‘Should I stay or should I go?’ Bacterial attachment vs biofilm formation on surface-modified membranes

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A number of techniques are used for testing the anti-biofouling activity of surfaces, yet the correlation between different results is often questionable. In this report, the correlation between initial bacterial deposition (fast tests, reported previously) and biofilm growth (much slower tests) was analyzed on a pristine and a surface-modified reverse osmosis membrane ESPA-1. The membrane was modified with grafted hydrophilic polymers bearing negatively charged, positively charged and zwitter-ionic moieties. Using three different bacterial strains it was found that there was no general correlation between the initial bacterial deposition rates and biofilm growth on surfaces, the reasons being different for each modified surface. For the negatively charged surface the slowest deposition due to the charge repulsion was eventually succeeded by the largest biofilm growth, probably due to secretion of extracellular polymeric substances (EPS) that mediated a strong attachment. For the positively charged surface, short-term charge attraction by quaternary amine groups led to the fastest deposition, but could be eventually overridden by their antimicrobial activity, resulting in non-consistent results where in some cases a lower biofilm formation rate was observed. The results indicate that initial deposition rates have to be used and interpreted with great care, when used for assessing the anti-biofouling activity of surfaces. However, for a weakly interacting ‘low-fouling’ zwitter-ionic surface, the positive correlation between initial cell deposition and biofilm growth, especially under flow, suggests that for this type of coating initial deposition tests may be fairly indicative of anti-biofouling potential.

Keywords: anti-biofouling surfaces; membrane modification; graft-polymerization; membranes; initial deposition; biofilm

Introduction

Membrane-based water filtration processes often suffer from biofouling, ie uncontrolled colonization and clogging of the membrane surface with biofilms. Presently, mitigation and prevention of biofouling and associated loss of performance (Herzberg & Elimelech 2007; Radu et al. 2010) and increased operation costs (Flemming 1997) are some of the major remaining challenges in membrane desalination and water treatment (Mansouri et al. 2010).

Since biofilms develop in distinct stages, reduction in biofilm formation may be accomplished throughout its different succession stages (Yang et al. 2011). Usually, biofilms are periodically removed by cleaning when the performance has suffered (Liikanen et al. 2002; Hjinen et al. 2012). It has also been proposed to minimize biofouling via continuous or intermittent addition of biocides (Kim et al. 2009; Bereschenko et al. 2011) or control of the nutrient level in the feed water (Flemming 1997). An attractive approach that is the main focus and motivation of the present study is inhibition of biofilm formation by reducing the propensity of the membrane to biofouling via modified surface chemistry (Mansouri et al. 2010). It is presumed that in this way biofilm growth may be prevented at its early stages without extensive use of chemicals and at low cost.

The first stage in biofilm formation is the reversible primary adhesion of cells onto a surface, known as initial deposition (Stoodley et al. 2002). Surface modification mainly targets this stage. It may be expected that by minimizing adhesive interactions between the surface and microorganism at this stage, the density of deposited bacteria and subsequent biofilm growth may be eliminated or substantially decreased. In addition, if a biofilm is formed, weaker interactions with the surface will make it easier to remove (Cheng et al. 2007; Mansouri et al. 2010). Finally, since biofilm formation requires a threshold concentration of signal molecules (autoinducers) produced by the bacteria following their deposition (Davies et al. 1998), for a lower concentration of deposited bacteria the autoinducer concentration may remain low enough and biofilm formation will be altered (Kim et al. 2011).

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The initial bacterial deposition on surfaces is influenced by various variables such as hydrodynamic conditions (Vrouwenvelder et al. 2010), solution chemistry, surface roughness and topography (Kang et al. 2004; Subramani & Hoek 2008), and interfacial forces governed by the physico-chemical characteristics of the bacteria and surface (Busscher & Weerkamp 1987; Speranza et al. 2004). Since the hydrodynamic conditions or water chemistry in membrane systems vary in a limited range, optimization of membrane surface characteristics to reduce its propensity to bacterial deposition offers much room for control of biofouling and fouling in general. This can be accomplished through coating, graft-polymerization, self-assembly, chemical coupling, irradiation, etc. (Kang & Cao 2011). The ultimate purpose of modification is to reduce the strength of interactions between the membrane and foulants. Ideally, the modification should prevent attachment of the broadest spectrum of potential foulants including various bacterial strains, extracellular polymeric substances (EPS), dissolved organic matter (DOM), precipitating salts etc.

