QCM-D Study of β-casein Adsorption on Bimodal PEG Brushes

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Abstract—Adsorption of proteins onto a solid surface is believed to be the initial and controlling step in biofouling. A better knowledge of the fouling process can be obtained by controlling the formation of the first protein layer at a solid surface. A number of methods have been investigated to inhibit adsorption of proteins. In this study, the adsorption kinetics of β-casein onto bimodal PEG brushes on a stainless steel surface has been studied by means of a quartz crystal microbalance with dissipation (QCM-D). Bimodal brushes consist of long and short PEG chains were prepared in situ using a physisorption method. The adsorption kinetics of β-casein were monitored with different PEG combinations and concentrations. β-casein adsorptions onto monomodal PEG brushes were determined for comparison. β-casein adsorption was also studied on a bare stainless steel surface as a control. All adsorptions were conducted at 23°C and pH 7.2. The results obtained showed that depositing PEG from solutions with increasing concentrations from 0.1 to 5.0 g / L decreased adsorption of β-casein by about 40% on monomodal surfaces. Bimodal PEG surfaces appeared to be less effective than monomodal PEG brushes surfaces in preventing adsorption of β-casein. It is suggested that bimodal PEG surfaces behave like a 'solid' which leads to β-casein adsorption at the surface via hydrophobic interaction for this observation. The difference between PEG combinations in suppressing the adsorption of β-casein was not very significant, indicating the existence of an optimal value of chain density to effectively suppress the adsorption of β-casein.

Keywords—β-casein, QCM-D, stainless steel, bimodal brush, PEG

I. INTRODUCTION

THE formation of an unintended biofilm at a surface may cause many problems such as in medical applications (artificial implants, catheters, contact lenses), in the food industry (contamination of process equipment), in water purification plants, on ship hulls and in the dairy industry. The formation of a biofilm starts with the adsorption of proteins at a surface, followed by deposition of biological cells, bacteria, or other microorganisms [1-3]. Research has shown that covering a (hydrophobic) surface with a PEG brush can be effective in preventing or retarding the adsorption of proteins at the surface and suppressing deposition of biological cells and bacteria.

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The presence of end-tethered poly(ethylene glycol) (PEG) at solid interfaces effectively impedes nonspecific protein adsorption [4-6].

This "protein resistance" always relates to PEG chain length, interface grafting density (the number of PEG chains per unit area) and hydration and conformation [2,5,7-9]. 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 [10-12]. 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 results in a low areal density in practice [5,8]. Poly(ethylene glycol) (PEG) is a synthetic non-toxic polymer that 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 [10,13].

Three different ways of protein deposition on the PEG layer can be distinguished as suggested in Helparin's model [14]; primary, secondary and tertiary adsorption. The term primary adsorption is used when the adsorbing particle is smaller than the separation distance between the PEG chains, allowing diffusion into the brush and adsorption at the surface. In the case of secondary adsorption, the particle is bigger than the distance between the PEG chains, so it cannot enter the brush, but it may adsorb at the brush–solvent interface. Ternary adsorption meanwhile occurs from compression of protein molecules towards the PEG layer and is a variation of primary

adsorption for very large proteins. However, [12] describes ternary adsorption as the situation when the particle can diffuse into the brush, where it is captured.

In this study, the adsorption of β-casein onto several bimodal PEG coated stainless steel surfaces was studied. The objective of this investigation was to test the antifouling properties of bimodal PEG surfaces by measuring the adsorption of β-casein at such surfaces. There appears to have been no work done on bimodal PEG layers on a stainless steel surface to date. Most of the published work has used gold surfaces, involving only PEG (MW5000+MW2000) combinations [6,11,15]. In this work, β-casein adsorption onto monomodal PEG surfaces was also determined for comparison. The adsorption of β-casein was performed on a quartz crystal diaphragm coated first with gold and then stainless steel. The adsorption and desorptions for both PEG and β-casein were done in-situ and monitored in real time using a quartz crystal microbalance.

II. METHODOLOGY

A. Materials

Branched polyethylenimine (PEI) with MW 25000 Da and β-casein (MW 23,000 Da) from bovine milk were purchased from Sigma-Aldrich (St.Louis,Mo,USA). Polyethylene glycol monomethyl ether (OH-PEG-CH$_3$, MW 350, 550, 2000 and 5000 Da) was 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, Na$_2$HPO$_4$ and NaH$_2$PO$_4$ (from Sigma Aldrich, St.Louis,Mo,USA). 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 concentrations of protein and PEG solution were 0.1 and 0.1 to 5.0 g / L, respectively for all runs. Stocks of protein solution were kept in the freezer at 4°C. Protein solutions not used within 48 hours of thawing were discarded. The PEI solutions were prepared in milliQ water at concentration of 30 g / L. All experiments were conducted at 23°C and pH 7.2.

