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ORIGINAL PAPER

Antifouling ability of polyethylene glycol of different molecular weights grafted onto polyester surfaces by cold plasma

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Abstract Different molecular weights of polyethylene glycol (PEG, MW 200, 400, 600, 2000, and 4600) were grafted onto silicon tetrachloride ($SiCl_4$) plasma functionalized polyethylene terephthalate (PET) surfaces. Dramatic increase of the C–O peak in the C1s high-resolution spectra determined by electron spectroscopy for chemical analysis suggests that PEG was successfully grafted. PEG-grafted PET showed significant inhibition of attachment and biofilm formation by *Salmonella enterica* sv. Typhimurium compared to unmodified PET. The antifouling ability of PEG-grafted PET surfaces was affected by the molecular weight of PEG and PEG2000 was the most effective. Both PEG600- and PEG2000-grafted PET also significantly inhibited biofilm formation by *Listeria monocytogenes*. Stability tests showed that over 2-month storage under ambient conditions PEG2000-grafted PET demonstrated reduced antifouling ability, but still significantly reduced biofilm formation by *S. enterica* sv. Typhimurium.

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Introduction

In natural environments, the prevailing lifestyle of microorganisms is associated with surfaces. The surface-attached microorganisms often grow aggregated with each other and produce a matrix of extracellular polymeric substances to form biofilms. Biofilms are more resistant to treatment by antimicrobial agents than their planktonic counterparts and often cause undesirable economic and health problems in many environments [1–3]. Bacterial adhesion and subsequent biofilm formation on implanted medical devices can cause device-associated infections and often require device removal [4, 5]. In the food industry, biofilm formation is a serious concern because it can cause cross contamination of food products, leading to decreased food quality or transmission of foodborne diseases [6–8].

It has been shown that coating surfaces with non-charged hydrophilic polymers resulted in reduced protein, cellular and bacterial adsorption on a variety of surfaces [9–12]. Most of the research in this area involved polyethylene glycol (PEG) or its derivatives [13–19]. PEG is composed of $–CH_2CH_2O–$ repeating units with a hydroxyl group at each end of the polymer chain. The mechanisms of the protein and cell repellent effects of PEG-modified surfaces remain unclear. However, several factors have been hypothesized to be at least partially responsible for the nonfouling property of PEG-modified surfaces. These include hydrophilicity, high mobility, large excluded volume, and steric hindrance effects [19–21]. PEG-modified surfaces have been extensively studied for their protein repelling properties and many have demonstrated significantly reduced or complete prevention of protein adsorption [10, 15, 17]. In theory, PEG molecular weight (chain length) can be one of the factors affecting the grafting density, the number of hydrogen bond donor/acceptor, and other PEG properties [22]. Several researchers have confirmed that longer PEG chains have stronger particle repellency [15, 23, 24]. As shown by Benhabbour et al. [23], PEG monomethyl ether (PEG-OMe) covalently bound to the dendronized surface of gold-coated silicon wafers produced molecular weight-dependent reduction of protein adsorption. In another study by Zhu et al. [24], surface attachment of 3T3 cells was also inhibited by PEG in a molecular weight-dependent manner. These studies indicate that the PEG molecular weight plays an important role in affecting protein and cell adsorption.

Over the last decade, there has been increased research interest in PEG coatings for reducing bacterial adhesion [14, 18, 25–27]. However, there are large discrepancies in the data available on the reduction of bacterial adhesion by various PEG-modified surfaces, varying from not effective to several orders of magnitude of reduction [28]. These disparities can be attributed to the different approaches applied for PEG modification as well as the experimental conditions and bacterial species used. Consequently, it is often difficult to compare results reported by different research groups. In addition, only a few studies have examined the effect of PEG molecular weight on bacterial adhesion and the results are

inconsistent. For example, Park et al. [18] suggested that longer PEG grafting chains are more effective to reduce adhesion of *Staphylococcus epidermidis* and *Escherichia coli* to polyurethane surfaces. However, Cunliffe et al. [29] demonstrated that poly(ethyleneoxide)-monomethylether (MeO-PEO-5000) and MeO-PEO-3 were equally effective in inhibiting adhesion of *Listeria monocytogenes*.