At present, a universal ‘non-fouling’ surface still remains a challenge due to the immense diversity of chemistries and interactions involved, especially for biofilms. Selected surface chemistries meeting certain criteria do show general low-fouling characteristics in aqueous media. One such criterion first proposed for proteins and later found to apply to cells and biofilms as well, includes the combination of hydrophilicity, zero net charge and absence of H-bond-donating groups with H-bond-accepting groups allowed (Ostuni et al. 2001; Norde 2007). An alternative chemical criterion suggests kosmotropicity as a unifying feature of low-fouling surfaces (Kane et al. 2003). Poly(ethylene glycol) (PEG) is perhaps the most efficient of known antifouling materials that fully meets these criteria. However, for some applications its hydrolytic and oxidative stability is insufficient and zwitter-ionic blocks such as sulfobetaines and carboxybetains have been suggested as more stable alternatives (Cheng et al. 2010; Jiang & Cao 2010). Engineering of ‘non-fouling’ membrane surfaces (Rana & Matsura 2010; Eshet et al. 2011) may utilize additional factors as well, eg purely physical effects of surface hydration (Eshet et al. 2011; Herzberg et al. 2011), use of active antibacterial moieties (Roy et al. 2007; Tiraferri et al. 2011) or combination of these different factors (Fu et al. 2005; Liu et al. 2010).

Assessing anti-biofouling efficiency is a critical step in developing ‘low-fouling’ surfaces. Unfortunately, genuine biofouling experiments, in typical conditions of membrane water treatment, are tedious, time-consuming and not very suitable for the testing of a large number of different surfaces. One alternative is measurement of the kinetics of bacterial deposition, which simulates the critical initial stage of biofouling in well-defined hydrodynamic conditions. This mimics the actual flow conditions in membrane elements and presumably provides a direct measure of the adherence between the surface and bacteria. Different setups have been used for such experiments, eg parallel plate flow cells (Rijnaarts et al. 1993; Kang et al. 2004; Eshet et al. 2011), radial stagnant point (Walker et al. 2004; Kerchove & Elimelech 2008) and the quartz crystal microbalance (Sweity et al. 2011; Marcus et al. 2012). In a previous study (Bernstein et al. 2011a) deposition kinetics in a parallel plate flow cell were employed for evaluating the effects of membrane surface chemistry and charge on initial deposition using the GFP-tagged bacterium *Pseudomonas fluorescens*. It was demonstrated that for this negatively charged strain, the rate of initial bacterial deposition could be inhibited or accelerated, depending on the surface hydrophilicity and charge. In particular, hydrophilic negative and zwitter-ionic surfaces showed a significantly reduced deposition rate and positive surface charge led to enhanced deposition, as compared with the pristine negatively charged and mildly hydrophobic polyanide surface. The results could be explained by a combination of hydrophobic and double-layer surface interactions and were well correlated with the strength and irreversibility of adhesion of surrogate particles to these surfaces.