B. QCM-D experiments

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. For adsorption of protein onto a single PEG chain layer, the surface was modified in situ by pumping the PEI solution first, followed by PEG solution and finally protein solution. Meanwhile, for the adsorption of protein onto bimodal PEG layers, the steps taken were the same as on the single PEG layer except instead of using PEG chains of a single length, chains with two lengths were used. Three combinations were used; PEG (5000+2000), PEG (5000+350) and PEG (550+350). The kinetics of sample adsorption and desorption were followed by changes in the resonant frequency of the crystal and dissipation of the crystal vibrations. The frequency and dissipation changes were recorded simultaneously at different overtones (n = 3 (15 MHz), 5 (25 MHz), 7 (35 MHz), 9 (45 MHz) and 11 (45 MHz)). All measurements reported in this paper were done with the system temperature stabilized at 23±0.5°C. The crystals were cleaned 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 [16].

C. QCM-D modelling

Four overtones (fifth, seventh, ninth and eleventh) were used to model the viscoelastic properties of the adsorbed layer using 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. It was assumed that the effective density of the layer is 1200 kg/m^3.

III. RESULTS

Fig. 1 shows the number density of tightly bound PEG molecules on the PEI coated stainless steel surface (those PEG molecules remaining after desorption with fresh buffer solution) as a function of PEG molecular weights and concentrations. Increasing concentration from 0.1 to 1.0 g/L showed no significant increase in PEG number density, especially shown by PEG2000 and 5000 Da. As concentration increased to 5.0 g/L, the PEG chain density rapidly increased especially shown by PEG 350 and 550 Da, up to 60 and 50%, respectively. Generally the number density of PEG350 was the highest followed by PEG 550, 2000 and 5000 Da.
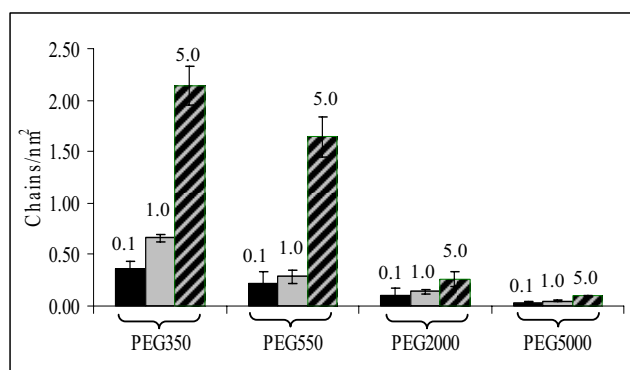
Fig. 1: Number density of tightly–bound PEG molecules on PEI coated stainless steel surfaces as a function of PEG molecular weights and concentrations. The data was obtained using the Voigt model. Figures above bar refer to the concentration of PEG solution

Fig. 2 shows the number density of long chain (first column) and then short chain (second column) on PEI coated stainless steel surfaces for combination of PEG (550+350), PEG (5000+350) and PEG (5000+2000). Introducing short PEG chains (especially PEG350) on a pre-constructed long PEG layer enhanced the total PEG number density. Generally, combination between PEG550 and 350 gave the highest chain density, followed with PEG (5000+350) and PEG (5000+2000). As expected PEG grafting density increased as PEG concentration increased, especially shown by PEG (550+350) and PEG (5000+350).
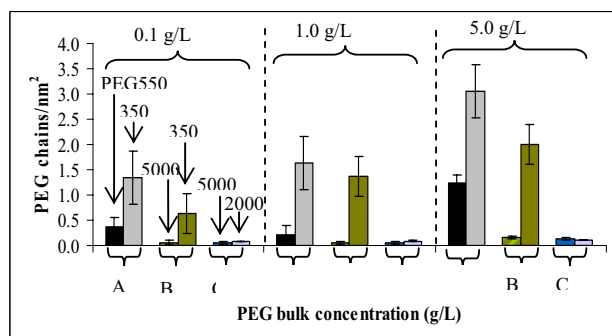

Fig. 2: Number density of tightly–bound PEG molecules on PEI coated stainless steel surfaces. First and second column refers to long and short chains, respectively for combination of PEG (550+350) (A), PEG (5000+350) (B) and PEG (5000+2000) (C). The data was obtained using the Voigt model.

