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Annu. Rev. Biophys. Biomol. Struct. 1996. 25:55–78
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USING SELF-ASSEMBLED MONOLAYERS TO UNDERSTAND THE INTERACTIONS OF MAN-MADE SURFACES WITH PROTEINS AND CELLS

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KEY WORDS: biocompatibility, biomaterials, biosurfaces

ABSTRACT

Self-assembled monolayers (SAMs) formed on the adsorption of long-chain alkanethiols to the surface of gold or alkylsilanes to hydroxylated surfaces are well-ordered organic surfaces that permit control over the properties of the interface at the molecular scale. The abililty to present molecules, peptides, and proteins at the interface make SAMs especially useful for fundamental studies of protein adsorption and cell adhesion. Microcontact printing is a simple technique that can pattern the formation of SAMs in the plane of the monolayer with dimensions on the micron scale. The convenience and broad application offered by SAMs and microcontact printing make this combination of techniques useful for studying a variety of fundamental phenomena in biointerfacial science.

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1056-8700/96/0610-0055$08.00

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PERSPECTIVES AND OVERVIEW

Man-made surfaces in contact with biological environments are important in biology, biotechnology, and medicine. These surfaces occur in tools and reagents for studies in molecular and cell biology; in substrates for enzyme-linked immunosorbent assay (ELISA), cell culture, and tissue engineering; in materials for contact lenses, dental prostheses, and devices for drug delivery; in coatings for catheters, indwelling sensors, and implant devices; and in materials for chromatography and storage of proteins (50, 65). The first event that usually occurs on contact of the synthetic material with a medium that contains dissolved protein is the adsorption of protein to the surface: other responses, such as the attachment of cells, are secondary and depend on the nature of the adsorbed layer of protein. Because of its central importance, the adsorption of protein to man-made surfaces has been studied extensively. Although much has been learned, there are still no mechanistic models that rationalize (or predict) the interaction of a protein with a surface in molecular detail. A broad goal of research in this area is to understand the interactions of proteins with surfaces at the level of detail that is now common for characterization of the interactions of proteins with water, ligands, and other proteins in solution.

Model systems designed to elucidate these mechanisms must have

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three components: (*a*) a protein with known high-resolution structure and properties (e.g. stability, conformational dynamics, and tendency to aggregate); (*b*) a structurally well-defined surface with properties that can be tailored and controlled simply and allows the complex functionality relevant to biochemistry to be introduced at the surface; and (*c*) one or several analytical techniques that can measure adsorption of protein in situ and in real time. Numerous proteins suitable for these types of studies are now available. Analytical methodologies appropriate for these studies (e.g. surface plasmon resonance spectroscopy and fluorescence spectroscopy) are becoming available. However, X-ray and electron diffraction studies of adsorbed crystalline monolayers are still the only techniques that can provide information at the molecular scale, and these techniques are applicable only in special cases. The absence of methods to prepare well-defined surfaces has been a problem; for those surfaces that are well defined, such as metals, metal oxides, and crystals, surface properties cannot be controlled precisely.

Self-assembled monolayers (SAMs)—particularly those formed by the adsorption of long-chain alkanethiols on gold—are a recently developed class of organic surfaces that are well suited for studying interactions of surfaces with proteins and cells. The ability to control the composition and properties of SAMs precisely through synthesis, combined with the simple methods that can pattern their functional groups in the plane of the monolayer, makes this class of surfaces the best now available for fundamental mechanistic studies of protein adsorption and cell adhesion. Here we review the use of two classes of SAMs, alkanethiolates on gold and alkylsiloxanes on hydroxylated surfaces, in the study of processes that occur at the interface between a man-made surface and a biological medium. We do not discuss much of the excellent work in which other classes of surfaces, such as Langmuir-Blodgett films, lipid bilayers, and polymers, have been used (for reviews, see 44, 60, 61, 75).

BACKGROUND

Man-Made Surfaces That Contact Biological Media

Many early studies of the interactions of artificial surfaces with biological media were motivated by problems associated with the formation of thrombus on foreign surfaces that contact blood—a process that is now relatively well understood and involves the adsorption of proteins intimately (73). For the past 3 decades, researchers have sought to understand the interactions of man-made materials with proteins (and

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processes dependent on protein adsorption), with applications in biocompatible materials as a central motivation. Although many materials have been identified that are compatible with tissue to varying degrees [e.g. titanium (implants), polymethylmethacrylate (contact lenses), ceramics (dental prosthesis), polyurethanes (artificial heart), pyrolytic carbon (heart valves)] there has been much less progress in the elucidation of the mechanisms by which these materials function.

Adsorption of Protein

A survey of the extensive literature on the adsorption of proteins to man-made surfaces is not practical in this review (for reviews, see 44, 61, 63, 75). The adsorption processes are complicated. Even in the simplest case, where a single, well-defined protein adsorbs to a uniform, well-defined surface, a substantial range of processes is usually involved (Figure 1). After an initial adsorption of a protein to a surface, the protein can (Figure 1*a*) dissociate from the surface and return to solution; (*d*) change orientation; (*d*) change conformation but retain biological activity; (*f*) denature and lose activity; or (*g*) exchange with other proteins in solution. These processes are complicated further by a range of conformationally altered and/or denatured states accessible to the adsorbed protein and by the many different microenvironments at the surface created by heterogeneities in the surface and the presence and conformations of other proteins. Lateral protein–protein interactions may dominate the protein–surface interactions. Because many of these processes are essentially irreversible, models must emphasize the kinetic aspects of protein adsorption (59).

