Germany) and distilled before use. Methoxy-terminated formyl-PEG (M-PEG, molecular weight 5000) was bought from Shearwater Polymers. Lysozyme and tear artificial fluid was purchased from Sigma.

2.2. Chemical functionalization

Graftcopolymerization: Plasma treatment was carried out with a microwave plasma unit of Eltron (Germany). PVDF films were argon plasma treated at a plasma power of 900 W for 30 s. Subsequently, the air-exposed PVDF-films were immersed in an aqueous solution containing 20% (v/v) of AAc monomer and heated at 90 °C to initiate graft polymerisation. The PAAc-grafted films were rinsed with distilled water for 24 h to remove nongrafted monomers and PAAc-homopolymers and stored in distilled water until use.

The carboxyl end groups of the grafted silicone were activated with 0.1 M EDC in sodium phosphate buffer with pH of 4.8 for 20 min at room temperature. Afterwards coupling of polyethylenimine was carried out in carbonate buffer pH 9.4 for 2 h at room temperature.

M-PEG was grafted onto aminated surface by reductive amination, using $NaCNBH_3$ as the reducing agent for the intermediate Schiff's base. Grafting was performed under marginal solvation conditions ('cloud point'). 1 mg/ml M-PEG was dissolved in 0.1 M sodium phosphate buffer at pH 6.3 containing 11% (w/v) K_2SO_4. The reaction was carried out at 60 °C [8].

All surfaces were incubated in 1 mg/ml lysozyme in PBS for 1 hour at 37°C. The samples were rinsed 3x with water and analysed immediately with MALDI-ToF-MS.

2.3. Physical and chemical surface characterization

All X-ray photoelectron spectra (XPS) were recorded on an X-Probe™ 206 spectrometer (Surface Science Instruments, Mountain View, CA). X-ray source is an aluminium anode which produces AlK_α X-rays at 1486.6 eV. The binding energies are referenced to hydrocarbon at 285.0 eV. The emission angle of electrons was set at 55° with respect to the sample normal, which results in an information depth of about 6 nm.

Contact angles were measured using the captive bubble method with pure water at room temperature on a G40 system (Krüss, Hamburg, Germany).

Surface-MALDI-ToF-mass spectra were obtained using a BRUKER BIFLEX™ III MALDI time-of-flight mass spectrometer (Bruker-Franzen Analytik GmbH, Bremen, Germany) equipped with a nitrogen laser. In this experiment a small sample piece cut from silicone and modified silicone were placed onto the stainless steel MALDI sample holder. Sinapinic acid in a 0.1% solution of trifluoroacetic acid in acetonitrile/water was applied onto the sample surface and the solvent was left to evaporate before the sample holder was inserted into the spectrometer.

3. RESULTS AND DISCUSSION

Plasma technology is applied for activation and functionalization of silicone surface. Figure 1 shows a schematic diagram of the silicone surface modifications by graft-co-polymerisation of AAc. Upon argon plasma treatment radicals are formed on the polymer surface, which react with oxygen in contact with air and form hydroperoxides. The subsequent thermal decomposition of hydroperoxides

produces secondary radicals that are able to initiate graft-co-polymerisation of acrylic acid (AAc) to produce carboxylic groups on the silicone surface (silicone-PAAc). In the next step polyethyleneimine (PEI) was grafted on the carboxyl groups on the silicone surface to create amino-groups (silicone-NH_2). Subsequently, M-PEG-ald was grafted onto the aminated surface. The graft density and length of PEG chains are essential for protein repellent properties. The high density of PEG chains is achieved by reaction of methoxy-polyethylenglykol-aldehyde (mPEG-ald) with amino groups of PEI layer at 'cloud point' reaction conditions (60 °C, 11% K_2SO_4). The resulting surface (silicone-PEG) has protein repellent properties.