After attachment to a surface, bacteria can grow and develop into biofilms. To our knowledge, there are no reports examining the effect of PEG molecular weight on biofilm formation. Recently, we described a new approach using silicon tetrachloride ($SiCl_4$) plasma to graft PEG onto poly(ethylene terephthalate) (PET) surfaces and demonstrated significant inhibition of biofilm formation by *L. monocytogenes* on the PEG (MW 600)-grafted substrates [30]. This approach focused on the implantation of $SiCl_x$ entities onto PET surfaces and allowed them to function as intermediate structures for the subsequent covalent grafting of PEG molecules. Our current study aims to investigate the effect of molecular weight of PEG on short-term bacterial attachment as well as biofilm formation using this grafting method. The effectiveness toward two foodborne pathogens, *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* and stability under room temperature storage of the PEG-grafted surfaces are presented.

Experimental

Materials

Polyester (PET, 0.28-mm thickness) films were a gift from Cargill (Minneapolis, MN). PET was cut into 1-inch diameter coupons and washed ultrasonically with methanol, acetone, and deionized water sequentially for 10 min each and dried under vacuum. Silicon tetrachloride ($SiCl_4$, 99%, packed under nitrogen) and polyethylene glycol (PEG; MW 200, 400, 600, 2000, and 4600) were purchased from Aldrich Chemical Company (Milwaukee, WI).

Surface functionalization of PET by $SiCl_4$ plasma and PEG grafting

PET substrates were first functionalized by $SiCl_4$ plasma in a parallel plate, capacitively coupled cylindrical stainless steel reactor as described previously [30]. In a typical experiment, after the substrates were placed on the lower electrode, the reactor was evacuated to base pressure (30 mTorr). After the desired $SiCl_4$ working pressure (150 mTorr) was established, the plasma was initiated by dissipating RF-power (100 W) to the electrodes and sustaining it for 1 min. At the end of the reaction, the chamber was evacuated to base pressure followed by re-pressurizing the system with argon gas to atmospheric pressure. The $SiCl_4$ plasma functionalized PET substrates were removed from the plasma reactor under the protection of argon and immediately immersed in liquid phase PEG (MW 200, 400, 600, 2000, or 4600), which had been melted (for MW 2000 and 4600) and degassed in a vacuum oven at 60 °C for 2 h. The grafting reaction was allowed to proceed in the vacuum

oven at 60 °C for 20 h, and then the substrates were rinsed with deionized water, dried under vacuum, and stored in petri dishes for analysis.

Surface analysis

The relative surface atomic concentrations and chemical states of unmodified and PEG-grafted substrates were analyzed by electron spectroscopy for chemical analysis (ESCA) using a Perkin Elmer Physical Electronics 5400 Small Area Spectrometer (Mg source; 15 kV; 300 W; pass energy, 89.45 eV; take-off angle, 45°; Perkin-Elmer, Palo Alto, CA). Surface morphologies of unmodified and PEG-grafted PET were evaluated by atomic force microscopy (AFM) using a Digital Instrument Nanoscope III microscope (scan rate, 1.9 Hz; sampling number, 512; Santa Barbara, CA) in the tapping mode. At least five images were collected for each treatment. Surface roughness was calculated using Ra (mean roughness). Contact angle measurements were performed using a contact angle goniometer from Ramé-Hart Inc. (Mountain Lakes, NJ).