Fig. 3 shows an example of adsorption and desorption kinetics of β-casein on bare, PEI-PEG2000, PEI-PEG5000 and PEI-PEG (5000+2000) surfaces. As can be seen, the mass density of β-casein on PEG (5000+2000) was the highest ($\cong$15 mg/m^2) followed by the adsorption on a bare surface, then on PEG2000 and PEG5000 surfaces at 11, 9 and 6 mg/m^2, respectively. Adsorption of β-casein on a PEG (5000+2000) reached a plateau slower than that on bare or monomodal PEG surfaces. After rinsing with buffer the remaining number of

molecules on the monomodal PEG surface was almost the same as those remaining on the bare surface. In contrast, about 40 % higher residual β-casein protein was found on PEG (5000+2000) surfaces as compared to that on the bare surface. Desorption appears to be the highest (when expressed as a percentage of the maximum) on the bare surface (36 %), followed by PEG 2000, PEG5000 and PEG (5000+2000) surfaces, 33, 16 and 13 %, respectively.
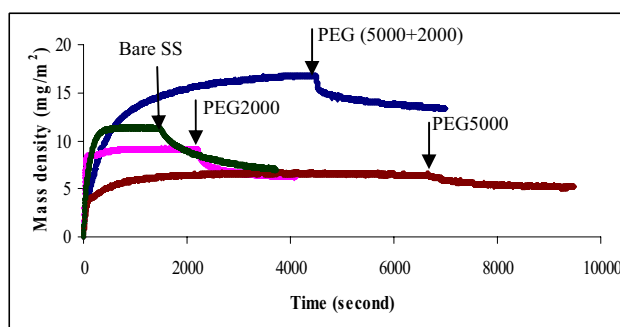

Fig. 3: Adsorption and desorption kinetics of β-casein on bare SS (black line), monomodal PEG2000 (pink line) and PEG5000 (brown line) and bimodal PEG (5000+2000) (blue line) surfaces. Arrows refer to the start of rinsing with buffer.

Fig. 4 shows the final steady state value of mass density of β-casein adsorbed on a bare stainless steel surface and on monomodal PEG coated stainless steel surfaces for PEG of various molecular weights and concentrations. Generally, the adsorption of β-casein on monomodal PEG surfaces ranged from about 4 to 6.5 mg/m^2. This corresponds to about 60 to 90 % adsorption of that on the bare surface.
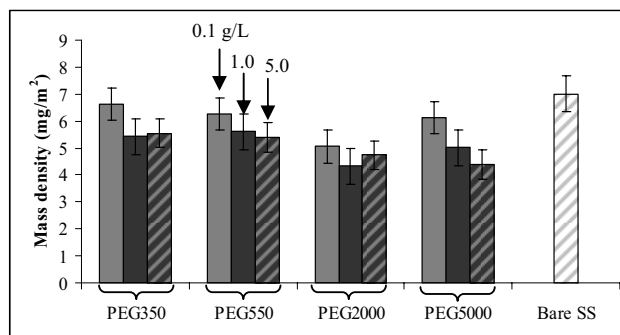

Fig. 4: Mass density of tightly bound β-casein on a bare and PEI-PEG surfaces for monomodal PEG of various molecular weights and concentrations.

Fig. 5 illustrates the final steady state values of β-casein mass density after rinsing with buffer on bimodal PEG surfaces as a function of PEG solution concentration and combinations. The effectiveness of bimodal PEG surfaces in inhibiting adsorption of β-casein was compared to that of monomodal PEG surfaces (mean mass density). Generally, adsorption of β-casein decreased with PEG films laid down

from solutions of higher concentration. The adsorption of β-casein was the lowest on PEG (550+350) followed by PEG (5000+2000) and PEG (5000+350) surfaces. However, the difference between these combinations in suppressing the adsorption of β-casein was not very significant. Overall, the adsorption of β-casein ranged from about 6 to 10 mg/m^2. Unexpectedly, the adsorption of β-casein was about 20 to 40 % *higher* on bimodal PEG surfaces than that on monomodal PEG surfaces.
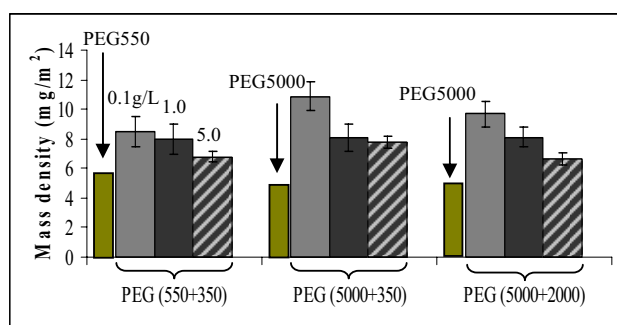

Fig. 5: Mass density of tightly bound β-casein on bimodal PEG coated surfaces as a function of PEG solution concentration and combinations, compared with the monomodal average value for the longer chains.

