QCM-D study of β-casein on silicate-PEG surfaces

Article *in* Journal of Applied Sciences · December 2010

DOI: 10.3923/jas.2010.3343.3348 · Source: DOAJ

4 authors, including:

Norzita Ngadi
Universiti Teknologi Malaysia
98 PUBLICATIONS **170** CITATIONS
SEE PROFILE

John Abrahamson
University of Canterbury
11 PUBLICATIONS **117** CITATIONS
SEE PROFILE

Ken Morison
University of Canterbury
40 PUBLICATIONS **474** CITATIONS
SEE PROFILE

Journal of Applied Sciences 10 (24): 3343-3348, 2010
ISSN 1812-5654

This article was presented in International Conference on Process Engineering and Advanced Materials 24th Symposium of Malaysian Chemical Engineers (15th-17th June, 2010)

QCM-D Study of β-casein on Silicate-PEG Surfaces

[1]Norzita Ngadi, [2]John Abrahamson, [2]Conan Fee and [2]Ken Morison
[1]Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia, Skudai, 81310, Johor, Malaysia
[2]Department of Chemical and Process Engineering, University of Canterbury, Christchurch, 8040, New Zealand

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 was performed on several surfaces where PEG was tethered onto stainless steel by silicate. Protein adsorption was also performed on the bare stainless steel (SS) surface for comparison. The adsorption was conducted at 23 and 40°C and pH 7.2. *In situ* Quartz Crystal Microbalance/Dissipation (QCM-D) was used to determine PEG adsorption kinetics, plateau PEG chain densities, protein adsorption kinetics and plateau protein adsorbed quantities. The results showed that the presence of PEG molecules reduced the adsorption of β-casein, more than 50% than that on the bare SS surface. Interestingly, the adsorption of β-casein on the PEG surfaces at 40°C was much lower than that at 23°C.

Key words: β-casein, silicate, PEG, stainless steel, biofouling

INTRODUCTION

The formation of an unintended biofilm at a surface may cause many problems particularly in medical applications (artificial implants, catheters, contact lenses), in the food industry (contamination of process equipment), in water purification plants, on ship hulls or in the dairy industry. The formation of a biofilm starts with the adsorption of proteins on a surface, followed by deposition of biological cells, bacteria, or other microorganisms (Visser and Jeurnink, 1997; Wei *et al*., 2005; Fukai *et al*., 2004). Previous 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 (Kingshott *et al*., 2003).

The presence of end-tethered poly(ethylene glycol) (PEG) at solid interfaces effectively impedes nonspecific protein adsorption (Kingshott *et al*., 2003; Unsworth *et al*., 2005; Satomi *et al*., 2007). This protein resistance always relates to PEG chain length, interface grafting density (the number of PEG chains per unit area) and hydration and conformation (Wei *et al*., 2005; Unsworth *et al*., 2005; Archambault and Brash, 2004; Roosjen *et al*., 2004; Norde and Gage, 2004). 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 (Prime and Whitesides, 1993; Uchida *et al*., 2005; Yoshikawa *et al*., 2006). 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 (Unsworth *et al*., 2005; Roosjen *et al*., 2004). Poly(ethylene glycol) (PEG) is a synthetic nontoxic polymer that has been approved by the US Food and Drug Administration (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

Corresponding Author: Norzita Ngadi, Faculty of Chemical and Natural Resources Engineering, Universiti Teknologi Malaysia, Skudai, 81310, Johor, Malaysia Tel: 006-019-6890870

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 (Prime and Whitesides, 1993; Wu *et al*., 2000).

Three different ways of protein deposition on the PEG layer can be distinguished as suggested in Helparin's model (Helparin, 1999) 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.

In this study, adsorption of proteins was performed on an AT-cut quartz crystal coated with gold and then a stainless steel surface. The adsorption and desorptions were done in situ and monitored in real time using a quartz crystal microbalance with the interpretation allowing also for dissipation (QCM-D). For modification of the surface, sodium silicate solutions were first adsorbed by physisorption onto bare stainless steel surface. Then PEG molecules were grafted physically (physisorption) onto the resulting silicate layers.

