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This Communication demonstrates that self-assembled monolayers (SAMs) of alkanethiolates on gold that present tri(propylene sulfoxide) groups prevent the nonspecific adsorption of protein and subsequent attachment of cells. A common strategy used to passivate surfaces against protein adsorption is to coat them with poly(ethylene glycol) (PEG).[1] It is not clear whether PEG is unique in its ability to confer resistance to adsorption, or if there are other materials with comparable (or superior) properties. SAMs of alkanethiolates on gold are a class of model organic surfaces that are well-suited to study the relationships between the structure of a surface and the adsorption of protein on the surface.[2] We have previously demonstrated that SAMs presenting short oligomers of the ethylene glycol group ($[-CH_2CH_2O-]_n$, $n = 2-7$) effectively resist the nonspecific adsorption of protein.[3,4] The goal of the present work was to *design* a new material that resists the adsorption of protein, but that has no counterpart in available biomaterials.

In designing a new "inert" material, we sought to preserve three characteristics of the oligo(ethylene glycol) chains: (i) a hydrophilic repeating unit; (ii) a unit that can hydrogen bond with water, and that is well-solvated; (iii) an oligomer that is conformationally flexible. We chose oligomers of the propylene sulfoxide group $[-CH_2CH_2CH_2S(O)-]$ as candidates that shared these properties with the oligo(ethylene glycol) chains. Both materials are hydrogen bond acceptors, but not donors. SAMs presenting tri(propylene sulfoxide) groups[5] are more hydrophilic than those presenting hexa(ethylene glycol) groups, as measured by advancing contact angles of water of 27° and 38°, respectively. A propylene linker was chosen in place of the ethylene linker because elimination reactions of the latter limit its stability. The biocompatibility of the propylene sulfoxide group is not known, but the parent functional group—dimethyl sulfoxide—is more biocompatible than is ethylene glycol.[6]

We used surface plasmon resonance (SPR) spectroscopy[4,7] to measure the adsorption of the proteins RNAse A and fibrinogen to mixed SAMs prepared from **1** and undecanethiol.[8] In the SPR experiment, p-polarized light is incident on the backside of a glass slide coated with a thin layer of gold that is modified with a SAM.[4,9,10] The angle of reflected light that shows a minimum in intensity (the resonance angle, θ_m) is related linearly to the amount of protein in the interfacial region. In the experiments described here, a buffer containing phosphate (10 mM) and sodium chloride (150 mM) (pH 7.4) was allowed to pass through the flow cell for 4 min, replaced with a solution of protein in the same buffer (1 mg/mL) for 6 min, and returned to buffer for 20 min.

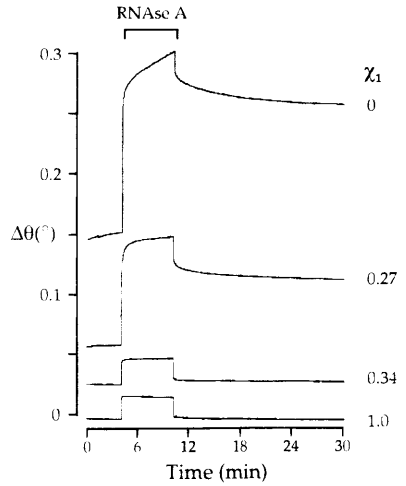

Figure 1. Data for the adsorption of RNase A to mixed SAMs comprising **1** and undecanethiolate. The *relative* change in the resonance angle (θ_m) is plotted versus time for each mixed SAM. The region of time during which protein was present in the buffer is indicated above the plot; the rapid increase (and decrease) in signal is due to dissolved protein in the bulk solution. The curves are offset vertically for clarity. The mole fraction of **1** in each SAM (χ_1) was determined by XPS and is indicated on the right side of the plot.

Both RNAse A and fibrinogen adsorbed quickly and irreversibly to hydrophobic SAMs presenting only methyl groups ($\chi_1 = 0$); a SAM prepared from only **1** ($\chi_1 = 1.0$) entirely resisted the adsorption of these two proteins (Figure 1). Even mixed SAMs containing as much as 60% undecanethiolate resisted the adsorption of both proteins; the amount of protein that adsorbed to SAMs having values of $\chi_1 < 0.4$ increased with the mole fraction of undecanethiolate (Figure 1). In all cases, greater than 90% of the protein remained adsorbed irreversibly during the final wash with buffer.

Figure 2 compares the adsorption of RNAse A and fibrinogen on mixed SAMs prepared from undecanethiol and either **1** or a hexa(ethylene glycol)-terminated alkanethiolate ($-S(CH_2)_{11}(OCH_2CH_2)_6OH$). SAMs presenting only tri(propylene sulfoxide) groups or hexa(ethylene glycol) groups are indistinguishable in their behavior: they entirely resist the *in situ* adsorption of protein—even "sticky" proteins such as fibrinogen.[4] Comparing the effectiveness of these groups when mixed with methyl-terminated alkanethiolates in the SAM is complicated for four reasons: (i) the lengths of the monomeric polymethylene units are different (propyl and ethyl); (ii) the number of oligomeric units differs for the two alkanethiolates; (iii) there is an error of ~5% in determining the value of χ in the SAM; (iv) the structures of mixed SAMs comprising alkanethiolates terminated in methyl groups, and these two

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(8) SAMs were prepared by immersing gold-coated glass slides in solutions of mixtures of the two alkanethiols in chloroform (2 mM total thiol) for 1 h. Experimental details are given in the supporting information.