ORIENTATION AND CONFORMATION OF ADSORBED PROTEIN Most studies have generated empirical models by analyzing the amount and rates of

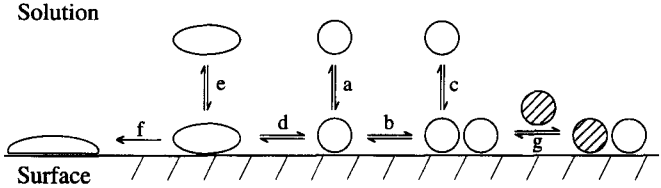

Figure 1 The complexities associated with studies of protein adsorption. Several equilibria must be considered on adsorption of a protein to a surface (*a*); lateral mobility of the adsorbed protein (*b*); dissociation of a protein adjacent to another protein (*c*); reversible denaturation and changes in conformation of the protein (*d*); dissociation of the altered protein (*e*); denaturation of the protein that results in irreversible adsorption (*f*); and exchange of the protein with a protein from solution (*g*). This scheme is not complete but is complicated further by the many different conformations and environments available to an adsorbed protein.

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protein adsorption; few have investigated adsorption at the molecular level. The information required for a detailed molecular description of these processes, including information concerning the orientation and conformation of adsorbed proteins as a function of time and conditions, has been difficult to obtain. Few analytical methods exist—and none with the power to reveal structure comparable to X-ray crystallography or multidimensional NMR spectroscopy—that can characterize the conformation and orientation of proteins adsorbed to surfaces. Infrared spectroscopy has been used to assess the degree of denaturation of fibronectin adsorbed to SAMs that present different functional groups (7). Lee & Belfort (37) correlated the activity of adsorbed RNase A to a model that involved conversion between two orientations of the protein at the surface. Darst et al (14) probed the orientation and conformation of myoglobin adsorbed to a polydimethylsiloxane surface by using a panel of five monoclonal antibodies that had known epitopes on the protein. This latter method and related footprinting methods that use proteases (87) or selective chemical reagents for modification of residues of proteins (69) are perhaps the most general methods for the direct characterization of unlabeled proteins adsorbed to surfaces. Kornberg and coworkers (13) have used electron diffraction to determine the structure of two-dimensional crystals of the protein streptavidin adsorbed to a biotinylated lipid layer. Rennie and coworkers (18) have used neutron reflection to determine the structure of β-casein adsorbed to hydrophobic alkylsiloxane monolayers. An emerging technique based on X-ray standing waves also provides direct structural information with near-atomic resolution for cases in which the layer of protein is ordered (6).

MEASURING PROTEIN ADSORPTION The most useful of the many analytical techniques that have been used to measure protein adsorption are those that are compatible with a variety of surfaces and that provide measurements in situ and in real time (59). Methods that measure the dielectric properties of an interface [surface plasmon resonance (SPR) spectroscopy (41), waveguide interferometry (62), and ellipsometry (45)] and those that measure changes in the resonance frequency of a piezoelectric material [quartz crystal microbalance (77), surface acoustic wave (77), and acoustic plate mode (12) devices] are particularly well suited. The primary drawback of these methods is that they measure bulk properties of the interface and provide little or no detail about atomic-level interactions. Surface plasmon resonance spectroscopy is particularly well suited for use in conjunction with SAMs, because both techniques use thin films of gold as substrates: A commercial SPR

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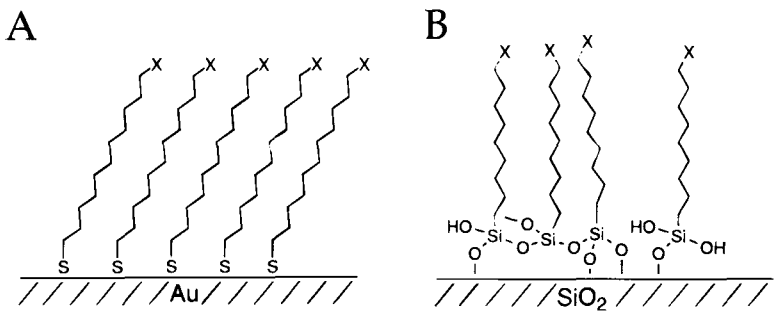

Figure 2 Models for SAMs of alkanethiolates on gold and alkylsiloxanes on hydroxylated surfaces. (*A*) The thiol groups coordinate to the hollow threefold sites of the gold (111) surface, and the alkyl chains pack in a quasi-crystalline array. (*B*) The conformations of alkylsilanes and the details of their bonding to surface hydroxyl groups are less clear; a mixture of possible conformations and geometries is probably involved. The surface properties of both SAMs are controlled by controlling the terminal function group X.

instrument has demonstrated many of the characteristics required for efficient studies of adsorption of proteins to functionalized SAMs on gold (53).

Self-Assembled Monolayers of Alkanethiolates on Gold

Self-assembled monolayers of alkanethiolates on gold form on the adsorption of a long-chain alkanethiol [$X(CH_2)_nSH$, $n = 11 - 18$) from solution (or vapor) to a gold surface (Equation 1). The structure of these SAMs is now well established (16, 79):

$$RSH + Au(0)_n \rightarrow RS^-Au(I)\ \ Au(0)_n + \tfrac{1}{2}H_2(?).$$

The sulfur atoms coordinate to the gold atoms of the surface, and the trans-extended alkyl chains are tilted approximately 30 degrees from the normal to the surface (Figure 2). The properties of the interface depend on the terminal functional group X of the precursor alkanethiol; even structurally complex groups can be introduced onto the surface through straightforward synthesis. The surface chemistry of SAMs can be controlled further by forming so-called mixed SAMs from solutions of two or more alkanethiols. Self-assembled monolayers are stable in air or in contact with water or ethanol for periods of several months; they desorb at temperatures greater than 70°C or when irradiated with UV light in the presence of oxygen. They have been used in cell culture for periods of days. Self-assembled monolayers that are supported on gold 5–10 nm in thickness (on glass slides) are transparent, and those

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supported on gold of thickness greater than 100 nm are opaque and reflective (15); even the thin films of gold are electrically conductive.