Antifouling evaluation of PEG-grafted PET surfaces

Bacterial attachment and biofilm formation were investigated to evaluate the antifouling ability of unmodified and PEG-grafted PET surfaces using *L. monocytogenes* or *S. enterica* sv. Typhimurium. Five strains of *L. monocytogenes*, Scott A (serotype 4b, human isolate), JBL 1157 (serotype 4b, processed meat), CLIP 23485 (unknown serotype, liver pâté), F6900 (serotype 1/2a, human), and F8964 (serotype 1/2b, human), and two strains of *S. enterica* sv. Typhimurium, AW9-1 and 101, were used. The strains were grown individually for 24 h in 5 mL trypticase soy broth (BBL Microbiology Systems/Becton–Dickinson, Cockeysville, MD) at 37 °C, and then 500 μL from each culture were combined to form a 5-strain cocktail of *L. monocytogenes* or 2-strain cocktail of *S. enterica* sv. Typhimurium. A 100 μL portion of the cocktail was used for inoculation of 50 mL EPS [30], a low nutrient medium, in a 250 mL beaker to achieve an initial bacterial concentration of 106 colony-forming units (CFU)/mL.

For biofilm development, PET samples were placed inside the beakers after inoculation and the cultures were incubated at 27 °C at 100 rpm in a gyratory water bath shaker (model G76, New Brunswick Scientific, Edison, NJ) for 48 h. For attachment studies, the inoculated cultures were incubated at 27 °C at 100 rpm for 6 h, PET samples were placed inside the beakers, and the cultures were then incubated for another 1 h. After attachment or biofilm formation, PET samples were aseptically removed from the beakers and put in a petri dish containing 25 mL 10 mM phosphate-buffered saline (PBS, pH 7.2). The samples were rinsed by manually rotating the petri dish 10 times clockwise and 10 times counterclockwise to remove unattached bacteria. Each surface was swabbed with a sterile swab pre-wetted with PBS 10 times in one direction from left to right. The sample was rotated 90° and swabbed another 10 times in the same way. Two swabs were used for each sample. The swab tips were broken off into a tube containing 5 mL PBS and glass beads and votexed for 30 s to remove the bacteria. The number of bacteria was

evaluated by plating the appropriate dilutions onto duplicate trypticase soy agar plates. The plates were incubated at 30 °C for 48 h and the numbers of CFUs developed were counted. Data were collected from at least three independent experiments with two samples for each treatment per experiment.

Stability evaluation

PEG2000-grafted PET samples were stored in petri dishes sealed with paraffin film at room temperature for 0, 1, and 2 months. After storage, samples were analyzed by ESCA and tested for biofilm formation by *S. enterica* sv. Typhimurium.

Statistical analysis

Data were analyzed by Student's *t* test and analysis of variance (ANOVA) using Microsoft Excel. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

We recently reported a novel method to graft PEG onto PET surfaces using $SiCl_4$ plasma [30]. Generation of C–Si–Cl_x functionalities on plasma-exposed PET surfaces allows the covalent attachment of PEG macromolecules through a condensation reaction mechanism. PET surfaces grafted with PEG600 had antifouling ability and reduced the number of *L. monocytogenes* biofilm cells by 1.6 log/cm^2 (96% reduction) compared to the unmodified PET. We showed by chemical derivatization of hydroxyl groups with hexafluoroglutaric anhydride that PEG600 was grafted to the surfaces with only one end of the molecule, forming a "brush"-type structure. Previous studies have demonstrated that surfaces with long-chain PEG resulted in less protein adsorption than surfaces with short-chain PEG. It would be interesting to determine the effect of molecular weight of PEG on bacterial adhesion. In this article, we investigated the influence of PEG molecular weight on surface grafting and bacterial attachment and biofilm formation by *S. entericia* sv. Typhimurium and *L. monocytogenes*.

The relative surface atomic concentrations and the relative surface areas of the deconvoluted C1s high-resolution peaks of unmodified and PEG-grafted PET surfaces derived from survey and high-resolution ESCA are shown in Table 1. A decrease in relative surface carbon atomic concentration and an increased relative surface oxygen atomic concentration were noted in all PEG-grafted substrates, in addition to the presence of a significant relative surface silicon atomic concentration. A significant increase of the C–O bond content and a related decrease of the O–C=O group was observed on all PEG-grafted PET surfaces. It is noteworthy that the C–C/C–Si bond concentration decreased with an increase in molecular weight of the grafted PEG molecules. This suggests that the longer grafted PEG chains masked the Si-based linkages.

