International Journal of Chemical, Materials and Biomolecular Sciences
ISSN: 2415-6620
Vol:2, No:11, 2008

QCM-D Study on Relationship of PEG Coated Stainless Steel Surfaces to Protein Resistance

Norzita Ngadi, John Abrahamson, Conan Fee, and Ken Morison

Abstract**—**Nonspecific protein adsorption generally occurs on any solid surfaces and usually has adverse consequences. Adsorption of proteins onto a solid surface is believed to be the initial and controlling step in biofouling. Surfaces modified with end-tethered poly(ethylene glycol) (PEG) have been shown to be protein-resistant to some degree. In this study, the adsorption of β-casein and lysozyme was performed on 6 different types of surfaces where PEG was tethered onto stainless steel by polyethylene imine (PEI) through either OH or NHS end groups. Protein adsorption was also performed on the bare stainless steel surface as a control. The adsorption was conducted at 23 °C and pH 7.2. In situ QCM-D was used to determine PEG adsorption kinetics, plateau PEG chain densities, protein adsorption kinetics and plateau protein adsorbed quantities. PEG grafting density was the highest for a NHS coupled chain, around 0.5 chains / nm^2. Interestingly, lysozyme which has smaller size than β-casein, appeared to adsorb much less mass than that of β-casein. Overall, the surface with high PEG grafting density exhibited a good protein rejection.

Keywords**—**QCM-D, PEG, stainless steel, β-casein, lysozyme.

I. Introduction

THE presence of end-tethered poly(ethylene glycol) (PEG) at solid interfaces effectively impedes nonspecific protein adsorption [1-3]. This "protein resistance" always relates to PEG chain length, interface grafting density, hydration and conformation [2, 4-7]. However, these factors are interdependent and hence it is difficult to elucidate the mechanisms of PEG-based protein resistance. Protein resistance has been shown to improve as the length of the PEG chains and grafting density increases [8-10]. Higher chain length results in larger excluded volumes, higher conformational entropy and more pronounced steric repulsion whereas higher grafting density results in difficulty of protein to diffuse to the underlying substrate. However, higher chain length practically results in a low areal density [2, 6]. The 'brush' condition which is normally higher in density is a better conformation than 'pancake' or 'mushroom' in hindering protein adsorption. Yet, if too dense, the chains will dehydrate and lose their flexibity to sweep the area where protein may adsorb [2]. At this stage the graft itself may become an adsorbent for protein and hence *increase* the adsorption of protein.

Authors are with Department of Chemical and Process Engineering, University of Canterbury, Christchurch, New Zealand (e-mail: john.abrahamson@canterbury.ac.nz).

Poly(ethylene glycol) (PEG) is a synthetic non-toxic polymer and has been approved by the FDA for internal consumption. The structure of PEG (or PEO when the molecular weight is larger than 10,000 Daltons) is OH-(-CH_2-CH_2-O-)$_n$-H. It is linear or branched and is available with a range of molecular weights. It is neutral and possesses no acidic sites (excluding the hydroxyl end-group which acts as a weak hydrogen-bond acid) and only weakly basic ether linkages. PEG is highly water soluble and has a good structural fit with water molecules, which assures a strong hydrogen bonding between the ether oxygen atoms of PEG and hydrogen atoms of the water molecules. Large numbers of hydrogen bonds with water molecules produce large repulsive forces with proteins, promoting protein resistance. The mechanisms commonly invoked to describe the protein resistant nature of end-tethered PEG surfaces have been (a) steric repulsion and (b) a hydration or water structuring layer. The former theory requires that the PEG chain length be larger than a minimum value while the latter is in accord with the observation that grafts of very short PEG (of two or three monomers) can give protein resistant surfaces [8, 11].