However, direct correlation between the rate of bacterial deposition on a surface and its propensity to host biofilm growth might be questionable. During deposition, a bacterium can be treated as a physical particle with the ability to move actively in the liquid–solid interface using its flagella and pili, and synthesize EPS (Kohler et al. 2000). For the short term of cell deposition (less than an hour), these active processes are only at their initial stage yet they progress when the biofilm starts to develop. These major differences in the interaction between the bacterium and the surface at these two stages (deposition vs biofilm maturation) can explain a few recent studies, which indicated little or no correlation between surface properties of ultrafiltration (UF) and reverse osmosis (RO) membranes (Pasmor et al. 2001; Lee et al. 2010; Baek et al. 2011; Miller et al. 2012). The present study attempts to explore this point in a systematic way using a series of modified membrane surfaces and several bacterial strains: *Escherichia coli* O157:H7 43895, *Pseudomonas aeruginosa* PAO1 and *Pseudomonas fluorescens* F113. Since membrane processes may involve various hydrodynamic conditions and both deposition and biofilm growth may be affected by hydrodynamics, biofilm formation was tested in both stagnant and cross-flow conditions. The main purpose of this research was to understand whether and under which conditions initial deposition of bacteria may correlate with biofilm development on membrane surfaces.
Materials and methods

RO membrane

Fully aromatic polyamide (PA) membrane ESPA-1 (Hydranautics, Oceanside, CA, USA) was kindly supplied by the manufacturer as flat sheets. The membranes were stored at 4°C and cleaned prior to the modification, as described elsewhere (Bernstein et al. 2010).

Bacterial strains and chemicals

Serotype enterohemorrhagic *E. coli* O157:H7 (ATCC43895, EDL933 strain; Strockbine et al. 1986), *P. aeruginosa* PAO1 (the sequenced Holloway strain; Stover et al. 2000) and *P. fluorescens* F113 (Tombolini et al. 2006) were used in this study. All experiments were conducted at 30°C, and Luria–Bertani (LB) medium was used for the culture of all three bacterial strains. All monomers (presented in Table 1) and the initiators K$_2$S$_2$O$_8$ and K$_2$S$_2$O$_5$ were purchased from Aldrich-Sigma (Rehovot, Israel) and used as obtained.

Membrane modification

The modification with METMAC, SPE and GMS was carried out in a dead-end cell as described elsewhere (Bernstein et al. 2011a, 2011b) in the conditions described in Bernstein et al. (2011a) with different modification time for METMAC and SPE of 45 min in order to obtain a higher degree of modification.

Membrane surface characterization

Attenuated total reflection (ATR)-FTIR spectra (average of 40 scans at 4 cm$^{-1}$ resolution) were recorded on a Vertex 70 FTIR spectrometer (Bruker, Billerica, MA, USA) using a Miracle ATR attachment with a one-reflection diamond-coated KRS-5 element (Pike, Madison, WI, USA). The contact angles were measured using a sessile drop of water on an OCA-20 contact angle analyzer (Data-Physics, San Jose, CA, USA). Every measurement was repeated and averaged for at least 5 drops (0.25 μl) on each membrane sample.

Static biofilm formation assay with modified membranes

Overnight bacterial cells were inoculated with an initial turbidity of 0.05 at 600 nm. A piece of a modified membrane of dimensions 0.3 × 0.3 cm was added into the bacterial culture and incubated at 30°C for 24 h without shaking in 96-well plates. Pieces of the membranes were firmly fixed into the bottom of the well with double adhesive tape. After incubation, the membranes were removed and washed three times with water in 15 ml tubes. Then, the membrane pieces were put in a new 96-well plate, stained with 0.1% crystal violet (300 μl) for 20 min and washed three times with water. Then, the membranes were removed and placed in a new 96-well plate, which was shaken in 95% ethanol for 2 min to extract all crystal violet from the biofilm cells. The total biofilm formation was measured by Thermo Scientific Multiscan EX (Thermo Fisher Scientific, Vantaa, Finland) at 570 nm. As a negative control, the modified membranes without cells were used. Each data point was averaged from at least three replicates.