Atomic force microscopy (AFM) analysis of unmodified and PEG-grafted PET surfaces (Fig. 1) clearly shows some morphology changes after the grafting of

Table 1 Relative atomic concentrations and C1s high-resolution peak areas of unmodified and PEG-grafted PET surfaces determined by survey and high-resolution ESCA

Sample	Relative atomic concentration (%)			C1s high-resolution peak (%)		
	C	O	Si	C–C/C–Si	C–O	O–C=O
PET	74.6	25.4	–	56.8	26.3	17.0
PET–PEG200	59.1	35.1	5.8	49.8	37.8	12.3
PET–PEG400	56.5	38.1	5.4	39.8	50.9	9.3
PET–PEG600	52.5	42.4	5.2	24.9	66.1	9.0
PET–PEG2000	60.9	36.6	2.4	8.5	88.9	2.6
PET–PEG4600	62.5	34.7	2.8	9.1	87.1	3.8

– Non-detectable

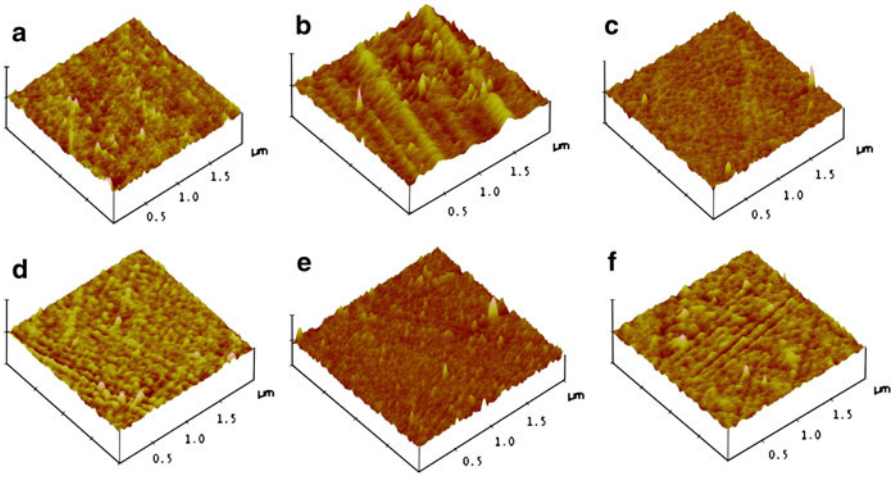

Fig. 1 AFM images of **a** unmodified PET (*Ra* 0.55 ± 0.12 nm), **b** PET–PEG200 (*Ra* 1.10 ± 0.51 nm), **c** PET–PEG400 (*Ra* 0.55 ± 0.08 nm), **d** PET–PEG600 (*Ra* 0.58 ± 0.14 nm), **e** PET–PEG2000 (*Ra* 0.82 ± 0.25 nm), and **f** PET–PEG4600 (*Ra* 0.51 ± 0.06 nm)

different molecular weights of PEG. However, the average roughness (*Ra*) calculated did not show significant difference between the unmodified and PEG-grafted PET surfaces.

Water contact angle measurements (Fig. 2) showed that all PEG-grafted PET surfaces exhibit lower contact angle values in comparison to unmodified PET. Water contact angle values decreased with an increase of the PEG molecular weight from 200 to 600. Further increase in molecular weight to 2000 and 4600 did not reduce the water contact angle further, suggesting full surface coverage by PEG of MW600 and above after grafting.

