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Single ether group in a glycol-based ultra-thin layer prevents surface fouling from undiluted serum†

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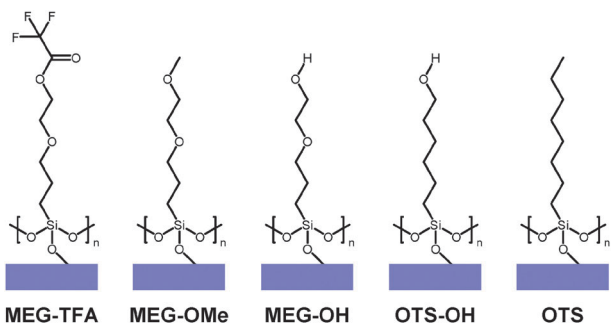
Through systematic structural modification, it is shown that the internal, single oxygen atom of simple monoethylene glycol-based organic films is essential for radically altering the fouling behaviour of quartz against undiluted serum, as characterized by the electromagnetic piezoelectric acoustic sensor. The synergy is strongest with distal hydroxyls.

Prevention of the fouling of surfaces by biological species circulating in the bloodstream and interstitial fluids is of utmost importance for the development of biocompatible and fully functional biomedical implants and devices with extended life expectancy. This phenomenon constitutes a challenging problem since it can result in implant malfunction, complete failure or cause adverse outcomes such as restenosis with respect to stent technology.[1] In the worst-case scenario, the host body triggers an immunological response, which results in post-operative complications such as peri-implant tissue inflammation with eventual rejection of the foreign object.[1] In biosensor technology, fouling is more often referred to as "non-specific adsorption" (NSA) and represents a serious prevailing concern for many detection platforms that could be potentially employed for the analysis of bodily fluids such as blood and serum. These biological matrices are composed of complex mixtures of interfering biomolecules, in particular proteins at high concentration (the reference interval for total serum protein is 60–80 g L^{-1}),[2] that have the propensity to adsorb non-specifically to the surface of the device thereby preventing the accurate measurement of target analytes present in considerably lower concentrations (down to ng L^{-1}). Tremendous efforts have been devoted over many years towards the development of protein-resistant surfaces and numerous types of organic coatings have been reported in the literature for such purpose, oligo- (OEG) and polyethylene glycol (PEG) polymers, historically, being the most prevalent.[3–5] Ultralow fouling (< 5 ng cm^{-2}) has now been achieved for single-protein buffered aqueous solutions as well as *diluted* blood plasma and serum.[3,4] Unfortunately, few coatings retain such level of performance when it comes to completely *undiluted* fluids,[3,4] and are, accordingly, not applicable in either biomedical or bioanalytical technology.

In order to examine the interactions of surfaces (*e.g.* SiO_2) with proteins, cells and various biomolecules, we have developed the highly sensitive electromagnetic piezoelectric acoustic sensor (EMPAS).[6,7] This device, which is operated in a flow-injection configuration, is capable of the detection of such chemistry in a real-time and label-free manner. This is demonstrated through use of the device as an immunosensor dedicated to the serological detection of anti-HIV antibodies with simultaneous assessment of cross-reactivity and serum fouling.[8] The system operates through the instigation of acoustic resonance at frequencies up to 1 GHz in a quartz substrate by an electromagnetic field associated with a flat spiral coil.[6–8] Adsorption of species from solution, whether from target analytes or fouling moieties, causes a change in the resonant frequency of the sensor. The response of the sensor in liquids is governed by interfacial effects such as alteration of viscoelasticity and slip phenomena rather than gravimetric effects.[6,7,9] Accordingly, the EMPAS system is ideal for the study of the antifouling *behaviour* of any coating imposed onto quartz.

The focus of this paper is a comparison of the anti-fouling behaviour, on quartz, of several organic monolayers (Scheme 1) constructed from structurally simple surface modifiers, characterized against *undiluted serum* with the EMPAS system.

Surface engineering using trichlorosilane self-assembling monolayer (SAM) chemistry for the development of selective biosensing platforms is currently the object of research in



Scheme 1 Surface modification of quartz.

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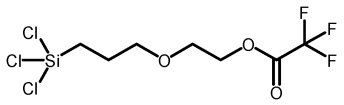
† Electronic supplementary information (ESI) available: Procedures for surface modifier synthesis, quartz cleaning, organic film formation and EMPAS measurements as well as film characterization by angle-resolved XPS and contact angle goniometry. See DOI: 10.1039/c2cc15692j



Scheme 2 Structure of MEG-TFA surface modifier.

our laboratory. In a recent study, that described combination of the EMPAS with such SAM chemistry, we reported low-fouling OEGylated mixed layers able to substantially decrease the adsorption of avidin from relatively concentrated (0.1 mg mL^{-1}) PBS-buffered solutions.[10,11] Distributed within these assemblies were shorter monoethylene glycolated (MEG) surface modifying building blocks (renamed MEG-TFA herein—Scheme 2), where the intended role was to space out the biorecognition element linking residues, causing these to protrude thereby offering enhanced overall binding ability.[10,11] This work led directly to the consideration that pure MEG-TFA films could possess important antifouling properties when exposed to complex, real-world bodily fluids such as undiluted serum. This feature of the work, in turn, led to a study of the related organic ultra-thin layers (Scheme 1), as well as bare quartz, in an attempt to gain better understanding of the structural parameters that influence antifouling.