Self-assembled Monolayers of Alkylsiloxanes

Alkylsiloxanes are obtained by reaction of a hydroxylated surface (usually the native oxide of silicon or glass) with a solution of alkyltrichlorosilane (or alkyltriethoxysilane) (48, 56, 72). The reactive siloxane groups condense with water and with hydroxyl groups of the surface and neighboring siloxanes to form a cross-linked network; the bonding arrangement is not well defined but depends on the conditions used to form the SAM (Figure 2). These SAMs are significantly more stable thermally than alkanethiolates on gold and do not require evaporation of a layer of metal for preparation of substrates. The siloxane monolayers are limited, however, in the range of functional groups that can be displayed at the surface by the reactivity of the alkyltrichlorosilane groups of the precursors and by the technical difficulty of introducing functional groups once the monolayer has formed.

The Physical-Organic Chemistry of Self-Assembled Monolayers

An important goal in interfacial science is to understand the relationship between the microscopic structure of a surface and its macroscopic properties; this relationship is particularly relevant in studies of protein adsorption where hydrophobic forces are dominant (71). Self-assembled monolayers on gold—and, to a lesser extent, alkylsiloxanes—offer the level of structural control required for detailed studies of adsorption processes. Studies of the influence of a terminal functional group X of a SAM on the wettability of the surface reveal that the hydrophobicity of the surfaces can be controlled precisely (2). Self-assembled monolayers that present polar functional groups (e.g. carboxylic acid and hydroxyl) are wetted by water. Those that present nonpolar, organic groups (e.g. trifluoromethyl and methyl) are autophobic and emerge dry from water. Monolayers that present fluorinated groups are more water repellent than Teflon.

INTERACTIONS OF PROTEINS WITH SELF-ASSEMBLED MONOLAYERS

Adsorption of Proteins to Self-Assembled Monolayers

The adsorption of several model proteins to SAMs that present different functional groups (e.g. alkyl, perfluoroalkyl, amide, ester, alcohol, ni-

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trile, carboxylic acid, phosphonic, boric acids, amines, and heterocycles) correlates approximately with the hydrophobicity of the surfaces (43, 57); the degree of denaturation, as inferred by the density of a layer of adsorbed protein, increases with the hydrophobicity of the surface and decreases with the concentration of protein in the contacting solution. Adsorption on hydrophobic surfaces often is irreversible kinetically, but the protein adlayer can be removed with detergents or replaced by other proteins in solution. Although SAMs that present ionic groups have been used extensively to control protein adsorption, less is known about the relationships between the properties of charged surfaces and the structure and properties of the layer of protein. Vroman (73) has used ellipsometry and immunologic identification of adsorbed proteins to characterize the exchange of plasma proteins at hydrophilic glass surfaces.

Surfaces That Resist the Adsorption of Proteins

Much effort has been directed toward the identification of biologically "inert" materials, i.e. materials that resist the adsorption of protein. The most successful method to confer this resistance to the adsorption of protein has been to coat the surface with poly(ethylene glycol) (PEG) (20, 22); a variety of methods, including adsorption, covalent immobilization, and radiation cross-linking, have been used to modify surfaces with PEG (22). Polymers that comprise carbohydrate units also passivate surfaces, but these materials are less stable and less effective than PEG (41, 76). A widely used strategy is to preadsorb a protein—usually bovine serum albumin—that resists adsorption of other proteins. This strategy suffers from problems associated with denaturation of the blocking protein over time (3) or exchange of this protein with others in solution. A further limitation of this strategy is the inability to present other groups (e.g. ligands, antibodies) at the surface in controlled environments.

Self-assembled monolayers that are prepared from alkanethiols terminated in short oligomers of the ethylene glycol group [$HS(CH_2)_{11}(OCH_2CH_2)_nOH$: $n = 2 - 7$] resist the adsorption of several model proteins, as measured by both ex situ ellipsometry and in situ SPR spectroscopy (53, 58). Even SAMs that contain as much as 50% methyl-terminated alkanethiolates, if mixed with oligo(ethylene glycol)-terminated alkanethiolates, resist the adsorption of protein. Self-assembled monolayers that present oligo(ethylene glycol) groups are useful as controls in studies of the adsorption of proteins to surfaces. The ability to prepare SAMs that present derivatives of these and other

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groups will be useful for investigating the mechanisms by which these surfaces resist adsorption.

De Gennes, Andrade, and coworkers (29, 30) have proposed that surfaces modified with long PEG chains resist the adsorption of protein by "steric stabilization." In aqueous solution, the PEG chains are solvated and disordered. Adsorption of protein to the surface causes the glycol chains to compress, with concomitant desolvation. Both the energetic penalty of transferring water to the bulk and the entropic penalty incurred on compression of the layer serve to resist protein adsorption. It is not clear that this analysis applies to thin, dense films of oligo(ethylene glycol) groups, as De Gennes and Andrade predicted that surfaces comprising densely packed, nearly crystalline chains of PEG might not resist the adsorption of protein. It is remarkable that SAMs presenting densely packed tri(ethylene glycol) groups resist the adsorption of protein. These layers are almost certainly different in comformational flexibility and solvation than are long, dilute PEG chains. We presume there is sufficient free volume in the glycol layer of the SAMs to allow solvation by water. An understanding of the properties of these SAMs may permit the design of new classes of inert surfaces.