In this study, β-casein and lysozyme proteins have been used to test the effectiveness of several surface layers attached or 'tethered' with polyethylimine (PEI) in preventing adsorption of protein. The PEG used had two different end-groups – OH and NHS (succinimidyl ester). The layers were; PEI-OH-PEG_{45}-OCH_3, PEI-OH-PEG_{113}-OCH_3, PEI-NHS-PEG_{45}-OCH_3, PEI-NHS-PEG_{113}-OCH_3, PEI-OH-PEG_{45+113}-OCH_3 and PEI-NHS-PEG_{45+113}-OCH_3. PEG_{45} and PEG_{113} refer to layers with alternatively 45 or 113 monomers in the PEG (molecular weight of 2000 or 5000 Da), while PEG_{45+113}, refers to a layer including both PEG_{45} and PEG_{113} (also known as bimodal PEG). The adsorption of proteins was performed on a quartz crystal diaphragm coated first with gold and then stainless steel. The adsorption/desorptions were done in-situ and monitored in real time using a quartz crystal microbalance. For modification of the stainless steel surface, cationic polyelectrolyte PEI solutions were first adsorbed by physisorption. Then PEG molecules were grafted onto the resulting PEI layer. For the bimodal PEG layer, PEG_{113} were introduced first followed by PEG_{45}.

II. Experimental

A. Material

Branched polyethylenimine (PEI) with MW 25000 Da, β-casein (MW 23,000 Da) from bovine milk and lysozyme (MW 14,600 Da) from chicken egg white were purchased from Sigma-Aldrich (St.Louis, Mo, USA). Polyethylene glycol monomethyl ether (OH-PEG-CH_3, MW 2000 and 5000 Da) and polyethylene glycol succinimidyl ester (NHS-PEG-CH_3, MW 2000 and 5000 Da) were purchased from Fluka (Darmstadt, Germany). All chemicals were used as received without further purification. Phosphate buffer (pH 7.2) was prepared in our laboratory with appropriate proportions of ultra high purity MilliQ water, sodium phosphate dibasic heptahydrate from Sigma–Aldrich and sodium dihydrogen phosphate monohydrate ($NaOH_2PO_4.H_20$) from Merck (Steinheim, Germany). The buffer solutions were degassed with helium prior to use to avoid bubble formation during QCM experiments. PEG and protein solutions were prepared in phosphate buffer solution. The concentration of protein and PEG solution was 0.1 and 1.0 g / L, respectively for all runs. A stock of protein solutions were kept in the freezer at 4°C. Protein solutions not used within 48 hours of thawing were discarded. The PEI solution with concentration of 30 g / L was prepared using milliQ water.

B. QCM-D Experiment

A Q-4 model QCM (Q-Sense, Goteborg, Sweden) with frequency and dissipation monitoring (QCM-D) and AT-cut quartz crystals with a fundamental resonant frequency of 5 MHz and a diameter of 14 mm were used. One side of each diaphragm crystal was coated by the manufacturer with 100 nm of gold and then 50 nm of stainless steel (SS2343). The composition of the stainless steel (SS) was carbon (0.03 %), chromium (16.5-18.5 %), nickel (11-14.5 %), molybdenum (2.5-3 %) and iron (64-70 %). The quartz crystal was mounted in a flow cell with the SS surface exposed to the solution. For adsorption of protein onto a bare surface, the protein sample solutions were pumped through the flow cell by a peristaltic pump at a flow rate of 100 μL / min. Desorption was performed immediately after the adsorption reached steady state, by replacing the protein solution with a pure buffer flow. Measuring the final frequency change in the presence of pure buffer (referred to the baseline in buffer) means that protein adsorption is found without involving changes in the liquid density and viscosity. In preparation for adsorption of proteins onto a modified surface, the surface was modified in situ. PEI solution was pumped through first, followed by PEG solution and finally protein solution with each step followed by desorption using the buffer (for PEI, MilliQ water was used). The kinetics of sample adsorption and desorption were followed by changes in the resonant frequency of the crystal and dissipation of the crystal vibrations. All measurements reported in this paper were done with the system temperature stabilized at 23±0.5 °C. The crystals were cleaned prior to runs by immersion in a 5:1:1 mixture of milliQ water, ammonia (25% v/v) and hydrogen peroxide (30% v/v) for 5 minutes at 75 °C, followed by thorough rinsing with milliQ water and drying with a moisture-free nitrogen gas stream. To finish the cleaning, the crystals were treated with UV light and ozone for 5-10 minutes to remove organic contamination. The general procedure for using this model of QCM has been reported elsewhere [11].

C. QCM-D Modeling

Four overtones (fifth, seventh, ninth and eleventh) were used to model the viscoelastic properties (viscosity, elasticity, thickness) using the Voigt model (Q-TOOLS software (301 version 2.1, Feb 2006), Q-Sense, Goeteborg, Sweden). Parameters assumed fixed were (i) layer density, 1200 kg / m^3, (ii) fluid viscosity, 0.001 kg / m s and (iii) fluid density, 1000 kg / m^3. Parameters fitted were (i) layer viscosity between 0.0001 and 0.05 kg / m s, (ii) layer shear between 10^4 and 10^8 Pa, and (iii) layer thickness between 10^{-10} and 10^{-6} m.