Both bacterial attachment and biofilm formation by *S. enterica* sv. Typhimurium on PEG-grafted PET surfaces were investigated and the results are shown in Figs. 3 and 4 respectively. Compared to the unmodified PET, all PEG-grafted surfaces

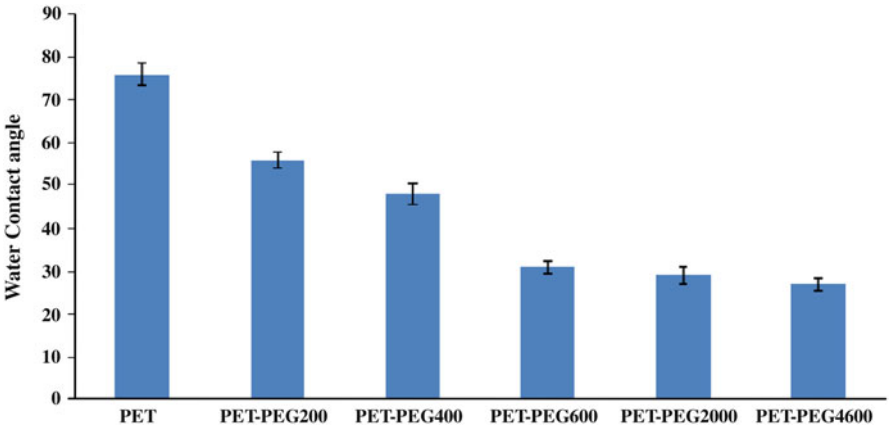

Fig. 2 Water contact angles of unmodified and PEG-grafted PET surfaces

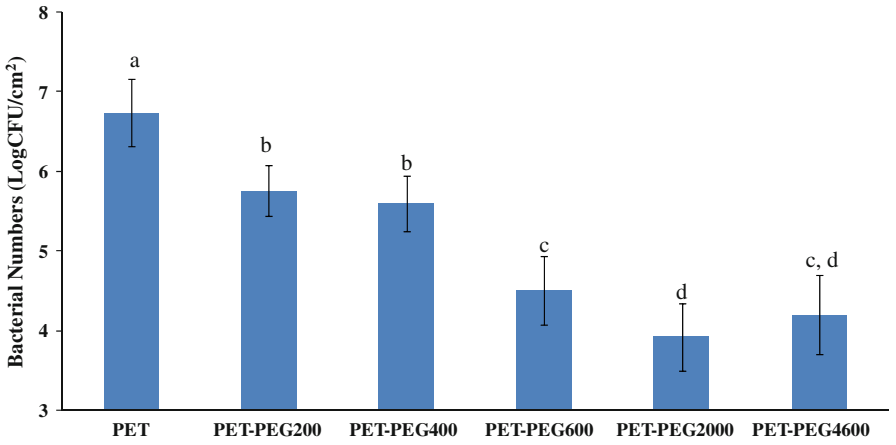

Fig. 3 Attachment of *S. enterica* sv. Typhimurium on unmodified and PEG-grafted PET. Means with *different letters* are significantly different at $p < 0.05$

demonstrated consistently lower levels of bacterial attachment (Fig. 3). Among PEG400, 600, and 2000, decreased bacterial attachment was found with an increase in molecular weight of PEG. PEG2000-grafted PET was the most inhibitory surface, and the numbers of attached bacteria were decreased by almost 2.8 log per cm^2 (99.8% decrease, $p < 0.05$). The PEG4600-grafted PET showed less inhibition than the PEG2000-grafted surface, but still in a significant manner when compared with the PET control (2.5 log, 99.7% decrease, $p < 0.05$). Our results on attachment are in agreement with Park et al. [18], who reported that long chain PEG could provide more bacterial repelling effect than short chain PEG. We showed that there is an upper limit to the effective molecular weight of PEG for bacterial repulsion. Norde and co-workers [27] also observed that long chain PEG was more effective in reducing 4-h adhesion of *Pseudomonas aeruginosa* to glass surfaces than short