IV. DISCUSSION

With the QCM-D technique, the whole process through surface modification to adsorption of proteins was possible to be monitored in real time. All the results presented in this study have been obtained using the Voigt model. 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 higher than the adsorbed protein amounts by the mass proportion of water in the adsorbed layers. 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 (or PEG) layer and that of water. Combination between QCM-D and optical techniques such as ellipsometry, and optical waveguide lightmode spectroscopy (OWLS) enable an estimate of this effective layer density [17,18] as the 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 about a 20 to 30 % 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 mass surface density of protein molecules presented here should then be multiplied by 50 % to get a

better estimate of protein molecules on the surface. A study done by [17] found out that for small and globular proteins such as hemoglobin (64.5 kDa) and albumin (65kDa) the measured mass of adsorbed protein is a factor of about 1.75 larger than that measured by optical techniques while it is about 2.0 to 3.2 times larger for large proteins (fibrinogen (MW340kDa) and antibodies). The mass density of β-casein adsorbed on a gold surface monitored using QCM-D meanwhile was greater by a factor of 3 to 5 compared with the optical devices [19]. The difference between QCM-D and optical techniques appears to depend on the nature and conformation of the adsorbed molecules and the liquid medium used.

The adsorption behaviour of β-casein was found to be similar between bare and PEG modified stainless steel surfaces. The adsorption of β-casein onto the stainless steel surface was believed to be driven mainly by hydrophobic interactions. The expected electrostatic repulsion between negatively charged β-casein and the surfaces was apparently compensated by hydrophobic forces. Less stable proteins ('soft' proteins) like β-casein have been found to be able to adsorb on both hydrophilic and hydrophobic surfaces even with the same charge [20], indicating that the hydrophobic forces dominate.

Theoretical and experimental approaches have demonstrated the importance of chain length and grafting density of PEG for imparting protein resistance to surfaces [2,7,21]. Protein resistance has been shown to improve as the length of the PEG chains and grafting density increases, although contradictory results are discussed below. It is generally accepted that higher chain length results in larger excluded volumes, higher conformational entropy and more pronounced steric repulsion whereas higher grafting density results in decreased protein diffusion to the underlying substrate.

The number density of PEG molecules on the surface was the highest for PEG350 followed by PEG 550, 2000 and 5000. This observation was expected since higher chain lengths result in low number density [5,7,8] compared to short chain lengths which are associated with high number density. Higher PEG chain density on a combination of PEG (550+350) than that on PEG (5000+2000) is consistent with the former expectation. The number density of bimodal PEG molecules on the surface was higher than that of the monomodal PEG by up to 50 % (at high concentration). This was apparently due to short chains filling the gap in the pre-constructed longer-chain PEG layer.

A mixture of long and short PEG chains (that is, bimodal PEG) has previously been shown to increase protein resistance of PEG coatings over those of monomodal PEG due to a combination of high mobility of the long chains and high density of the short chains close to the surface [3,11,22]. Work done by [15] for example, found that adsorption of fetal bovine serum (FBS) protein measured using surface plasmon resonance (SPR) was about 35 % lower on bimodal surfaces

than that on monomodal surfaces. The chain density for bimodal PEG (5000+2000 MW's) and monomodal PEG5000 achieved in their work were about 2.5 and 1.5 chains/nm^2, respectively, using PEG layers from PEG concentrations of 0.01 g/L. Nevertheless, in our study, the adsorption of β-casein on a bimodal PEG surface was about 20 to 40 % *higher* than that on monomodal PEG surfaces even though PEG chain density of our bimodal surfaces was almost 50 % higher than that of monomodal surfaces. This is probably because the grafting density achieved on our bimodal PEG surface was high. It was reported that if the density of the layer was high enough to reach the dense-brush regime, the graft itself may become adsorbent for protein and hence increase the adsorption of protein. Chains deposited in this regime are expected to dehydrate and lose their flexibity to sweep the incoming proteins [23, 24]. A study done by [25] showed that adsorption of lysozyme and fibrinogen was lowest on a PEO surface at a critical chain density ($\cong 0.43/nm^2$) but as PEO chain density increased above the critical value, the adsorption increased. This indicates that there is an optimal value of chain density to effectively suppress the adsorption of proteins. The mechanism postulated in this study is illustrated in Fig. 6. There are 2 regimes proposed; regime I where the deposited PEG chains are expected to be more flexible to sweep the incoming protein while in regime II, the deposited chains are dense enough to behave like a 'solid'. Some of the protein molecules are expected to diffuse through the regime I and deposited on the regime II via hydrophobic interaction.
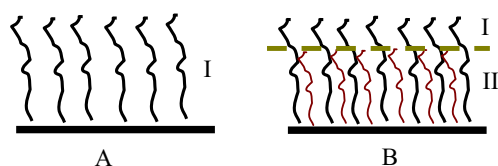