The thickness of the layer obtained from the Voigt model was multiplied by the density of the layer to estimate the mass adsorbed per unit surface area. The number density of protein molecules adsorbed was then calculated by dividing the mass adsorbed per unit area by the molecular weight of the protein and multiplying by Avogadro's number ($6.023x10^{23}$ molecules.mol^{-1}).

III. Results

Fig. 1 shows the mass density of PEI layers on a stainless steel surface as a function of time interpreted using the Voigt model. Adsorption of PEI onto the surface was fast and reached a plateau in less than 1 minute. The mass density adsorbed at steady state was about 20 mg / m^2 (that is, about 0.5 chains / nm^2). When the PEI layers were rinsed with phosphate buffer, the mass decreased and presumably weakly bound PEI molecules were desorbed. Almost 85 % of PEI mass was desorbed, leaving approximately 3 mg / m^2 (that is, 0.07 chains / nm^2), corresponding to around 4 nm spacing.

Fig. 2 shows the adsorption/desorption kinetics of PEG-OH (MW5000 Da) onto a PEI coated stainless steel surface. The adsorption of PEG was fast in the initial few seconds followed with a gradual decrease in rate before reaching plateau at about 1.2 mg / m^2. Rinsing the layer with buffer solution removed about 30 % of molecules.

Fig. 3 illustrates the number density of tightly bound PEG molecules on the stainless steel surface coated with a PEI layer (those PEG molecules remaining after desorption with fresh buffer solution). Generally, density of PEG-NHS chains was higher than that of PEG-OH. The chain density of bimodal layers for each PEG type was slightly higher than that of the same monomodal PEG. The highest PEG chain density was achieved using bimodal PEG-NHS (that is, combination between 5000 and 2000 Da), about 0.5 chains / nm^2. As expected, chain density of PEG5000 was lower than that of PEG2000 Da.

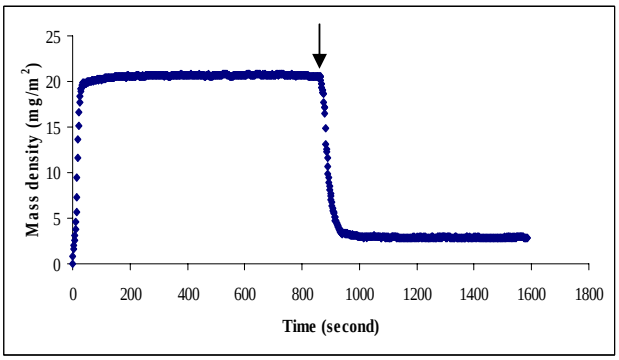

Fig. 1 Adsorption and desorption kinetic of PEI on a stainless steel surface. The arrow refers to the start of rinsing with buffer

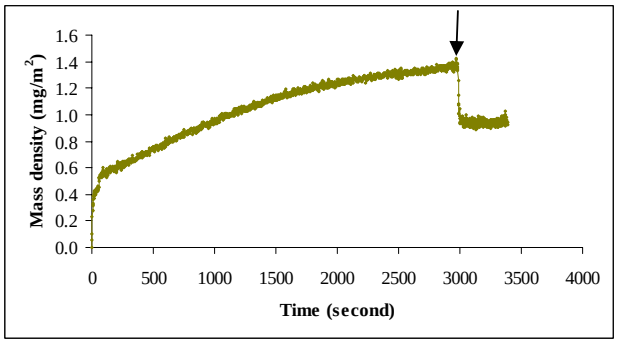

Fig. 2 Adsorption and desorption kinetic of PEG5000-OH on a PEI coated stainless steel. The arrow refers to the start of rinsing with buffer

.