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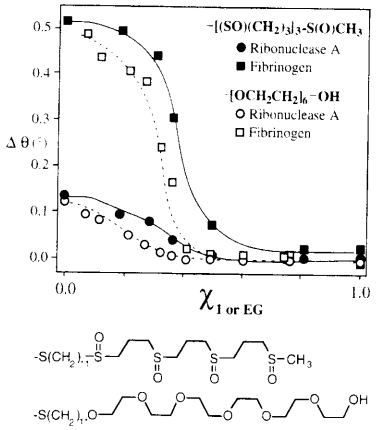

Figure 2. Quantitation of the adsorption of RNAse A (circles) and fibrinogen (squares) to mixed SAMs prepared from undecanethiol and either **1** (filled symbols, solid curves) or the hexa(ethylene glycol)-terminated alkanethiol (EG) (open symbols, dashed curves; data taken from ref 4). The structures of the two alkanethiols are shown at the bottom. The net change in resonance angle 10 s after protein was removed from the flow cell is plotted versus χ_1 or χ_{EG} for each protein. The curves serve only to organize the data visually and do not represent a physical model.

groups may differ, including the sizes of phase-separated domains of single alkanethiolates and the conformational order of the hydrocarbon chains. The data suggest that the $(EG)_6OH$ group is only slightly better than the tri(propylene sulfoxide) group in resisting the adsorption of RNAse and fibrinogen.

SAMs terminated in the oligo(ethylene glycol) group resist the adsorption of matrix proteins and subsequent cell attachment;[11,12] SAMs of **1** show a similar capability. Microcontact printing[13] was used to pattern SAMs into lines of hexadecanethiolate 60 μm in width and separated by regions of **1** 120 μm in width. These substrates were exposed to a solution of fibronectin (FN; 25 μg/mL) for 1 h to coat the methyl-terminated regions of the SAM with protein. When these substrates were placed in defined media containing a suspension of bovine capillary endothelial cells, the cells attached and spread only on the hydrophobic lines (Figure 3). The attached cells were confined to the underlying pattern of SAM for a period of 1−2 days, after which the cells began to invade regions of **1**; for patterned SAMs presenting methyl and oligo(ethylene glycol) groups, the cells remain confined to the underlying pattern for periods of 5−7 days.[12] These data again suggest that the $(EG)_6$-OH group is slightly more inert than the tri(propylene sulfoxide) group.

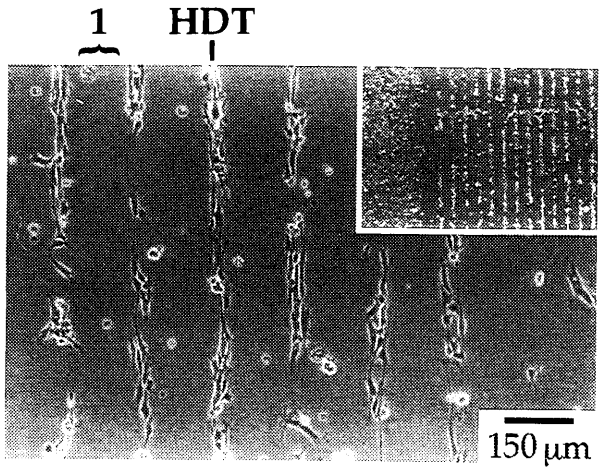

Figure 3. Attachment of bovine capillary endothelial cells to SAMs patterned into regions of hexadecanethiolate (HDT) and **1**. These regions are indicated above the figure. Regions of **1** of the patterned SAM resisted the adsorption of fibronectin and subsequent attachment of cells. The inset shows a lower-magnification view containing an unpatterned region (left) and the patterned region (right). This photograph was taken after 24 h in culture.

The most important result from this work is the demonstration of a successful process for the *de novo* design and testing of a new material that resists the nonspecific adsorption of protein; the properties of the tri(propylene sulfoxide) group indicate that the oligo(ethylene glycol) group is not unique in its ability to serve as an inert surface. The sulfoxide group is not used commonly in biomaterials, but was chosen by mimicking characteristics of the structurally unrelated oligo(ethylene glycol) group. This work was made possible by the structural order of SAMs of alkanethiolates on gold, and the ability to precisely control the properties of these interfaces through synthesis of precursor alkanethiols. This class of model surfaces is especially well-suited for relating the molecular structure of interfaces to responses when placed in biological media (e.g., protein adsorption and cell attachment). The findings of this Communication establish a protocol for the design and evaluation of new surfaces with properties tailored for their interactions with biological systems.

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Supporting Information Available: Synthetic scheme, experimental procedures, and characterization data for the synthesis of **1** and preparation of SAMs (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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