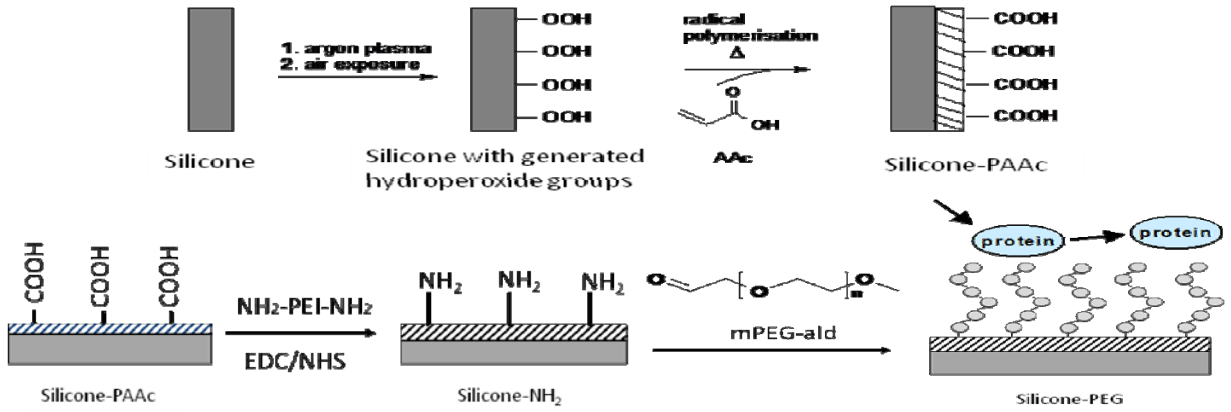

Figure 1: Activation and functionalisation of silicone surface

Hydrophilicity of the surfaces, reflected by the contact angle is expected to be significantly reduced for the modified surfaces. The results of the contact angle measurements by the captive bubble method confirmed these expectations. Unmodified silicone has a contact angle of 83±5°, which indicates a high hydrophobe surface. After graft-co-polymerisation of acrylic acid, an increase in wettability with water is achieved, contact angle was considerably reduced to 29±2°. Highly hydrophilic surface is produced due to the carboxylic groups. After grafting of PEG chains, the contact angle is further reduced to 19±4°.

Quantitative information about the chemical composition of the outermost (10 nm) surface layer of the unmodified and modified silicone surfaces has been obtained by means of XPS. All modifications steps were reflected in clear alterations of the surface element composition. XPS data showing the elemental composition as well as the different carbon species are listed in Table 1.

High resolved C1s-spectra from surfaces showed three different carbon species originating from aliphatic, ether and carboxyl carbon. The results showed successful grafting of acrylic acid on the plasma activated silicone surface. After graft copolymerisation of PAAc, newly formed O–C=O groups are detected at 289.1 eV, whereas silicium content decreased from 27.5 to 21.6 atom-%. Covalent grafting of a PEI layer results in an introduction of amino groups on the silicone-PAAc surface. As shown in Table 1, surface nitrogen content of 2.3 atom-% was registered and is an indicator of PEI presence on the surface. Also, the carbon position at 286.5 eV is attributed to C-N carbon of amino groups, overlaps with C-O signals and increased from 2.0 to 3.5 atom-%.

The efficiency of grafting M-PEG chains onto the silicone-PEI surface was derived from the ratio of the XPS intensities of ether carbon atom (originating from PEG) and alkyl carbon atom (originating from silicone-PEI). Deconvolution of the C1s-XPS of the silicone-PEG surface (Table 1) shows two dominant contributions with respective binding energies of 285.0 eV and 286.5 eV. The strong 286.5 eV contribution from the ether carbon atom of M-PEG-ald indicates a successful binding reaction between M-PEG-ald and the PEI coated surface. Pure PEG samples exhibit a single C1s peak centred at 286.5 eV.

The XPS data of Table 1 also show that M-PEG-ald coatings is invariably thinner than the information depth of the XPS measurements as the analysis does not match the theoretical composition of pure PEG (a single C-O peak and no N from underlying amine PEI layer). Thus, XPS represent superpositions from the M-PEG-ald coatings and the underlying layers.

Table 1. Untreated and modified silicone surfaces characterized by means of XPS for elemental composition and binding energy

Surface	Si atom-%	Carbon (C1s) atom-%			O atom-%	N atom-%
		285.0 eV	286.5 eV	289.1 eV		
		C-C C-H, C-Si	C-O C-N	O-C=O N-C=O		
Silicone	27.5	45.5	1.9		25.2	
Silicone-PAAc	21.6	41.3	2.0	5.4	28.9	
Silicone-PEI	18.4	51.5	3.5	1.1	23.9	2.3
Silicone-PEG	13.6	29.8	27.9	1.2	27.1	0.4

After all modification steps a relatively high content of silicium is still registered. Reasons for detected silicium are reorganization effects on the surface. In order to minimize free surface energy, the hydrophobic methyl groups settle outwards, as the XPS spectra are measured from dried samples in high vacuum.