Immobilization of Proteins to Self-Assembled Monolayers

The immobilization of proteins to substrates is important in many areas, ranging from ELISA and cell culture to biosensors; consequently, many strategies have been developed to confine proteins to surfaces (51, 63). Methods that rely on noncovalent association of proteins with surfaces—with the use of both hydrophobic and electrostatic interactions—are the most common and experimentally simplest, but they are also the least well controlled.

Methods that rely on covalent coupling of proteins to surfaces are inherently more controlled and give layers of protein that cannot dissociate from the surface or exchange with other proteins in solution. A variety of surface chemistries have been used; the most successful have been based on the formation of amide and disulfide bonds (26, 74, 78). The selectivity and rapid reaction of thiols with α-haloacetyl groups constitutes a particularly attractive protocol (38). The use of well-defined surfaces and proteins that have only a small number of reactive groups permits a high degree of control over the attached protein. For example, genetic engineering was used to construct a mutant of cytochrome c that had only a single cysteine group; immobilization of this protein to a SAM terminated in thiol groups gave a uniformly oriented layer of protein (26).

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A common problem that limits the use of immobilized proteins is denaturation of the protein, with concomitant loss in activity. Methods to increase the lifetimes of these proteins have involved coupling to ''inert'' materials (76). A commercial SPR instrument that quantitates protein–protein interactions uses a gel layer of carboxylated dextran to stabilize immobilized proteins (41). Self-assembled monolayers that are terminated in oligo(ethylene glycol) groups may have broad usefulness as inert supports, because a variety of reactive groups can be incorporated in SAMs in controlled environments.

Biospecific Adsorption of Proteins to Self-Assembled Monolayers

The design of surfaces to which analytes bind specifically is important for biosensors and other technologies, e.g. affinity chromatography, cell culture, coatings for implants, and artificial organs. These surfaces must possess specificity for a particular protein and simultaneously resist the nonspecific adsorption of other proteins.

Immobilization schemes based on the biotin–streptavidin interaction have been investigated widely. Spinke et al (68) studied the recognition of streptavidin by SAMs that present biotin ligands. The effectively irreversible complexation in this system is useful for many applications, such as immobilization of proteins or nucleic acids, but is not relevant to the weak, reversible recognition that is more common in biology. In an early model system, Mosbach and coworkers (45) used ellipsometry to characterize the reversible binding of lactate dehydrogenase to SAMs that present analogues of nicotinamide adenine dinucleotide (NAD). Several groups have studied the recognition of immobilized antigen by antibodies, because of the availability of antibodies to a variety of antigens and the high specificity displayed by antibodies (35).

Self-assembled monolayers that present oligo(ethylene glycol) groups were used as supports to which ligands for proteins were attached (Figure 3). With the use of SPR spectroscopy, Sigal et al (64) measured the binding of a His-tagged T-cell receptor to a SAM presenting a Ni(II) complex. Likewise, carbonic anhydrase bound to SAMs that presented a benzenesulfonamide group (Figure 4) (52). In both cases, the amount of protein that bound increased with the density of ligand on the SAM. Both SAMs also resisted the nonspecific adsorption of other proteins. The SAM terminated in benzenesulfonamide groups could be used to measure the concentration of carbonic anhydrase (CA) in a complex mixture that contains several other proteins (52). The effectiveness of the oligo(ethylene glycol) groups to resist nonspecific

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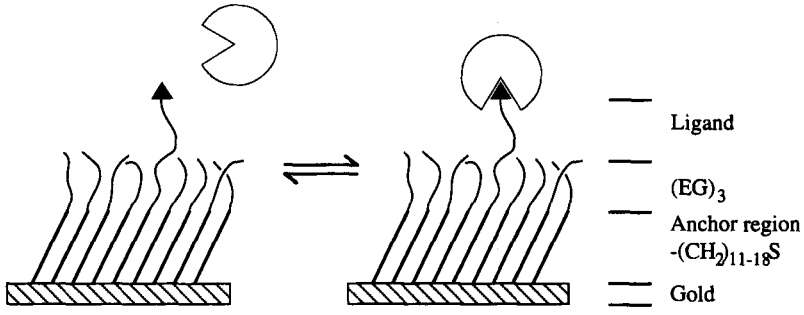

Figure 3 Design of SAMs for biospecific adsorption. Mixed SAMs that present ligands and oligo(ethylene glycol) groups permit control over the density of adsorbed protein. The glycol layer is effective at preventing nonspecific adsorption of protein.