Continuous biofilm formation assay using parallel plate flow cells and confocal microscopy

A continuously growing biofilm was analyzed in a custom-made flow cell device. Figure 1 describes the device with multiple slides placed in a polypropylene tub

| Table 1. The monomers used in this study. |
|------------------------------------------|
| **Monomer name** | **Monomer abbreviation** | **Monomer structure** | **Functional group** | **Wave number of IR band (cm$^{-1}$)** |
| [2(Methacryloyloxy) ethyl]dimethyl-(3 sulfopropyl) ammonium | SPE | | Quaternary amine sulfonic acid | 945, 1,040 |
| [2(Methacryloyloxy) ethyl]trimethylammonium chloride | METMAC | | Quaternary amine | 945 |
| Sulfonatedglycidyl methacrylate$^*$ | GMS | | Sulfonic acid | 1,040 |

$^*$Obtained by post-sulfonation of grafted poly(glycidyl methacrylate) layer (Bernstein et al. 2011a).
providing equal growth conditions, which would otherwise be possible only in a multi-channel flow cells.

Glass slides (25 × 75 mm) (Marienfeld, Lauda-Königshofen, Germany) were placed in the grooves for biofilm cultivation. All four membranes were simultaneously placed on the side of a glass slide with double adhesive tape as shown in Figure 1. The biofilm formation developed on the membrane was microscopically observed and analyzed.

The flow rate of LB medium containing 20 μg ml⁻¹ kanamycin to the flow cell was set to 2 ml min⁻¹. To study the effect of surface modification, four different membranes (ESPA-1, GMS, METMAC and SPE) were simultaneously placed in the multi-channel flow cell. For all flow cell experiments, the total number of living cells in the inoculum was 3.0 (± 0.6) × 10⁷ per ml (OD₆₀₀ nm = 0.1) were injected to the flow cell as the initial inoculum for biofilm growth. The experiment was performed four times with two different flow cells, one accommodating four glass slides held in grooves (Figure 1) and the other accommodating eight glass slides (Figure S1 – Supplementary information). [Supplementary material is available via a multimedia link on the online article webpage.] For each of the experiments with four slides (Figure 1), nine random positions were chosen from each membrane for microscopic analysis. A stack of 25 planar images was processed for each three-dimensional image. CLSM images were generated using the Nikon eclipse (Tokyo, Japan) image browser and analyzed with the COMSTAT biofilm program (Heydorn et al. 2000) and Imaris Software (Imaris Bitplane, Zurich, Switzerland). A three-dimensional reconstruction of the CLSM image stacks was carried out with Imaris software and the calculation of the specific biovolume, average thickness, and roughness coefficient (Murga et al. 1995) of the observed biofilm sample was carried out with COMSTAT. A pristine membrane was processed similarly and only negligible fluorescent signal was observed. For the experiment conducted in the eight-groove flow cell (Figure S1), four random positions were chosen for microscopic analysis and the image stacks were analyzed with the Imaris Bitplane software statistical tool box.

Results and discussion

Surface characteristics and chemistry of modified membranes

The presence of surface-modifying graft-polymer on the membrane surface was verified and quantified by ATR-FTIR spectroscopy and measurements of the contact angle using a sessile drop of water that provided an indication of the surface hydrophilicity. Figure 2 presents ATR-FTIR spectra of the pristine and modified membranes. The carbonyl group of the acrylic monomers at 1,726 cm⁻¹, common for all monomers, and monomer-specific bands that appeared in the IR spectra after modification and served as quantitative indicators of grafting, are specified in Table 1. The degree of modification was similar to that reported in a previous study (Bernstein et al. 2011a), except for the case of quaternary amine (METMAC), which was higher. Since in this and previous studies the membranes were used as only substrata for deposition and biofilm growth, the amount of the graft-polymer was not critical, as long as the desired change in the surface characteristics was achieved.

The contact angles of the modified membranes were similar to those reported in previous results (Bernstein et al. 2011a): 30 ± 4° were measured for the membranes Japan).
modiﬁed with SPE and METMAC, and the still smaller value of ca10° was measured for membrane modiﬁed with GMS. These values indicate that, owing to the presence of ionic groups, the modiﬁed membranes were more hydrophilic than the original (unmodiﬁed) membrane, having a contact angle of 45 ± 3°. The characteristics of the modiﬁed membranes including those measured previously for similarly modiﬁed membranes (Bernstein et al. 2011a) are summarized in Table 2. The Table also shows the deposition rates for P. ﬂuorescens expressed as deposition coefﬁcients. In brief, on METMAC-modiﬁed membranes containing positively charged quaternary amine groups the rate of P. ﬂuorescens attachment signiﬁcantly increased relative to non-modiﬁed membrane. On the other hand, a signiﬁcant decrease in cell attachment was observed on GMS- and SPE-modiﬁed membranes containing, respectively, negatively charged sulfonate and overall neutral zwitter-ionic groups (Bernstein et al. 2011a). These results are compared below to bioﬁlm development on these surfaces.