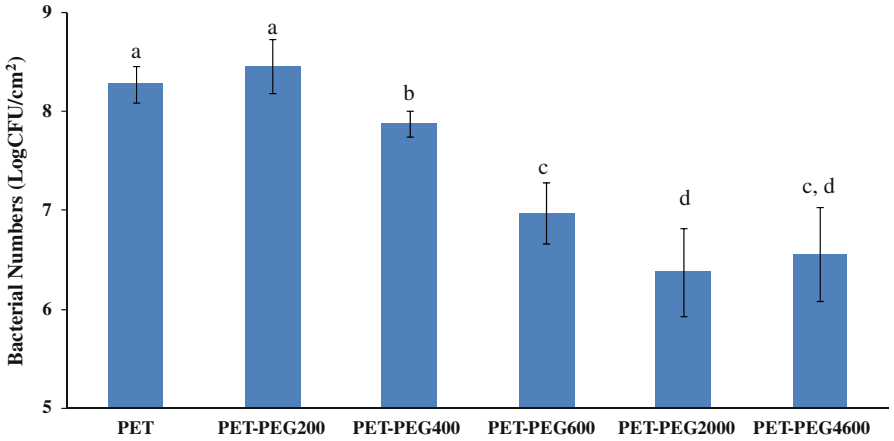

Fig. 4 Biofilm formation by *S. enterica* sv. Typhimurium on unmodified and PEG-grafted PET. Means with *different letters* are significantly different at $p < 0.05$

chain PEG with no significant difference on reduction between the two longer chains (PEG2000 and PEG9800).

All PEG-grafted PET, with the exception of the PEG200-grafted surface, demonstrated decreased biofilm formation by *S. enteric* sv. Typhimurium compared to the unmodified PET (Fig. 4). The reduction in biofilm formation was even more significant when longer PEG chains were grafted, reaching a maximum decrease with PEG2000 (98.7% decrease, $p < 0.005$). As in the case of attachment, when the molecular weight was further increased to 4600, no further reduction of biofilm formation was observed.

The observed effect of PEG molecular weight on attachment and biofilm formation by *S. enterica* sv. Typhimurium could be attributed to the larger excluded volume and higher degree of flexibility associated with the longer PEG chains. Several reports have shown that protein adsorption is affected by the molecular weight of grafted PEG, with higher molecular weight PEG resulting in less protein adsorption [15, 20, 31]. Interestingly, PEG2000 has been reported also by several other groups as the optimum molecular weight for reduction of protein adsorption. Benhabbour et al. [23] grafted PEG monomethyl ether (PEG-OMe) to dentronized surfaces and compared different chain lengths of PEG (MW 350, 750, 2000, and 5000) on protein adsorption. They found that protein adsorption decreased with increasing PEG-OMe molecular weight up to 2000. Studies by Gombotz et al. [15] claimed surfaces containing PEG with molecular weight between 2000 and 3500 as the optimal range for reducing protein adsorption. The authors suggested that the water structuring around these PEG molecules may create an appropriate "excluded volume," contributing to the observed low protein adsorption. Whether the same proposed mechanisms apply to bacterial attachment and biofilm formation is not known.

Biofilm formation by another foodborne pathogen, *L. monocytogenes*, was evaluated using PEG600- and PEG2000-grafted PET (Fig. 5). Compared to the unmodified control, PEG600-grafted PET inhibited *L. monocytogenes* biofilm

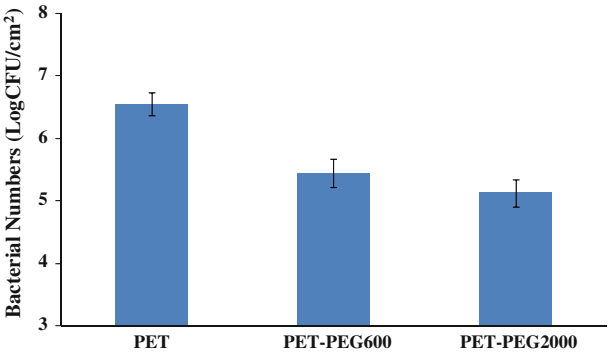

Fig. 5 Biofilm formation by *L. monocytogenes* on PEG600 and PEG2000-grafted PET surfaces

formation by 1.1 log (92.1% decrease, $p < 0.05$), whereas the inhibition by PEG2000-grafted PET was about 1.4 log (96.1% decrease, $p < 0.05$).