MEG-TFA, MEG-OMe and OTS films were prepared *via* immersion of cleaned quartz discs into 1/1000 (v/v) solutions of the appropriate surface modifier in anhydrous toluene, for 60 min at room temperature.† *All* films were next soaked over-night in a 1/1 (v/v) solution of methanol and Milli-Q water, generating in the process the desired MEG-OH and OTS-OH coatings by cleaving the labile trifluoroacetyl (TFA) terminal groups of respectively MEG-TFA and OTS-TFA (not shown)† surfaces. The formation of the MEG-TFA films and subsequent complete removal of the TFA groups were successfully determined using X-ray photoelectron spectroscopy (XPS) following the appearance (respectively the loss) of a peak for fluorine at 689 eV, the one element unambiguously attributable to MEG-TFA (Fig. 1). Unlike fluorine however, the signal for carbon (at 285 eV) was not affected by the following mild aqueous treatment, clearly demonstrating that the latter had effectively cleaved the TFA groups without etching the organic backbone from the quartz substrate. The intensity of the substrate Si signal, given the escape depth of these electrons, strongly implies the formation of ultra-thin films. This result is consistent with ellipsometric measurements, which gave a thickness of ~5 Å for MEG-TFA film.†

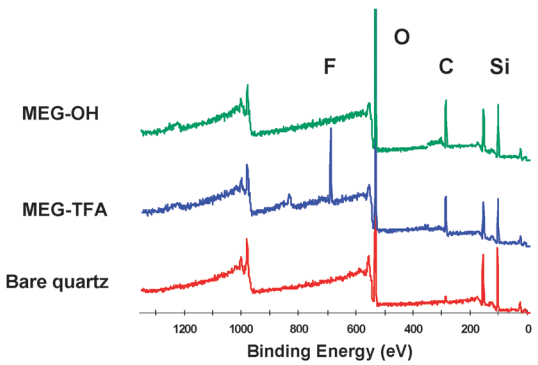


Fig. 1 XPS surveys for bare quartz, MEG-TFA and MEG-OH films.

The antifouling behaviour of bare and derivatized quartz surfaces against undiluted goat serum (45–75 mg protein/mL) was studied with the EMPAS at the ultra-high frequency of 0.94 GHz. From Fig. 2, it is immediately obvious that *all* organic adlayers yield a decrease in the shift in resonant frequency compared to the value observed for unmodified quartz (−31 kHz). Fully alkylated OTS films perform poorly (−22 kHz). Substitution for distal hydroxyl groups in OTS-OH provides no improvement (−23 kHz). Remarkably, the incorporation of an internal, single atom of oxygen in an MEG-type film results in a highly significant reduction in the resonant frequency shift associated with surface adsorption (−2 kHz for MEG-OH). This represents a >15-fold decrease relative to the result for bare quartz. In a number of experiments, no shift is observed. Other MEGylated films (MEG-TFA and MEG-OMe) also exhibit such an effect, although to a lesser extent. This result confirms the observation that the internal atom of oxygen in the β position is not only essential, but also acts synergistically in tandem with the distal hydroxyl group. These results may reveal why EGylated surfaces are so effective for the prevention of fouling. EMPAS measurements generally display reproducible behaviour with the exception of the MEG-TFA films. Random removal of the labile TFA groups during the EMPAS experiment, by the running PBS buffer or/and most certainly serum, is likely responsible for such an observation.

The shape of the EMPAS response profiles is also notable. For bare quartz, the resonant frequency first drops sharply (~−25 kHz) then gradually decreases (~−7 kHz in ~600 s) before final stabilization (Fig. 3A). While the former phase is undoubtedly due to serum reaching the quartz surface, producing a high level of fouling, the second phase likely reflects the passage of the viscous sample over the surface, in the flow-injection configuration. No final rinse-off by the uninterrupted buffer flow is observed, clearly indicating that species responsible for fouling accumulate irreversibly. With respect to MEG-OH films, the EMPAS profiles display radically different behaviour, with a progressive and comparatively limited initial drop in resonant frequency (>−5 kHz in ~250 s) typically followed by a gradual and extensive (up to 1500 s and +2 kHz) rinse-off (Fig. 3B). This observation seems to indicate that, in this case, the majority of serum species are adsorbed in a reversible fashion involving transient interaction with the substrate surface. Interestingly, this could represent the signature of a sequential "Vroman-like" process,