The effect of membrane surface modiﬁcation on bioﬁlm formation

According to the current bioﬁlm formation paradigm, this is a developmental process, which proceeds as a succession of stages starting from initial cell attachment, followed by proliferation of sessile microcolonies,

| Membrane  | Contact angle (°) | Zeta potential at pH 6 (mV) | Initial deposition coefﬁcient (μm min⁻¹) | Static bioﬁlm assay relative to ESPA-1 | Speciﬁc biovolume assay under ﬂow relative to ESPA-1 |
|-----------|------------------|-----------------------------|------------------------------------------|----------------------------------------|---------------------------------------------------|
| ESPA-1    | 50 ± 2           | −30                         | 1.67 ± 1                                 | 1                                      | 1                                                 |
| SPE       | 28 ± 4           | −10                         | 0.33 ± 0.16                              | 0.41                                   | 0.22                                              |
| GMS       | 10 − 15          | −39                         | 0.20 ± 0.03                              | 1.68                                   | 2.76                                              |
| METMAC    | 30 ± 4           | 12                          | 4.31 ± 1.36                              | 0.39                                   | 1.37                                              |

Data taken from Bernstein et al. (2011a).
maturation and detachment. As all of these steps may be affected by hydrodynamic conditions, biofilm formation was examined on all types of surfaces both in a stagnant medium (in 96-well plates, batch growth for 24 h) and under flow conditions. The effect of surface characteristics on biofilm growth on membranes in stagnant medium for the three examined model bacterial strains is presented in Figure 3. Considering the difference between modified and unmodified membranes, a weak correlation between bacterial deposition rates (Table 2) and biofilm growth was obtained only for the SPE-modified zwitter-ionic surface. However, a striking difference was obtained for charged surfaces. While the negatively charged GMS surface reduced bacterial deposition, it enhanced biofilm formation. An opposite effect, ie enhanced initial deposition and major inhibition of biofilm formation, was observed for the positively charged METMAC-modified membrane. Notably, the trend of biofilm formation by all the three strains was similar in respect to a specific surface, regardless of the difference in the surface charge of each strain, as reported by others (Van Loosdrecht et al. 1987). For example, METMAC modification is shown to inhibit biofilm formation for all strains, namely, by 90, 61, and 63% for E. coli O157:H7, P. aeruginosa PAO1, and P. fluorescens, respectively (Figure 3).

Figure 3. Biofilm formation by three bacterial model strains: E. coli O157:H7 43895, P. aeruginosa PAO1, and P. fluorescens F113, under static growth conditions for 24 h, at 30°C, in LB medium. Four membranes (ESPA-1, GMS, METMAC and SPE) were placed in 96-well plates.