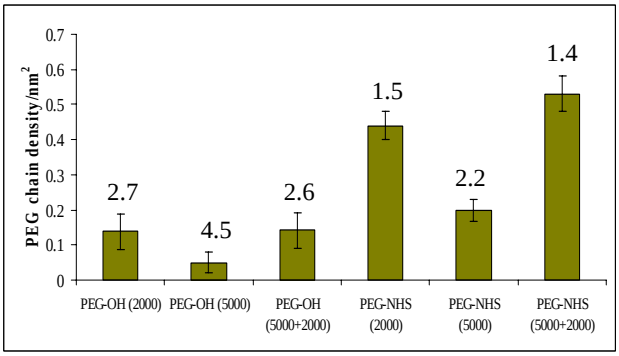

Fig. 3 Chain density of tightly bound PEG molecules on PEI coated stainless steel. Values above bars are chain spacing in nm

Fig. 4 shows an example of adsorption/desorption kinetics of β-casein on bare PEI-PEG5K-OH and PEI-PEG5K-NHS surfaces. As can be seen, the number density of β-casein on modified surfaces was lower than that on an unmodified stainless steel surface, especially lower on a PEI-PEG5K-OH surface. Adsorption of β-casein on a bare stainless steel surface reached a plateau faster than that on modified surfaces. The number density of β-casein in equilibrium with the protein solution was the lowest on PEI-PEG5K-OH surface, at approximately 40 % lower than that on bare surfaces. After rinsing with buffer the remaining number of molecules on the PEI-PEG5K-NHS surface was almost the same as those remaining molecules on the bare surface. In contrast, about a 40 % reduction in residual protein was found on PEI-PEG5K-OH surfaces. Desorption appears to be faster and approaches steady state faster on modified surfaces than on the bare surface.

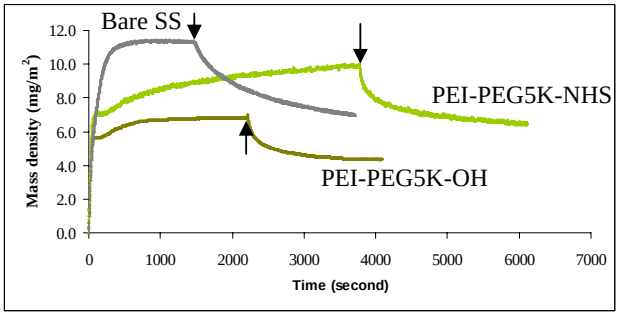

Fig. 4 Adsorption and desorption kinetics of β-casein on bare, PEI-PEG5K-OH and PEI-PEG5K-NHS surfaces. Arrows refer to the start of rinsing with buffer

Figs. 5 and 6 show the corresponding final steady state values of mass density after rinsing with buffer, of β-casein and lysozyme adsorbed on a bare stainless steel surface and on PEI, PEI-PEG-OH and PEI-PEG-NHS layers. The horizontal line was drawn at the bare SS value to make an easy comparison between modified and unmodified surfaces. As can be seen in Fig. 5, the mass of β-casein adsorbed on PEG-OH (2000), PEG-OH (5000), and PEG-NHS (5000+2000) was about 40 % lower than that on a bare stainless steel surface whereas no significant reduction occurred on the rest of the PEG coated surfaces. By contrast, the mass density adsorbed was about 35 % *higher* on the PEI surface.

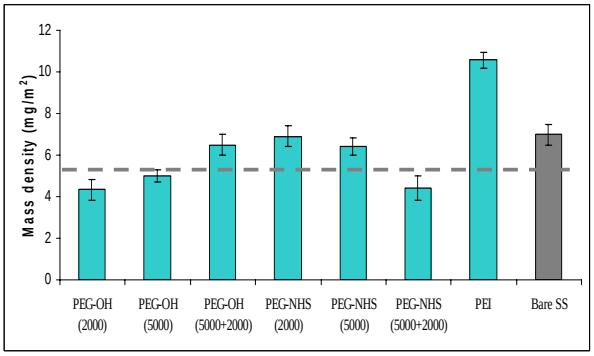

Fig. 5 Number density of tightly bound β-casein on a bare, PEI and PEI-PEG surfaces for PEG of various molecular weights

Fig. 6 shows a huge decrement in mass of lysozyme adsorbed on PEG modified stainless steel surfaces as compared to that on the bare surface. It was intriguing to note that almost no adsorption of lysozyme occurred on the PEI

layer. Stainless steel modified with bimodal PEG layer (PEI-PEG-OH (5000+2000) and PEI-PEG-NHS (5000+2000)) had less than 1 % of the bare surface adsorption.