The ability of M-PEG layer to repel *in vitro* lysozyme adsorption was assessed by surface-MALDI-ToF mass spectrometry. Surface-MALDI-ToF-MS detects small amounts of adsorbed material [9] but is difficult to quantify. Lysozyme is a relatively small protein with isoelectric point at 11.35 and with a net positive charge at pH 7.4 (physiological condition). It is one of mayor proteins in human tear fluids. Thus, its adsorption on a hydrogel material should be promoted as the density of negatively charged groups increases. On the other hand, lysozyme uptake into positively charged hydrogel matrices should be reduced due to charge repulsion effects.

Due to the high hydrophilic nature of silicone, unmodified silicone surface strongly adsorbs lysozyme (Figure 2). The peaks observed at m/z values of 14229 and 7072 are assigned to the protonated molecular ion $(M+H)^+$ and the doubly charged molecular ion $(M+2H)^{2+}$ of lysozyme. Carboxylic groups from silicone-PAAc surface are negatively charged at pH 7.4. Electrostatic attraction between the negatively charged carboxyl groups of ionised polyacrylic acid and the overall positive charge of lysozyme is the cause of lysozyme uptake by such a hydrogel [10]. Figure 2 shows that PAAc layer on a silicone film enhanced the adsorption of lysozyme whereas grafting of the cationic PEI yields a surface that strongly rejects lysozyme.

Surface–MALDI-ToF mass spectrum measured on the silicone-PEG coating after lysozyme adsorption shows no peaks of adsorbed lysozyme. It indicates that grafting density of the PEG chains is sufficient to prevent lysozyme adsorption.

XPS results of adsorption of 1 mg/ml lysozyme (PBS-buffer pH 7.4 for 1 hours at 37 °C) on the nonmodified and modified silicone surfaces are shown at Figure 3.

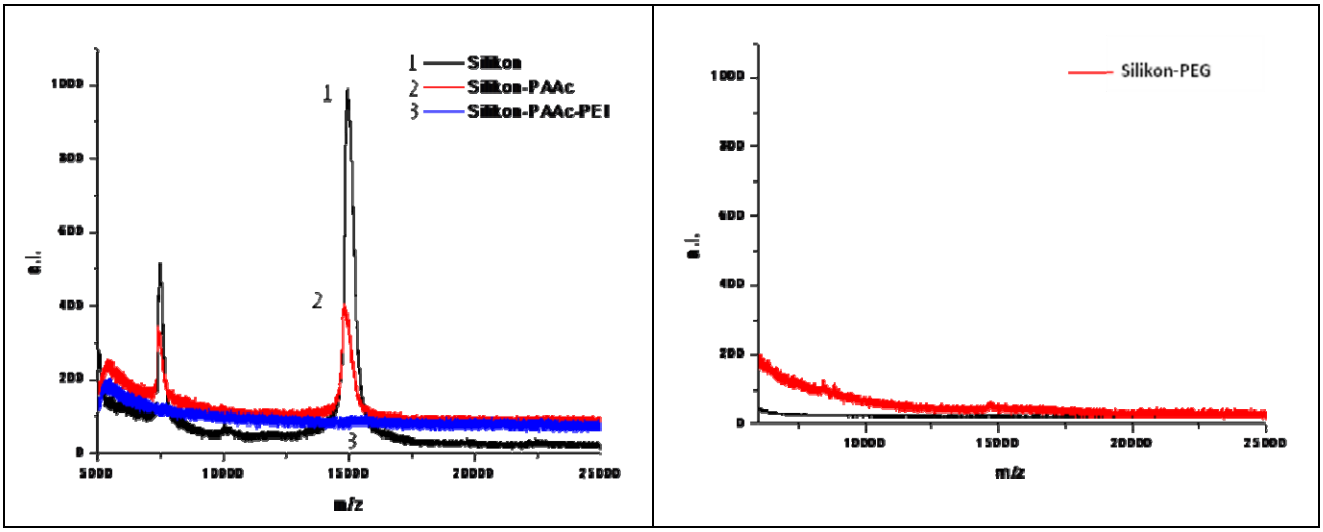

Figure 2. Surface-MALDI-TOF-MS spectra of lysozyme adsorbed on the silicone (1), silicone-PAAc (2) and silicone-PEI (3) surfaces (left) and on the silicone-PEG surface (right)