Figure 4 presents the CLSM analysis of biofilm formation under flow conditions in a multi-channel flow cell (Figure 1) at the average cross flow velocity of 0.44 cm min\(^{-1}\) and corresponding wall shear rate of 0.14 s\(^{-1}\). Regardless of the hydrodynamic conditions, the results of biofilm growth in stagnant or flow setups correlate well (Figures 3 and 4), except for the METMAC membrane, which did not show biofilm inhibition effects under flow but rather a slight increase in biofilm formation. Estimation of the biomass volume per area (termed as a specific biovolume), average thickness and roughness coefficient for GFP-expressing P. fluorescens cells was performed using COMSTAT biofilm program and is presented in the right panel of Figure 4. It confirms a significant decrease in biofilm formation on the SPE modified membrane and a significant increase in biofilm formation on the negatively charged GMS surface. Again, the results for GMS are opposite to their effect on bacterial deposition rates. One notable difference, however, is that in flow conditions, even under relatively low wall shear rate conditions (0.07 s\(^{-1}\)) the SPE surface showed a more significant reduction of biofilm growth (Supplementary material – Figure S2). Intriguingly, as the shear rate was elevated (from 0.07 to 0.14 s\(^{-1}\)), the effect of biofilm reduction became more pronounced (compare specific biovolume Figure S2 and Figure 4), probably due to the low affinity of the biofilm to the SPE coating and its more facile removal at a
higher shear. The effect of SPE then well correlates with the initial deposition rate for this surface. Hence, the preliminary experiments under a slower flow rate (corresponding to 0.22 cm min\(^{-1}\) and a shear rate of 0.07 s\(^{-1}\)) resulted in similar differences in the grown biomass on the membrane for both SPE and GMS surfaces (Figure S2). Estimation of the biomass volume per area for the GFP-expressing cells using different software for biofilm analysis (Imaris-Bitplane statistics tool) is presented in the right panel of Figure S2 and provides consistent results at different CLSM magnification and at lower velocity and shear rate.

In contrast, the positively charged METMAC surface showed inconsistent behavior as a substratum for biofilm growth. While at the lower velocity of 0.22 cm min\(^{-1}\) consistent biofilm inhibition was observed (Figure S2) as in the static biofilm assay (Figure 3), at higher velocity of the medium (0.44 cm min\(^{-1}\)), biofilm growth was slightly elevated (Figure 4). A plausible speculation can attribute induced EPS production under higher shear rate conditions also observed by others (Kim et al. 2001), which protects the bacterial cells from the antimicrobial properties of the surface.

The roughness coefficient, determined by COMSTAT as suggested by Murga et al. (1995), is calculated from the thickness distribution of the biofilm and provides a measure of how much the thickness of the biofilm varies, which can be used as an indicator of uniformity of surface coverage by biofilm. The coverage of the membrane with the biofilm and the roughness coefficient, a measure of thickness variations, should be negatively correlated, as the latter should become smaller as the patches merge and the surface coverage become more uniform. Indeed, as seen in Figure 4, the scattered spots of the biofilm formed on the SPE coated membrane, yielding a relatively low surface coverage, showed the highest roughness, while for the GMS coated surface, which was nearly fully covered with biofilm, the thickness distribution of the biofilm was more homogenous and the roughness coefficient was reduced. It should be noted that at the microscopic scale the surface modification is not perfectly homogenous (Bernstein et al. 2010, 2011b). Likely, the areas where biofilm did grow on the SPE-modified membranes might be areas where the modification was lower. This suggests that the efficiency of the zwitter-ionic SPE-modified surface for reducing biofilm formation could be even larger than the present results indicate.

**Biofilm formation vs initial bacterial deposition**

The relevant data summarized in the last three columns of Table 2 clearly show that while both initial bacterial deposition and biofilm formation can be affected by the physico-chemical properties of the substratum, the reduced initial deposited rate does not necessarily result in a smaller biofilm growth and the correlation may even be negative. The negative correlation observed for the surface modified with GMS is consistent with others studies (Gottenbos et al. 2001; Nejadnik et al. 2008). Short-term effects of bacterial attachment may last for a period of ca 60–120 min and are attributed mostly by the physico-chemical interactions related to properties such as hydrophobicity and charge of the substratum and bacterial cells. As expected, the negatively charged bacteria are attracted to the positively charged MATEMC surface and repelled from the negatively charged GMS and zwitter-ionic SPE surfaces.

The results for the GMS surface indicate that such physico-chemical interactions can only delay biofilm formation (Nejadnik et al. 2008). Most likely, during the extended incubation period of 24 h, bacteria synthesize EPS, which forms an adhesive layer that can override the charge and hydrophilicity of the underlying membrane (Azeredo & Oliveira 2000). It may reverse the initial repulsion and allow the bacteria to attach irreversibly to the surface (Dufrene et al. 1996; Gómez-Suárez et al. 2002). Moreover, the charge of the surface can lead to enhanced interaction with the EPS matrix, which may explain why the highly charged repulsive GMS surface becomes eventually a more favorable substratum for biofilm formation than the original weakly charged ESPA-1 membrane.