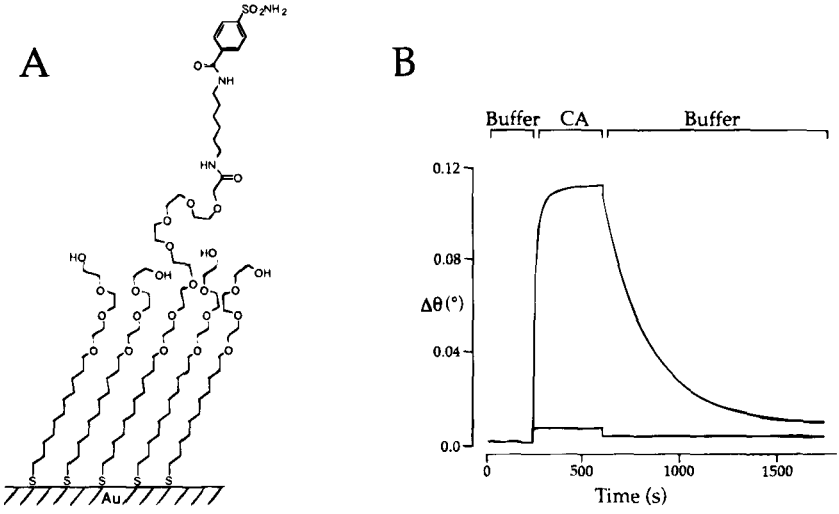

Figure 4 Surface plasmon resonance spectroscopy was used to measure the rate and quantity of binding of CA to a SAM terminated in EG_3 groups and benzenesulfonamide groups (*A*). The change in resonance angle ($\Delta\theta$) of light reflected from the SAM/gold is plotted against time; the time over which the solution of CA (5 μM) was allowed to flow through the cell is indicated at the top of the plot (*B*). (*upper curve*) Binding (and dissociation) of CA to a SAM containing approximately 5% of the ligand-terminated alkanethiolate. Carbonic anhydrase did not adsorb to a SAM that presented only ethylene glycol groups (*lower curve*). A response caused by the change in index of refraction of the CA-containing solution was observed on introduction of protein into the flow cell (evident in *lower curve*). The difference between the measured response and this background signal represents binding of the CA to the SAM.

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adsorption, combined with the ability of SAMs to present a range of groups in controlled environments, makes this system well suited for other studies of biospecific adsorption and for applications dependent on specific adsorption.

Attachment of Cells to Self-Assembled Monolayers

The attachment and spreading of anchorage-dependent cells to surfaces are mediated by proteins of the extracellular matrix, e.g. fibronectin, laminin, and collagen. A common strategy for controlling the attachment of cells to a surface therefore relies on controlling the adsorption of matrix proteins to the surface. Both hydrophobic (42, 46), and ionic (34) SAMs have been used as substrates for cell culture. A significant problem with these preparations is the lack of control over the adsorption process. It generally has been assumed that the density of matrix protein is the parameter that influences the behavior of attached cells. Studies of the differentiation response of fibroblasts and neuroblastoma cells on siloxane SAMs terminated in different groups that had been coated with fibronectin suggested that cell behavior depended on the conformation of fibronectin and not on the density of protein (7, 40). The role of protein adsorption in most instances remains poorly understood.

Cell attachment to and spreading on fibronectin involve binding of integrin receptors of the cell to the tripeptide RGD of the matrix. Massia & Hubbell (47) demonstrated that a siloxane SAM that presents the RGD peptide supported the attachment and spreading of fibroblast cells. These synthetic culture substrates have advantages over the traditional matrix-coated substrates of increased reproducibility in culture and utility for fundamental studies of cell-matrix interactions. Corresponding work has focused on the development of substrates for serum-free cell culture by immobilizing essential growth factors at the surface of the substrate (86).

CONTROL OVER SPATIAL ADSORPTION OF PROTEIN

Patterning Self-Assembled Monolayers

MICROCONTACT PRINTING Microcontact printing (μCP) (36, 54, 81) provides a new and convenient method for patterning SAMs of alkanethiolates on gold with features of sizes ranging down to 1 μm (Figure

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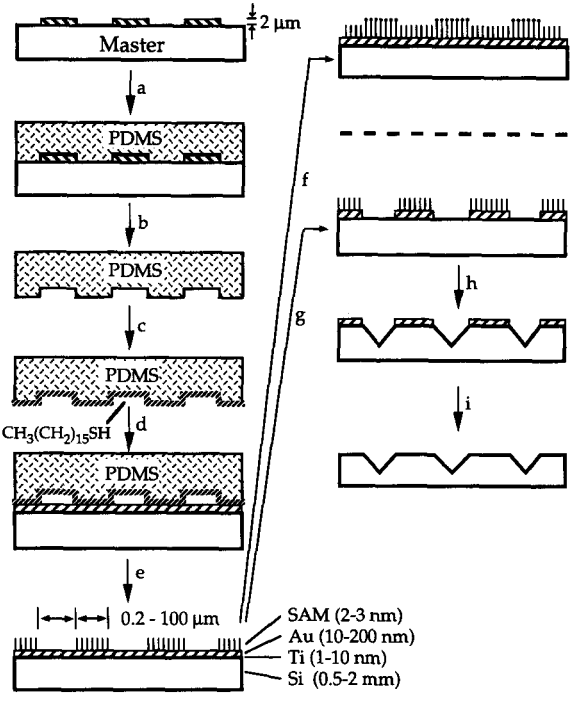

Figure 5 Microcontact printing starts with a master template containing a pattern of relief (*a*); this master can be fabricated by photolithography or by other methods. A PDMS stamp cast from this master (*b*) is inked with a solution of alkanethiol in ethanol (*c*) and used to transfer the alkanethiol to surface of gold (*d*); a SAM is formed only at those regions where the stamp contacts the surface (*e*). The bare regions of gold can then be derivatized with a different SAM by rinsing with a solution of a second alkanethiol (*f*). The initial patterned SAM can also be used to protect the underlying gold from dissolution in a corrosive etchant (*g*). Anisotropic etching of the exposed silicon gives contoured surfaces (*h*). The gold mask can be removed by washing with *aqua regiai*; the resulting silicon substrates are useful as new masters from which stamps can be cast or as substrates for a variety of applications.