MATERIALS AND METHODS

Materials: β-casein (MW 23,000 Da) from bovine milk and sodium silicate solution were purchased from Sigma-Aldrich. Polyethylene glycol monomethyl ether (OH-PEG-CH_3, MW 350, 550, 2000 and 5000 Da) was purchased from Fluka. 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_2HPO_4 and NaH_2PO_4 (from Sigma Aldrich). 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 10.0 g L^{-1}, respectively for all runs. Stocks of protein solution were kept in the freezer at 4°C. Protein solutions not used within 48 h of thawing were discarded. The sodium silicate solutions were prepared in milliQ water at concentration of 50 g L^{-1}. All experiments were conducted at 23 and 40°C and pH 7.2.

QCM-D experiments: A Q-4 model QCM 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 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^{-1}. 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 PEG chain layer, the surface was modified in situ by pumping the sodium solution first, followed by PEG solution and finally protein solution. 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)). 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 min at 75°C, followed by thorough rinsing with milliQ water and drying with a moisture-free nitrogen gas stream. Finally, the crystals were treated with UV light and ozone for 5-10 min to remove organic contamination.

RESULTS

Figure 1 shows adsorption and desorption kinetics of silicate from 50 g L^{-1} solution onto and off a stainless steel surface at temperatures of 23 and 40°C. There was no significant difference between the silicate adsorption and desorption kinetic profiles at the two temperatures. The adsorption was fast initially followed by a gradual

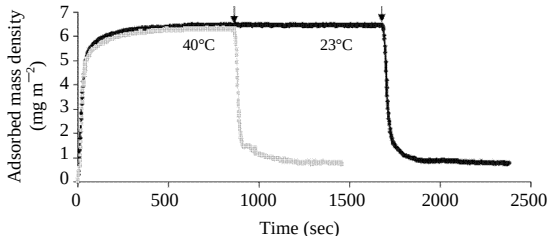

Fig. 1: The Voigt mass density of silicate adsorbed from 50 g L^{-1} solution and desorbed onto and off a stainless steel surface as a function of time

increase before leveling off at time less than 500 sec. The mass density adsorbed at steady state was about 6.5 mg m^{-2}, corresponding to 21 molecules/nm^2 at the two temperatures. When the silicate layers were rinsed with phosphate buffer, the mass decreased and weakly bound silicate molecules were desorbed. Almost 90% of the silicate mass were desorbed, leaving approximately 0.8 mg m^{-2} (≅2.5 chains/nm^2) bound on the surface at the two temperatures.

Figure 2 and 3 display the tightly-bound PEG molecules on the stainless steel surface coated with a silicate layer at 23 and 40°C, respectively. We represent a layer of silicate on a SS surface as a SS-silicate (50), where 50 refers to the silicate solution concentration in g L^{-1}. At the two temperatures, the trend of the PEG grafting density towards the PEG molecular weight and solution concentration was the same; the PEG grafting density increased as concentration increased and decreased as PEG molecular increased. The PEG grafting density on the SS-silicate (50) surface had shown a similar trend as on the SS-PEI (30) surface with the rise of the temperature; the PEG grafting density increased with the temperature only on the surfaces prepared using PEG concentrations of 0.1 and 1 g L^{-1} whereas it decreased at high concentration.

Figure 4 shows the final steady state values of mass density of β-casein before and after rinsing with buffer on a bare SS, SS-silicate (50) and SS-silicate (50)-PEG surfaces for PEG of various molecular weights and solution concentrations at a temperature of 23°C. Bars with plain and stripe color refer to mass density adsorbed

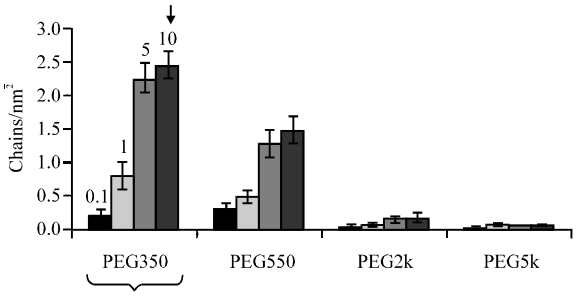

Fig. 2: Number density of tightly-bound PEG molecules on SS-silicate (50) surfaces as a function of PEG molecular weight and solution concentration. Figures above bars are concentration of PEG solution, g L^{-1}