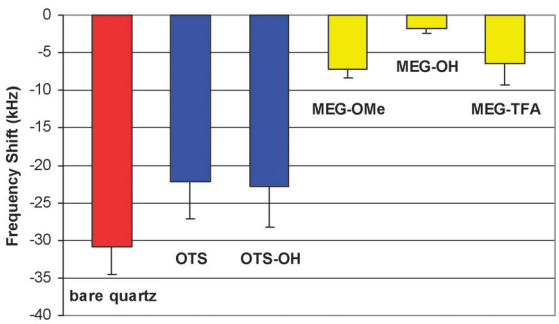


Fig. 2 EMPAS frequency shift upon injection of undiluted serum onto bare and derivatized quartz surfaces (4 to 8 replicates per data set).

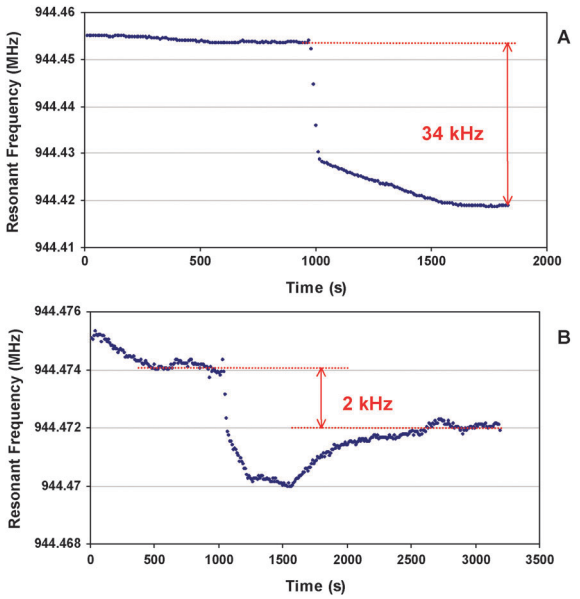


Fig. 3 EMPAS profiles for (a) bare quartz and (b) MEG-OH film.

wherein higher mobility, more abundant proteins first adsorb before being ultimately displaced by less motile, higher affinity entities.[12] Protein adsorption is a complex, multi-step process[12] and, accordingly, the increase in resonant frequency may reflect the occurrence of rigidifying viscoelastic phenomena within the fouling adlayer, such as those arising from protein structural rearrangments. Such structural and conformational reorganizations have been reported for serum albumin,[13] calcium-binding calmodulin[14] and HIV envelop glycoprotein gp120[15] using the conventional bulk acoustic wave sensor employed for measurement of both series resonant frequency and energy dissipation or motional resistance.

Finally, when MEG-OMe films are *not* submitted to the subsequent aqueous treatment, the EMPAS profiles show different response upon serum injection (−13 *vs.* −7 kHz, Fig. 4). These results appear to corroborate the widely proposed hypothesis that surface hydration is intimately involved in protein repellency. This argument proposes the concept of a "water barrier", wherein embedded and interfacial water molecules are tightly bound into permeated structures that have an energy cost in terms of disturbance.[16] Such barriers have been elegantly highlighted for several types

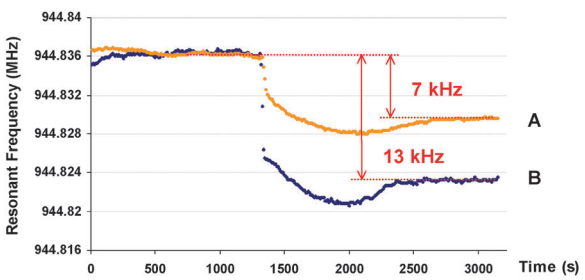


Fig. 4 EMPAS profiles for MEG-OMe coatings (a) soaked (15% RSD, $n = 6$) or (b) not soaked (8% RSD, $n = 5$) into MeOH/H_2O, overnight.

of surfaces and shown to be physically distinct, solute-free exclusion zones projecting up to several hundred microns into the contiguous aqueous phase.[17] Quartz also has long-range water structuring properties but only the molecules within a few monolayers are rigidly bound to the surface.[18] This explains why quartz presents a surface that is fouled with facility by serum.

In conclusion, we have presented evidence that short MEGylated organic monolayers, constructed from structurally simple surface modifiers in a straightforward two-step sequence, are able to radically alter the fouling behaviour of quartz against undiluted serum. A key feature is the observation that the internal ether oxygen acts in tandem with the distal –OH moiety, likely through a mechanism involving the instigation of a special intramolecular zone of hydration, which compromises the ability of the latter to engage in H-bonding. From a practical point of view, we believe that this work may prove to be applicable to other medically and bioanalytically relevant hydroxylated (bio)materials such as medical grade stainless steel and ceramics for implantable devices, as well as silicon and indium-tin oxides for biosensor applications.

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