For stability evaluations, PEG2000-grafted PET surfaces were stored at room temperature for up to 2 months and tested for biofilm formation by *S. enterica* sv. Typhimurium. The results are shown in Fig. 6. The newly prepared PET–PEG2000 surfaces could inhibit *S. enterica* sv. Typhimurium biofilm formation by about 2 log, while the inhibition was reduced to about 1 log after 1- and 2-month storage, although the reduction was still significant ($p < 0.05$). ESCA analysis showed that the surface area of the C–O peak of the PEG2000-grated PET was reduced during storage at room temperature (Fig. 7). The reduced antifouling property of the PEG2000-grafted PET surfaces might be caused by adsorption of hydrocarbon moieties from environment during storage, which could partially "mask" the PEG molecules. Alternatively, conformational changes of the grafted PEG molecules during storage may have altered their antifouling ability.

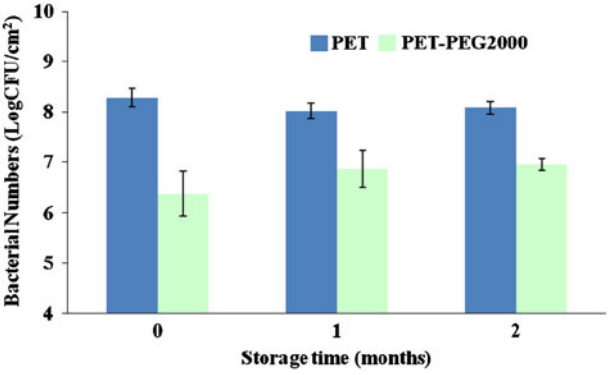

Fig. 6 Influence of storage time on biofilm formation by *S. enterica* sv. Typhimurium on PEG2000-grafted PET surfaces

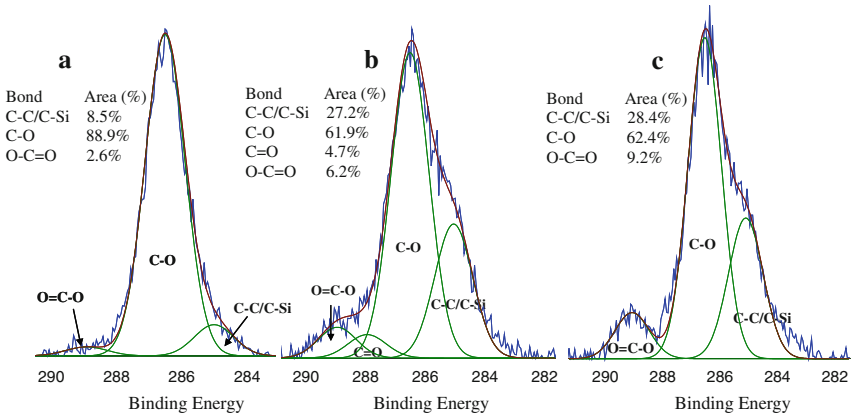

Fig. 7 ESCA high resolution C1s spectra of PET–PEG2000 stored at room temperature for (*a*) 0, (*b*) 1, and (*c*) 2 months

Conclusions

PEG was grafted onto $SiCl_4$ plasma functionalized PET surfaces and the influence of molecular weight of PEG on grafting was investigated. PEG-grafted PET surfaces demonstrated increased surface area C–O peaks and increased hydrophilicity when compared to the unmodified PET. All PEG-grafted surfaces showed significant inhibition of attachment by *S. enterica* sv. Typhimurium, while inhibition of biofilm formation was observed with PEG with molecular weight $\geq$400. There was an association of increased antifouling ability of PEG with increasing molecular weight up to 2000. PEG2000-grafted PET surfaces showed about 3 log of decrease in *S. enterica* sv. Typhimurium attachment and 2 log of decrease in biofilm formation compared to the unmodified control. Both PEG600- and PEG2000-grafted PET demonstrated significant decrease of biofilm formation by *L. monocytogenes*. After 2-month storage under ambient conditions, PEG2000-grafted PET surfaces demonstrated less antifouling ability but still significantly reduced biofilm formation by *S. enterica* sv. Typhimurium.

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