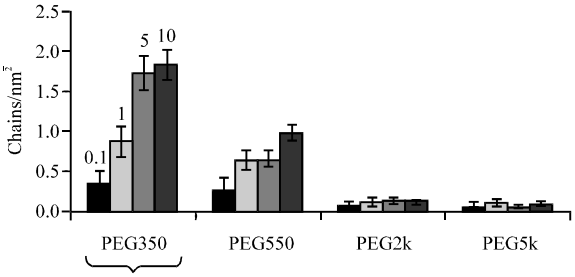

Fig. 3: Number density of tightly-bound PEG molecules on SS-silicate (50) surfaces as a function of PEG molecular weight and solution concentration. Figures above bars are concentration of PEG solution, g L^{-1}

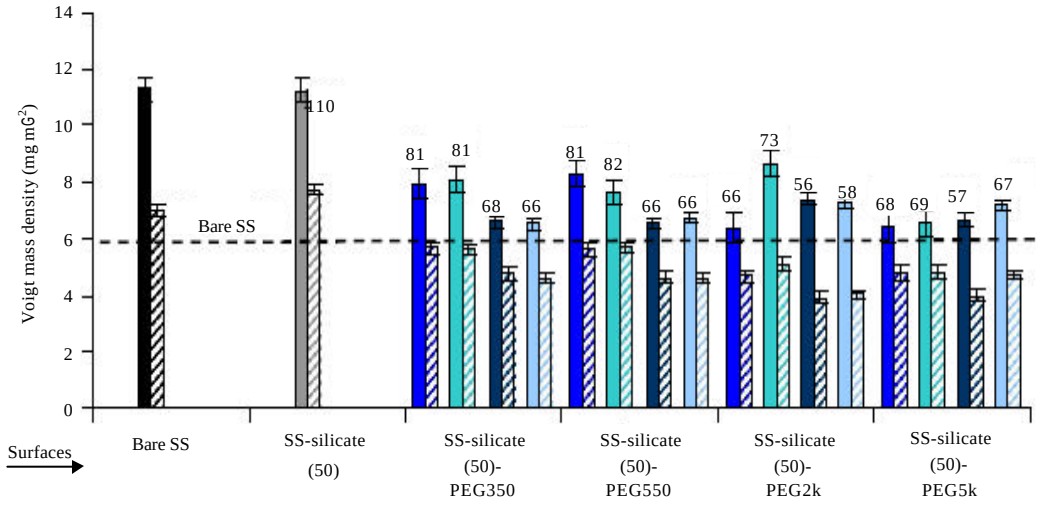

Fig. 4: The Voigt mass density of β-casein adsorbed on a bare SS, SS-silicate (50) and SS-silicate (50)-PEG surfaces for PEG of various molecular weights and solution concentration at a temperature of 23°C

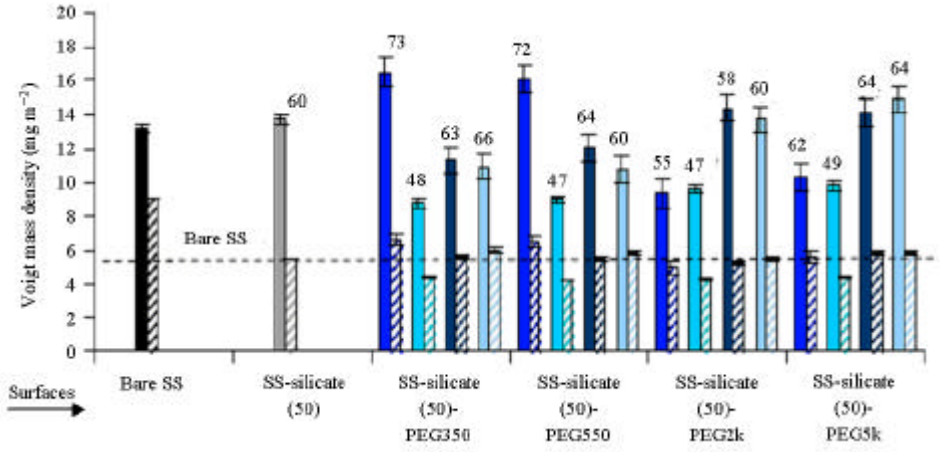

Fig. 5: The Voigt mass density of β-casein adsorbed on a bare SS, SS-silicate (50) and SS-silicate (50)-PEG surfaces for PEG of various molecular weights and solution concentration at a temperature of 40°C

at final steady state before and after rinsing with buffer solution, respectively. Values above bars are percentage of strong adsorption compared to that on the bare SS surface. As can be seen, the adsorption was lower on the SS-silicate (50)-PEG surfaces than that on the bare SS surface; the adsorption down to less than 60%. The adsorption of β-casein was less on the surfaces prepared using high PEG solution concentration (5 and 10 g L^{-1}). Nevertheless, the surfaces which were prepared using short PEG chains (PEG350 and 550 Da) and low PEG solution concentration (0.1 and 1 g L^{-1}) were still capable to reduce the adsorption of β-casein.