5); features as small as 200 nm have been formed with the use of this technique (85). Microcontact printing starts with an appropriate relief structure from which an elastomeric stamp is cast; this ''master'' template usually is generated photolithographically, but any substrate that has an appropriate pattern of relief can be used. The polydimethylsiloxane (PDMS) stamp is ''inked'' with a solution of alkanethiol in ethanol, dried, and manually brought into contact with a surface of gold. The alkanethiol is transferred to the surface only at those regions where the stamp contacts the surface. This process produces a pattern of SAM

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that is defined by the pattern of the stamp. Conformal contact between the elastomeric stamp and surface allow surfaces that are rough (at the scale of 100 nm) to be patterned over areas several square centimeters in size with edge resolution of the features better than 50 nm. Multiple stamps can be cast from a single master, and each stamp can be used hundreds of times. Microcontact printing also was used to pattern siloxanes on the surfaces of SiO_2 and glass (84) and to pattern SAMs on nonplanar and contoured surfaces (27). Because μCP relies on molecular self-assembly and does not require stringent control over the laboratory environment, it can produce μm-scale patterns conveniently and at low cost relative to methods that use photolithography.

PHOTOLITHOGRAPHY Photolithographic methods illuminate a surface with UV light through a mask (5, 17, 34). In the common "lift-off" method (34), a silicon oxide substrate is coated with a thin layer of photoresist. The resist is exposed to UV light through a mask, and the exposed regions are subsequently removed in a developing bath; this process creates a pattern of silicon dioxide that can be derivatized with an alkylsiloxane SAM. The remaining regions of photoresist are then removed, and a different SAM is formed on the complementary regions. Other variants of photolithography create patterns by using UV light to damage, or modify, a SAM. Wrighton and coworkers (19) prepared SAMs of alkanethiolates terminated in an aryl azide group; near-UV irradiation of the SAM through a lithographic mask and a thin film of an amine resulted in the attachment of the amine in the exposed regions. Hickman et al (24) irradiated thiol-terminated siloxanes through a mask in the presence of oxygen to form sulfonate groups. These methods are less well controlled and less general than such methods as μCP, which pattern the adsorption of preformed components. Photolithographic methods can produce patterns that have features down to 1 μm conveniently. Capital costs for the equipment and controlled environment facilities, however, make this technique expensive and inconvenient for the biological researcher.

FABRICATION OF CONTOURED SURFACES Both μCP (32) and photolithography (10) have been used to pattern silicon substrates with a layer of resist that protects the substrate from dissolution in a chemical etchant (Figure 4). Chemical etching of these patterned substrates produces contoured features whose shapes depend on the orientation of the silicon and the time of etching; anisotropic etching of a silicon $\langle 100 \rangle$ surface produces controlled V-shaped grooves. The properties of these etched substrates can be tailored either by forming an alkylsilox-

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ane SAM or by evaporating a layer of gold and forming a SAM of alkanethiolates. Alternatively, the topographic pattern can be transferred to other substrates (e.g. prepolymers) with the use of a PDMS stamp cast from the etched master.

Patterning Adsorption of Protein on Self-Assembled Monolayers

Patterned SAMs on gold have been used extensively to control the adsorption of protein to surfaces (Figure 6). This method relies on the ability of a SAM terminated in oligo(ethylene glycol) groups to resist the adsorption of protein. Microcontact printing was used to pattern a SAM into regions terminated in methyl groups and oligo(ethylene glycol) groups (42, 54). Immersion of these SAMs in aqueous solutions that contain proteins resulted in the adsorption of a monolayer of protein only on the methyl-terminated regions; this pattern of protein could be imaged by scanning electron microscopy (43). Bhatia et al (4) patterned a siloxane film terminated in thiol groups by irradiation with UV light through a mask. The fluorescent protein phycoerythrin was immobilized to the thiol groups in regions that were protected from the UV light with the mask; photo-induced oxidation of the thiol groups in regions of the surface that were irradiated presumably gave negatively charged sulfonate groups, which resisted the adsorption of protein.

Patterned Attachment of Cells on Self-Assembled Monolayers

The same methods used to pattern the adsorption of proteins to surfaces have been used to direct the attachment of cells to surfaces (5, 23, 34, 66, 67). In an early example, Kleinfeld et al (34) used photolithography to pattern siloxane SAMs into regions terminated in methyl and amino groups. When plated in the presence of serum, neural cells attached and spread selectively on the amino-terminated regions; in the absence of serum, the cells attached to all regions. Others have also found that amino-terminated siloxanes are excellent substrates for culture of neural cells (24). We presume that proteins of the serum that do not support cell attachment adsorbed to the hydrophobic areas. Regions terminated in perfluoro groups have also been used to resist the attachment of cells (3, 67, 70).

Self-assembled monolayers patterned into regions terminated in methyl and oligo(ethylene glycol) groups permit spatial control over the attachment of cells. Microcontact printing was used to pattern SAMs into adhesive lines ranging from 10 to 100 μm in width; after coating these substrates with fibronectin, endothelial cells were confined to