It was expected that the METMAC surface bearing a strong positive charge would enhance adhesion of EPS and, ultimately, biofilm growth, similar to GMS. In contrast, reduced biofilm formation was observed on the METMAC coating, correlated to the static biofilm assay only at low shear (Figure S2), while at higher shear, a slight increase in the specific biovolume and a slight decrease in the biofilm thickness were observed (Figure 4). Surprisingly, while the METMAC coating show marked enhancement of bacterial deposition, an unexpected inhibition of biofilm formation was observed in both the static biofilm assay and also under flow at low shear of 0.07 s\(^{-1}\) (Figure S2). No clear observation of enhanced biofilm formation and no correlation to the bacterial deposition assay was detected for any of the cases. Most likely, the case of METMAC is a result of an entirely different phenomenon, the antimicrobial activity of quaternary amine groups (Kugler et al. 2005). This well-known effect is still not fully understood, but it has been suggested that quaternary amines may destabilize cell membranes (Ninham & Nostro 2010) and suppress the elongation and division of the bacteria (Gottenbos et al. 1999, 2001). It has been reported that surface modification with quaternary amines enhances bacterial deposition, provided the amine concentration surpasses a certain threshold (Huang et al. 2008). It is likely that in the present case the high amine concentration overrode the effect of enhanced deposition.
Intriguingly, SPE modification successfully reduced both initial bacterial attachment and biofilm growth. The consistent effect of the SPE on both initial deposition and biofilm growth, different from the two other monomers, implies a different inhibition mechanism. It can be hypothesized that in this case, inhibition of biofilm growth as well as bacterial attachment is due to minimization of bacteria–surface and EPS–surface interactions of all types rather than maximization of repulsion of bacteria, which may be readily reversed by EPS. As mentioned in the “Introduction”, zwitter-ionic monomers are hydrophilic and bear no net charge and thus they formally meet the ‘chemical’ criteria for ‘low-fouling’ surfaces (Ostuni et al. 2001; Norde 2007). In addition, swelling of hydrophilic grafted poly-SPE minimizes the thermodynamic driving force for adhesion via purely ‘physical’ dilution of all possible interactions between either bacteria or EPS and surface (Eshet et al. 2011; Herzberg et al. 2011). These general ‘chemical’ and ‘physical’ effects provided by an SPE coating may persist throughout all stages of biofilm growth, thus adhesion may be weak both before and after EPS release. This explains the good correlation between initial deposition and biofilm growth. In the latter case, the facilitated removal of both the cells and EPS by the flow-induced shear shifts the dynamic equilibrium between detachment and growth towards less accumulated biofilm, which may not happen in stagnant conditions when biofilm settles and grows on an undisturbed surface. This mechanism seems to be consistent with the more significant reduction of biofilm formation when shear was elevated (Figure S2 and Figure 4).

It can be concluded that in general there is no universal correlation between the initial bacterial deposition rates and biofilm growth on surfaces. The former then have to be used and interpreted with great care for assessing the anti-biofouling activity of surfaces. Curiously, the specific reasons for the observed correlations for each of the three analyzed surfaces were presumably different. For a negatively charged surface, the charge repulsion of the bacteria could be easily reversed upon biofilm growth probably due to secretion of EPS, whereas, for a positively charged surface, short-term charge attraction by amine groups, which enhanced bacterial deposition, could be overridden by their antimicrobial activity that suppressed biofilm growth. In contrast, the weakly interacting ‘low-fouling’ zwitter-ionic surface showed a good correlation between initial deposition and biofilm growth, especially under flow. This suggests that for the latter type of coatings both tests may be fairly indicative of anti-biofouling activity.

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