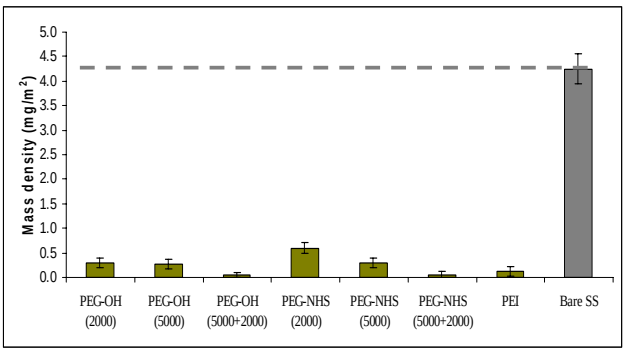

Fig. 6 Number density of tightly bound lysozyme on a bare, PEI and PEI-PEG surfaces for PEG of various molecular weights

IV. Discussion

All the results presented in this study have been obtained using the Voigt model [12]. It is important to note that the mass estimated by the Voigt model from QCM-D data is the mass of the total layer next to the crystal surface, which includes both protein (and/or PEG and/or PEI) molecules plus water that is bound or trapped in the layer. Thus all the data presented here for adsorbed amounts (including the number density values) are too high by the mass proportion of water in the adsorbed layer. From QCM-D measurements alone, the contribution of the solvent to the total mass is not easy to establish. Also uncertain is the assumed layer density of 1200 kg / m^3. The density of the layer should lie approximately between that of a protein layer and that of water. It was reported that the density of typical protein is 1400 kg / m^3 [13]. A combination between QCM-D and optical techniques such as ellipsometry and optical waveguide lightmode spectroscopy (0WLS) enable an estimate of this effective layer density [13,14] as these latter techniques are sensitive to only the 'dry mass' of a substance adsorbed onto the surface. Changing the assumed layer density from 1000 to 1400 kg / m^3 (40 % increase) gave only about 20 % increase in the estimated mass of the layer, indicating that the thickness calculated by the model is also affected by the layer density assumed, and reduces with an increase in density. Our assumption of 1200 kg / m^3 should therefore lead at most ± 10 % error in total layer mass. This value also corresponds to about 50 mass % protein (or other polymer) molecules in the layer. Thus, the number of protein molecules presented here should then be multiplied by 50 % to get a better estimate of protein molecules on the surface. Attachment of PEI molecules onto the stainless steel was expected to be based on electrostatic interaction (interaction between positive charge of PEI and negative charge of bare stainless steel). However, the electrostatic interaction force was not strong enough to bind much PEI on the surface as shown by a huge desorption into buffer (Fig. 1). A cross-linking modifier such as glutaraldehyde was found to be able to reduce the desorption of PEI [15].

The grafting of PEG-NHS molecules to the PEI anchor layer was believed to be achieved through ester-amine reaction; NHS ester groups reacting with amine groups on PEI. Hydrogen bonding, electrostatic attraction and van der Waals interaction meanwhile were believed to constitute the interaction between PEI and PEG-OH molecules. With such physically adsorbed PEG containing layers, there is always a risk of displacement of adsorbed polymer layers by the protein. The number density of PEG molecules on the surface was the highest for bimodal PEG (MW 5000+2000) (consistent with [3, 9] followed by PEG (MW2000) and 5000 (in the same order as work in [2,5,6]).

Up to 99 % reduction of lysozyme, a small protein, on PEI-OH-PEG_{45+113}-OCH_3 and PEI-NHS-PEG_{45+13}-OCH_3 surfaces was a significant achievement for surface treatments which were done simply by passing solutions through. Considering only protein size as a factor, proteins with smaller size have generally a higher adsorption. For example, the amount of lysozyme (MW 14.6kDa) adsorbed on a poly(ethylene terephthalate) (PET) surface modified with PEG was higher than that of collagen (285 kDa) on the same surface [16]. In another study, myoglobin (16kDa) adsorbed more on PEGylated surfaces than did bovine serum albumin (BSA) (67kDa) or fibrinogen (MW 340kDa) [17]. Indeed, prevention of small protein adsorption is a great challenge [1].