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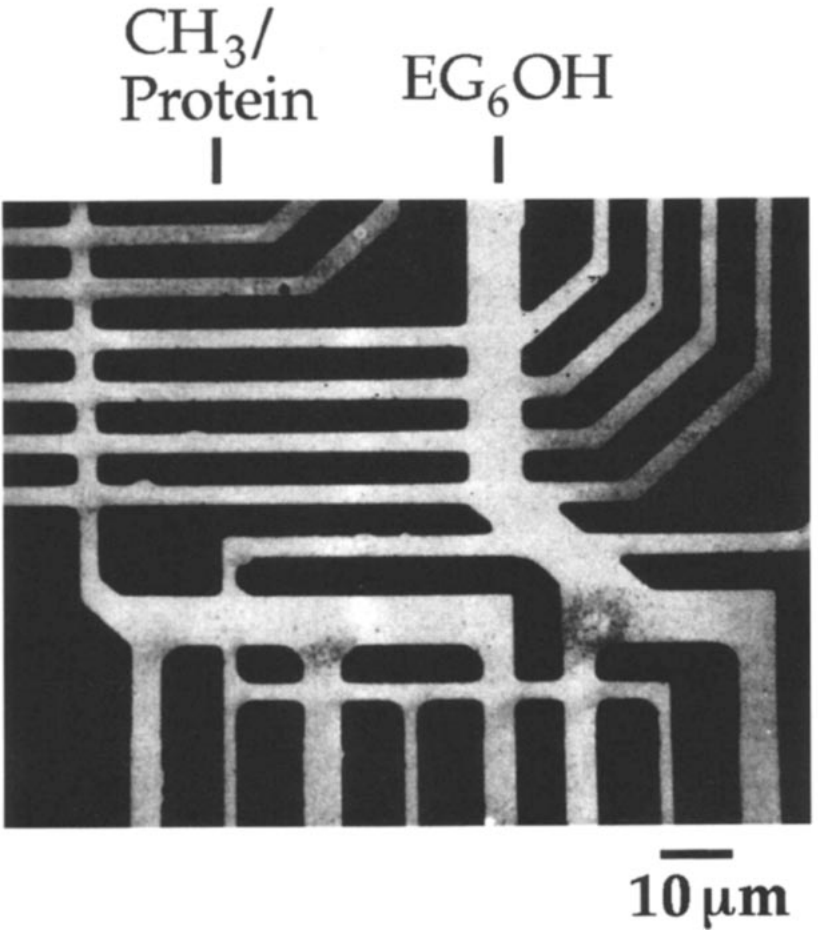

Figure 6 Scanning electron micrograph of fibrinogen adsorbed on a patterned SAM. A patterned hexadecanethiolate SAM on gold was formed by μCP, and the remainder of the surface was derivatized by immersion in a solution containing a hexa(ethylene glycol)-terminated alkanethiol [$HS(CH_2)_{11}(OCH_2CH_2)_6OH$]. The patterned substrate was immersed in a solution of fibrinogen (1 mg/mL) in phosphate-buffered saline for 2 h, removed from solution, rinsed with water, and dried. Fibrinogen adsorbed only to the methyl-terminated regions of the SAM, as illustrated by the dark regions in the micrograph: Secondary electron emission from the underlying gold is attenuated by the protein adlayer.

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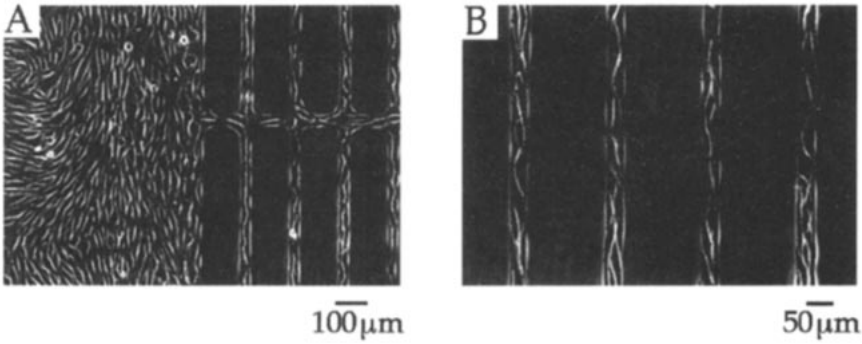

Figure 7 Control over the attachment of bovine capillary endothelial cells to planar substrates that were patterned into regions terminated in methyl groups and tri(ethylene glycol) groups using μCP. The substrates were coated with fibronectin before cell attachment; fibronectin adsorbed only to the regions of methyl-terminated SAM. (*A*) An optical micrograph showing attachment of endothelial cells to a nonpatterned region (*left*) and to lines 30 μm in width. (*B*) A view at higher magnification of cells attached to the lines.

grow on these lines (Figure 7). This technique was also used to pattern SAMs into adhesive regions approximately 20 $\times$ 50 μm in size that were surrounded by EG_6-terminated SAM (66). After the hydrophobic regions were coated with laminin, hepatocytes attached to the rectangular islands and conformed to the shape of the underlying pattern. The size of the islands controlled DNA synthesis, cell growth, and protein secretion of the attached cells. The ability to pattern the attachment of individual cells may be useful for single cell manipulation, toxicology and drug screening.

Attachment of Cells on Contoured Surfaces

Several groups have used surfaces contoured into grooves and ridges, which were fabricated with the use of photo- or electron-beam lithography, to study their effects on the behavior and growth of attached cells (9, 10, 25, 49). Chou et al (9) found that human fibroblasts adherent to surfaces contoured into V-shaped grooves had increased levels of fibronectin synthesis and secretion. Surfaces with arrays of grooves of varying dimensions controlled the alignment and orientation of attached mammalian cells (10, 49). Surfaces with arrays of ridges directed the motility and induced differentiation of the fungus *Uromyces* (25).