Figure 5 shows the Voigt mass density of β-casein adsorbed at a steady state adsorption and desorption on a bare SS, SS-silicate (50) and SS-silicate (50)-PEG surfaces for PEG of various molecular weights and solution concentrations at a temperature of 40°C. Bars with plain and stripe color refer to mass density adsorbed at final steady state before and after rinsing with buffer solution, respectively. Values above bars are percentage of strong adsorption compared to that on the bare SS surface. The horizontal dashed line was drawn at the bare SS value to make an easy comparison between modified and unmodified surfaces. It was interesting to note that the adsorption of β-casein on the SS-silicate (50)-PEG surfaces at 40°C was much lower than that at 23°C. At glance, the SS-silicate (50)-PEG surfaces prepared using low PEG molecular weight and low PEG solution concentration were still competent to reduce the adsorption of β-casein. Also, interesting to note, a SS-silicate (50) surface (without presence of PEG molecules) was able to reduce the adsorption of β-casein as good as SS-silicate (50)-PEG surfaces. Overall, the percentage of adsorption of β-casein on the modified surfaces was down to less than 50 % than that on the bare SS surface.

DISCUSSION

QCM-D measurement: All the results presented in this study have been obtained using the Voigt model (Weber *et al*., 2005). 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} (Hook *et al*., 2002). 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 (Hook *et al*., 2002; Muller *et al*., 2005) 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.

Silicate on SS surface: The attachment of silicate on a SS surface was expected to be based on physisorption interactions (hydrogen bonding and Van der Waals) (Bardina *et al*., 2001). The mean spacing between silicate molecules was about 0.6 nm (from QCM-D measurements). The diameters of oxygen and silicon atom respectively are about 0.15 and 0.23 nm. The area of one molecule of silicate is about $0.15\times0.28 = 0.042$ nm^2. Thus the fractional area coverage is $(0.04/(0.6)\times(0.6) = 0.12)$ (i.e., 12% covered). The presence of silicate solution provides a bare SS surface with silanol groups (Si-OH) (Bardina *et al*., 2001) and hence may transform the surface from a hydrophobic to a hydrophilic surface.

PEG on silicate anchor layer: The tightly-bound PEG grafting density on SS-silicate surfaces obtained in this study ranged from 0.02 to 2.45 chains/nm^2 (wet basis). The ratio of PEG to silicate varied from about 0.01 to 1. From the calculation above, a silicate layer occupied only about 12% of the total coverage. However, the PEG grafting density achieved on a SS-silicate surface was high (perhaps to 2.45/1.5 = 1.6 chains/nm^2 (MW for sodium silicate is 180 g mol/1)). Some of these PEG molecules may have attached to the SS surface directly, through its O- and OH groups. The grafting of PEG molecules to a SS-silicate surface is expected to be achieved through surface silanol groups (Bardina *et al*., 2001).

Adsorption of β-casein on SS-silicate and SS-silicate-PEG surfaces: Adsorption of β-casein generally was dependent on both the PEG chain length (MW) and grafting density. The adsorption of β-casein decreased as PEG MW was increased from 350 to 5000 Da and also decreased as the grafting density increased (Wei *et al*., 2005; Archambault and Brash, 2004; Yoshikawa *et al*., 2006; Fukai *et al*., 2004). A higher PEG layer (MW) implies a larger separation between the surface and the incoming proteins and hence a stronger attenuation of the long range Van der Waals interaction (Archambault and Brash, 2004; Roosjen *et al*., 2004). Thus, it is expected that the secondary adsorption is minimized. Furthermore, high chain lengths are with large excluded volumes to sweep away more incoming proteins than short chain lengths can do. High chain lengths appear to maximize the steric repulsion mechanism.