A mixture of long and short PEG chains (that is, bimodal PEG) has been shown to increase protein resistance of PEG coatings over those of monomodal PEG due to a combined of high mobility of the long chains and high density of the short chains close to the surface [3,9,17]. In our case, the grafting density rather than chain length was believed be the primary factor in blocking the adsorption of lysozyme because the distance between PEG chains was smaller than that of the dimension of lysozyme molecules. The grafting density achieved in this study appeared to be sufficiently high to inhibit adsorption of lysozyme. PEI surfaces (without the presence of PEG molecules) were also as good as bimodal PEG surfaces in hindering adsorption of lysozyme. This was apparently because of electrostatic repulsion between lysozyme and PEI molecules since both of them have net positive charges at pH 7.2.

The ability of PEG coated surfaces to inhibit adsorption of β-casein was not so impressive as compared to that for lysozyme. According to Halperin's model, [18] large proteins can adsorb at the outer surface of the PEO layer through van der Waals interactions. However, in the case of PEG coated surfaces, the layers formed are typically quite dilute (well hydrated), and hence the van der Waals interaction between proteins and the polymer layer is expected to be weak. β-casein's molecular weight is almost double to lysozyme. However, the viscosity radius of β-casein (2.3 nm) is smaller than that of the lysozyme dimension and the average spacing between the PEG molecules was generally slightly larger than that of β-casein's radius (see Fig. 3), hence enabling the

molecules to penetrate through the PEG layers and adsorb on the surface.

Mass density of β-casein adsorbed on a PEI surface was higher than that on the bare surface as can be seen in Fig. 5. Two main driving forces were believed to be involved; (i) hydrophobic interaction between proteins and surface (with the assumption that the density of PEI molecules probably was not sufficiently high enough to shield the stainless steel surfaces) and (ii) electrostatic attraction between proteins and PEI molecules. At ph 7.2, β-casein and PEI molecules have negative and positive net charges, respectively. However, for less stable proteins ('soft' protein) like β-casein, they are able to adsorb on both hydrophilic and hydrophobic surfaces even with the same charges. For 'soft' proteins, the adsorption does not generally behave accordingly to electrostatic dictates [19].

Generally, the PEG coated surfaces prepared in this study was able to inhibit adsorption of β-casein and lysozyme proteins. The method used was simple and cost effective yet promising (especially for lysozyme). It has been reported that the method of PEG attachment, either chemisorption or physisorption has little effect on the protein rejecting capacity once a sufficient interfacial chain density was achieved [20]. This may have practical significance as regards coating for long term stability (for physisorption method). A study done by [8] found out that the number of ethylene glycol (EO) chains per unit area required to eliminate adsorption was almost the same for lysozyme, ribonuclease, pyruvate kinase and fibrinogen. They suggested that the adsorbance of each mixed self-assembled monolayer (SAM) was dominated by the interfacial properties of the SAM and not the protein used. With the QCM-D technique, the whole process through surface modification to adsorption of proteins was possible to be monitored in real time.

V. Conclusion

From this work, it can be concluded that:

- The effectiveness of PEG based coatings to repel adsorption of protein was dependent on PEG grafting density
- Combination between short and long chains gave higher grafting densities
- The method of surface modification was simple yet promising
- All the data presented in this study represent 'wet mass', hence the data obtained are overestimated values

Acknowledgment

We acknowledge the Malaysian Government for supporting during her PhD.

References

[1] P. Kingshott, W. Jiang, G. B. Nikolaj and G. Lone, G, "Covalent attachment of Poly(ethylene glycol) to surfaces, critical for reducing bacterial adhesion", Langmuir. vol. 19, pp. 6912-6921, 2003

[2] L.D.Unsworth, H.Sheardown and J.L.Brash, "Protein resistance of surfaces prepared by sorption of end-thiolated poly(ethylene glycol) to gold:Effect of surface chain density", Langmuir. vol. 21. pp.1036-1041, 2005

[3] T.Satomi, Y.Nagasaki, H.Kobayashi, T.Tateishi, K.Kataoka and H.Otsuka, "Physicochemical characterization of densely packed poly (ethylene glycol) layer for minimizing nonspecific protein adsorption", Journal of Nanoscience and Nanotechnology, vol. 7, pp. 2394-2399, 2007

[4] J. Wei, D.B. Ravn, L. Gram and P. Kingshott, "Stainless Steel Modified with PEG can Prevent Protein Adsorption but not Bacterial Adhesion", Colloids and Surfaces B: Biointerfaces, vol. 32, pp. 275-291.2005

[5] J.G. Archambault and J.L. Brash, "Protein Repellent Polyurethane-Urea Surfaces by Chemical Grafting of Hydroxyl-Terminated Poly(ethylene oxide): Effects of Protein Size and Charge", Colloids and Surfaces B: Biointerfaces, vol. 33, pp. 111-120, 2004