Fig. 6: Proposed mechanism between monomodal surface (A) and bimodal surface (B)

Generally, the PEG coated surfaces prepared in this study were able to inhibit adsorption of β-casein protein. It has been reported that the method of PEG attachment chosen, either chemisorption or physisorption has little effect on the protein rejecting capacity once a sufficient interfacial chain density is achieved [26]. However, in this study, it appeared that bimodal PEG surfaces were not so effective in reducing adsorption of β-casein as compared to that of monomodal PEG surfaces.

V. CONCLUSION

From this work, it can be concluded that:

- All the data presented in this study represent 'wet mass', hence the data obtained are overestimated values
- Combination between short and long chains gave higher grafting densities
- The effectiveness of PEG based coatings to repel adsorption of protein was dependent on PEG grafting density
- The difference between PEG combinations in suppressing the adsorption of β-casein appeared not very significant, indicating the existence of an optimal value of chain density to effectively suppress the adsorption of β-casein
- Bimodal PEG surfaces appeared to be less effective in rendering adsorption of β-casein as compared to that of monomodal PEG surfaces. It is suggested that bimodal PEG surfaces behave like a 'solid' which leads to β-casein adsorption at the surface via hydrophobic interaction

REFERENCES

[1] J.Visser and T.J.M.Jeurnink, "*Fouling of heat exchangers in the dairy industry,*" Experimental Thermal and Fluid Science, vol. 14, pp. 407-424, 1997.

[2] 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.

[3] 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.

[4] 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 .

[5] 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 .

[6] 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.

[7] 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.

[8] 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.

[9] 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.

[10] 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.

[11] 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. 2005.

[12] 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.

[13] 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.

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

[15] K. Uchida, Y. Hoshino, A. Tamura, K.Yoshimoto S. Kojima, K.Yamashita, I. Ymanaka, H. Otsuka, K. Kataoka and Y.Nagasaki, "*Creation of a mixed poly(ethylene glycol) tethered-chain surface for preventing the nonspecific adsorption of proteins and peptides*," Biointerphases, vol. 2, pp. 126-130, 2007.

[16] M.Rodahl, F.Hook, C.Fredriksson, C.A. Keller, A.Krozer, P.Brzezinski, M.Voinova and B.Kasemo, "*Simultaneous frequency and dissipation factor QCM measurements of biomolecular adsorption and cell adhesion*", Faraday Discuss, vol. 107, pp. 229-246, 1997.

[17] 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 sSurfaces using in situ Ellipsometry, Optical Waveguide Lightmode Spectroscopy and Quartz Crystal Microbalance/Dissipation*". Colloids and Surfaces B: Biointerfaces, pp. 155-170, 2002.

[18] 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.

[19] M.Lee, S.K.Park, C.Chung and H.Kim, "*QCM study of beta-casein adsorption on the hydrophobic surface: Effect of ionic strength and cations*", Bulletin of the Korean Chemical Society, vol. 25, pp. 1031-1035, 2004.

[20] 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.

[21] M.Jonsson and H.O. Johansson, "*Effect of surface grafted polymers on the adsorption of different model proteins*", Colloids and Surfaces B: Biointerfaces, vol. 37, pp.71-81, 2004.

[22] M.A.Carignano and I.Szleifer. 2000, "*Prevention of protein adsorption by flexible and rigid chain molecules*", Colloids and Surfaces B: Biointerfaces, vol. 18, pp. 169-182, 2000.

[23] 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.

[24] P.Y. J.Yeh, R. K. Kainthan, Y. Zou, M. Chiao and J. N. Kizhakkedathu, Self-*assembled monothiol-terminated yyperbranched polyglycerols on a gold surface: A comparative study on the structure, morphology, and protein adsorption characteristics with linear poly(ethylene glycol*)s,"Langmuir, vol. 24, pp. 4907-4916, 2008.

[25] L.D.Unsworth, Z.Tun, H.Sheardown and J.L.Brash, "*In situ neutron relectrometry investigation of gold-chemisorbed PEO layers of varying chain density: Relationship of layer structure to protein resistance*," Journal of Colloid and Interface Science, vol. 296, pp.520-526, 2008.

[26] 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.