In general, the adsorption of β-casein on silicate surfaces, was about 0.55 times lower at 40°C than at room temperature. The results obtained contradicted to other works, for example, Prime and Whiteside (Prime and Whitesides, 1993) reported that the adsorption of pyruvate kinase on a self assembled monolayer (SAM) of $SC_{11}E_6OH$ surface was higher at 37°C than at room temperature. The possible reason for lower adsorption at high temperature in this study is most probably due to the percentage of desorption. The percentage of desorption of β-casein was about 1.4 to 2.3 times higher at 40°C than at room temperature. Another possibility is may be due to the PEG grafting density. Generally, the PEG grafting density on silicate surfaces was higher at 40°C than at room temperature. It has been reported that, if the chain density is too dense, the chains will dehydrate and lose their flexibity to sweep the area where protein may adsorb (Unsworth *et al*., 2005). At this stage the graft itself may become an adsorbent for protein and hence increase the adsorption of protein.

CONCLUSIONS

From this study, it can be concluded that:

- Coated the stainless steel surface with silicate or silicate-PEG layers improved β-casein protein resistance
- β-casein resistance was better at 40°C than at room temperature
- All the data presented in this study represent 'wet mass', hence the data obtained are overestimated values

REFERENCES

Archambault, J.G. and J.L. Brash, 2004. Protein repellent polyurethane-urea surfaces by chemical grafting of hydroxyl-terminated poly(ethylene oxide): Effects of protein size and charge. Colloids Surfaces B: Biointerfaces, 33: 111-120.

Bardina, I.A., N.V. Kovaleva and S. Nikitin, 2001. Surface properties of initial and polyethylene glycol modified silica gels. Russian J. Phys. Chem., 75: 442-445.

Fukai, R., P.H.R. Dakwa and W. Chen, 2004. Strategies toward biocompatible artificial implants: Grafting of functionalized poly(ethylene glycol)s to poly(ethylene terephthalate) surfaces. J. Polymer Sci. Part A: Polymer Chem., 42: 5389-5400.

Helparin, A., 1999. Polymer brushes that resist adsorption of model proteins: Design parameters. Langmuir, 15: 2525-2533.

Hook, F., J. Voros, M. Rodahl, R. Kurrat and P. Boni *et al.*, 2002. A comparative study of protein adsorption on titanium oxide surfaces using *in situ* ellipsometry, optical waveguide lightmode spectroscopy and quartz crystal microbalance/dissipation. Colloids Surfaces B: Biointerfaces, 24: 155-170.

Kingshott, P., W. Jiang, D. Bagge-Ravn, G. Nikolaj and G. Lone, 2003. Covalent attachment of poly(ethylene glycol) to surfaces, critical for reducing bacterial adhesion. Langmuir, 19: 6912-6921.

Muller, M.T., X. Yan, S. Lee, S.S. Perry and N.D. Spencer, 2005. Lubrication properties of a brushlike copolymer as a function of the amount of solvent absorbed within the brush. Macromolecules, 38: 5706-5713.

Norde, W. and D. Gage, 2004. Interaction of BSA and human blood plasma with PEO-tethered surfaces: Influence of PEO chain length, grafting density and temperature. Langmuir, 20: 4162-4167.

Prime, K.L. and G.M. Whitesides, 1993. Adsorption of proteins onto surfaces containing end-attached oligo (ethylene oxide): A model system using self-assembled monolayers. J. Am. Chem. Soc., 115: 10714-10721.

Roosjen, A., H.C. van der Mei, H.J. Busscher and W. Norde, 2004. Microbial adhesion to poly(ethylene oxide) brushes: Influence of polymer chain length and temperature. Langmuir, 20: 10949-10955.

Satomi, T., Y. Nagasaki, H. Kobayashi, T. Tateishi, K. Kataoka and H. Otsuka, 2007. Physicochemical characterization of densely packed poly (ethylene glycol) layer for minimizing nonspecific protein adsorption. J. Nanosci. Nanotechnol., 7: 2394-2399.

Uchida, K., H. Otsuka, M. Kaneko, K. Kataoka and Y. Nagasaki, 2005. 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. Anal. Chem., 77: 1075-1080.

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

Visser, J. and T.J.M. Jeurnink, 1997. Fouling of heat exchangers in the dairy industry. Exp. Thermal Fluid Sci., 14: 407-424.

Weber, N., H.P. Wendel and J. Kohn, 2005. Formation of viscoelastic protein layers on polymeric surfaces relevant to platelet adhesion. J. Biomed. Mater. Res. A. 72: 420-427.

Wei, J., D.B. Ravn, L. Gram and P. Kingshott, 2005. PEG can prevent protein adsorption but not bacterial adhesion. Colloids Surfaces B: Biointerfaces, 32: 275-291.

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

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