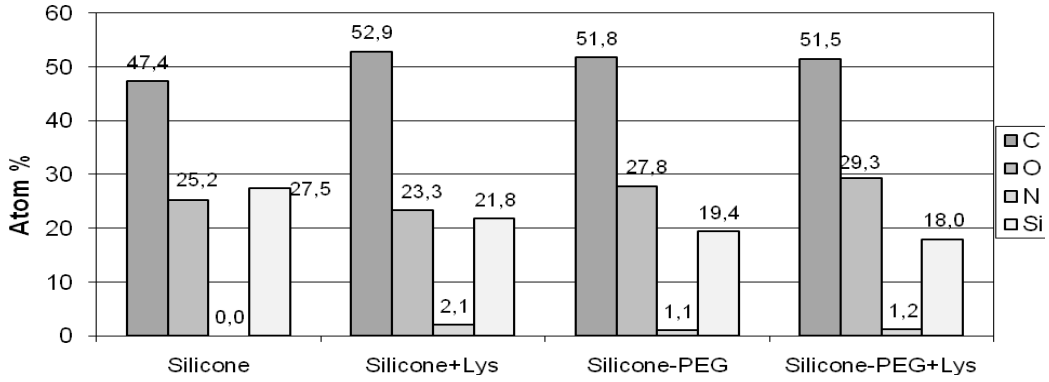

Figure 3. XPS results of 1 mg/ml lysozyme adsorption on silicone and silicone-PEG surfaces

Nitrogen content of 2,1 atom-% after adsorption of lysozyme is measured on nonmodified silicone surface. As silicone does not contain nitrogen, this can originate only from protein lysozyme, which indicated a strong adsorption of lysozyme onto silicone due to hydrophobic nature of the surface

After incubation of silicone-PEG surface with lysozyme, there is no increase of nitrogen content detected. PEG modified silicone surfaces inhibited protein adsorption onto surface.

4. CONCLUSIONS

In the present study plasma graft-copolymerisation was used to produce hydrogel coatings on the silicone contact lenses that can minimise protein adsorption. In the first step carboxylic groups were introduced to a silicone substrate by graft-copolymerisation of AAc. After attachment of PEI to the carboxylic groups, linear M-PEG chains were grafted on the surface. Maximal grafting density of M-PEG chains onto surface was achieved by grafting at the lower critical solution temperature of PEG. All surfaces modification steps were analyzed using contact angle measurements, and XPS. Protein adsorption measurements were performed with lysozyme using surface MALDI-TOF MS and XPS. Results showed that the high density of PEG chains on the silicone contact lenses surfaces can be produced thus inhibiting protein adsorption.

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ХИДРОГЕЛОВИ НА БАЗИ ПОЛИАКРИЛНЕ КИСЕЛИНЕ У СВРХУ СМАЊЕЊА БИОАДСОРПЦИЈЕ НА СИЛИКОНСКИМ КОНТАКТНИМ ЛЕЋАМА

Сажетак: Контактне леће имају два ограничења: ниска пропусност кисика и таложење протеина и липида. Како би се спријечила адхеЗија биолошких молекула на површину материјала, материјал мора да буде потпуно инертан на све биолошке реакције. Ово је могуће постићи модификовањем површине материјала. Како би се побољшала удобност ношења контактних лећа, површина мора да буде врло хидрофилна и лубриосна. У овом раду површина силиконских контактних лећа модификована је путем плазма индуковане кополимеризације акрилне киселине. На тај начин створена је веома хидрофилна површина, а поред тога карбоксилне скупине које су створене на површини представљају идеалну платформу за накнадно модификовање са полиетиленгликолом. Сваки корак модификације анализиран је путем XPS и мјерења контактнog угла. Адсорпција протеина лисозима на површину силиконских лећа анализирана је са *surface*-MALDI-TOF-MS и XPS. Након инкубације са лисозимом, анализа је показала смањење адсорбираног лисозима на хидрогел модификованим контактним лећама. Површинска модификација силиконских контактних лећа са PEG је прихватљива метода за смањење адсорпције протеина на контактне леће.

Кључне ријечи: хидрогелови, PEG, адсорпција протеина, контактне леће.

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