[6] A. Roosjen, H.C. Mei, H.J. Busscher and W. Norde, "Microbial Adhesion to Poly(ethylene oxide) Brushes: Influence of Polymer Chain Length and Temperature", Langmuir, vol. 20, pp. 10949-10955, 2004

[7] W.Norde and D.Gage, "Interaction of BSA and human blood plasma with PEO-tethered surfaces: influence of PEO chain length, grafting density and temperature", Langmuir, vol. 20, pp. 4162-4167, 2004

[8] K.L.Prime and G.M.Whitesides, "Adsorption of Proteins onto Surfaces Containing End-Attached Oligo(ethylene oxide): A Model System Using Self-Assembled Monolayers", Journal American Chemistry Society, vol. 115, pp. 10714-10721, 1993

[9] K.Uchida, H.Otsuka, M.Kaneko, K.Kataoka and Y.Nagasaki, "A reactive poly (ethylene glycol) layer to achieve specific surface plasmon resonance sensing with a high S/N: The substantial role of a short underbrushed PEG layer in minimizing nonspecific adsorption", Analytical Chemistry, vol. 77, pp. 1075-1080

[10] C.Yoshikawa, A.Goto. Y.Tsuji, T.Fukuda, T.Kimura, K.Yamamoto and A.Kishida, "Protein repellency of well-defined, concentrated poly (2-hydroxyethyl methacrylate) brushes by the size exclusion effect", Macromolecules, vol. 39, pp. 2284-2290, 2006

[11] Y.J.Wu, R.B.Timmons, J.S.Jen and F.E.Molock, "Non-fouling surfaces produced by gas phase pulsed plasma polymerization of an ultra low molecular weight ethylene oxide containing monomer", Colloids and surfaces B: Biointerfaces, vol. 18, pp. 235-248, 2000

[12] N.Weber, H.Peter, and J.Kohn, "Formation of viscoelastic protein layers on polymeric surfaces relevant to platelet adhesion", Wiley InterScience, pp. 420-427,2005

[13] F. Hook, J. Voros, M. Rodahl, R. Kurrat, P. Boni, J.J. Ramsden, M. Textor, N.D. Spencer, P. Tengvall, J. Gold and B. Kasemo, "A Comparative Study of Protein Adsorption on Titanium Oxide Surfaces Using in situ Ellipsometry, Optical Waveguide Lightmode Spectroscopy and Quartz Crystal Microbalance/Dissipation". Colloids and Surfaces B: Biointerfaces, pp. 155-170, 2002.

[14] M.T. Muller, X. Yan, S. Lee, S.S. Perry and N.D. Spencer, N.D. (2005). "Lubrication Properties of a Brushlike Copolymer as a Function of the Amount of Solvent Absorbed within the Brush". Macromolecules, pp. 5706-5713, 2005.

[15] I.M. Nnebe, R.D. Tilton, J.W. Schneider, "Direct Force Measurement of the Stability of Poly(ethylene glycol)-Polyethylenimine Graft Films", Journal of Colloid and Interface Science, vol. 276, pp. 306-316, 2004.

[16] R.Fukai. P.H.R.Dakwa and W.Chen, "Strategies toward biocompatible artificial implants:grafting of functionalized poly(ethylene glycol)s to poly(ethylene terephthalate) surfaces", Journal of Polymer Science:Part A:Polymer Chemistry. vol. 42, pp. 5389-5400, 2004.

[17] R. Michel, S. Pasche, M. Textor and D. G. Castner, "Influence of PEG Architecture on Protein Adsorption and Conformation", Langmuir, vol. 21, pp. 12327-12332, 2005.

[18] A.Helparin, "Polymer brushes that resist adsorption of model proteins:Design parameters", Langmuir, vol. 15, pp. 2525-2533, 1999

[19] N.Nath, J.Hyun, H.Ma and A.Chilkoti, "Surface Engineering for Control of Protein and Cell Interactions", Surface Science, vol. 570, pp. 98-110, 2004.

[20] M.Malmsten, K.Emoto and J.M.V.Alstine, "Effect of chain density on inhibition of protein adsorption by Poly(ethylene glycol) based coatings", Journal of Colloidal and Interface Science, vol. 202, pp. 507-517, 1998.