A simple technique based on μCP and micromolding (33) was

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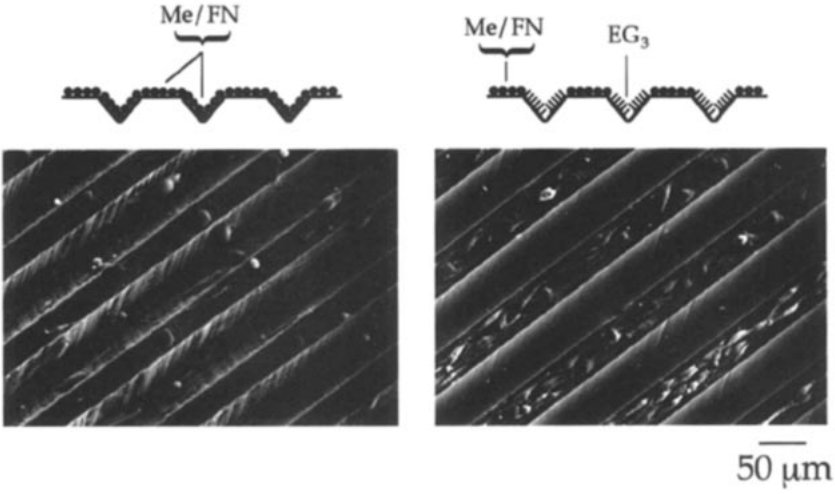

Figure 8 Control over the attachment of endothelial cells to contoured surfaces using SAMs. The substrates are films of polyurethane (supported on glass slides) that were coated with gold and modified with SAMs of alkanethiolates terminated in methyl groups and tri(ethylene glycol) groups; the substrates were coated with fibronectin before cell attachment. (*left*) Cells attached to both the ridges and grooves of substrates that present fibronectin at all regions. (*right*) Cells attached only to the ridges when the grooves were modified with a SAM presenting tri(ethylene glycol) groups.

used to fabricate contoured surfaces of optically transparent films of polyurethane on glass coverslips. After evaporating a thin film of gold onto these substrates, the ridges were derivatized with a SAM by stamping with a flat PDMS stamp; a different SAM was formed in the grooves by immersing the substrate in a solution of alkanethiol. By modifying the ridges with a SAM of hexadecanethiolate, and the grooves with a SAM terminated in oligo(ethylene glycol) groups, endothelial cells were confined to attach and spread only on the ridges (Figure 8). By the reverse process, cells were confined to attach and grow in the grooves.

APPLICATIONS OF SURFACES BASED ON SELF-ASSEMBLED MONOLAYERS IN BIOCHEMISTRY

Drug Design and Screening

Several combinatorial strategies for drug design screen mixtures of potential ligands to identify those with a desired property—usually the ability to bind a protein. Methods that use immobilized libraries have

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the primary advantage that the identity of each ligand is defined by its location in the matrix; amplification and sequencing of selected ligands are not required. Fodor and colleagues (28) used photolithography and solid-phase synthesis to pattern SAMs of siloxanes into arrays of hundreds of different peptides. Confocal microscopy was used to assay the in situ binding of a monoclonal antibody to each of these peptides in a single experiment. This technology also was used to create an array containing 256 octanucleotides that served as a hybridization probe for sequencing DNA. The demonstration of libraries that contain non-natural biopolymers (8), and the potential to screen for reactions at surfaces (78), widens the scope of this technology. Scanning probe microscopies may be useful for screening libraries, because these techniques can assay functionalized surfaces at the sub-micron scale rapidly (39, 80).

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Biosensors

Self-assembled monolayers are finding increasing use to tailor the molecular recognition properties of surfaces used in biosensing. The surface of a TiO_2–SiO_2 waveguide was modified with an alkylsiloxane monolayer terminated in amino groups to which the antibody anti-HBsAg was conjugated; this difference interferometer measured the binding of hepatitis B down to a concentration of 2×10^{-13} M in undiluted serum (62). Self-assembled monolayers have been used in similar ways to control the properties of sensors on the basis of the quartz crystal microbalance (77), acoustic plate modes (31), and surface plasmon resonance (52, 53, 64). Nonspecific adsorption of protein is a common problem with these devices. A commercial technology uses gel layers of dextran to control unwanted adsorption (41), although most applications use bovine serum albumin–coated surfaces. Recent work with ligands immobilized on SAMs terminated in oligo(ethylene glycol) groups provides a route to biospecific surfaces with a high control over the properties of the surfaces (52, 64).

Electrochemical Methods

Self-assembled monolayers can either mediate or inhibit the transfer of electrons from the underlying gold to electrolytes in solution. The properties of SAMs terminated in electroactive groups (e.g. ferrocene or quinone groups) could be switched reversibly by adjusting the potential at the underlying gold (1). Several groups have studied the transfer of electrons from gold to electroactive proteins immobilized to SAMs (11, 82). There is now commercial technology for analysis of biological

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analytes on the basis of electrochemiluminescence from tris-bipyridine ruthenium(II) tags (21): This technology is well suited for SAMs.

PROSPECTS FOR SELF-ASSEMBLED MONOLAYERS IN BIOLOGY

Self-Assembled Monolayers as Model Surfaces

Self-assembled monolayers of alkanethiolates on gold provide the best system available with which to understand interactions of proteins and cells with man-made surfaces. The ease with which complex and delicate groups of the sorts relevant to biochemistry can be presented in controlled environments, combined with simple methods that can pattern the formation of SAMs in the plane of the monolayer, make these surfaces well suited for studies of fundamental aspects of biointerfacial science. Other advantages with this system include the optical transparency of SAMs when supported on thin films of gold, the electrical conductivity of the underlying gold, the compatibility of these substrates with a range of analytical methodologies, the stability of these substrates during storage and in contact with biological media, and the range of surfaces, including curved and nonplanar substrates, that can be used.

Self-Assembled Monolayers in Cell Biology

Designed substrates permit strategies for the noninvasive control over the activity of attached cells. Langer and coworkers (83) cultured endothelial cells on optically transparent films of electrically conducting polypyrrole. Cells attached and spread normally on fibronectin-coated polypyrrole in the oxidized state; on application of a reducing potential, however, the extension of cells and the synthesis of DNA were both inhibited. Okano et al (55) grafted thermoresponsive gels of poly(*N*-isopropylacrylamide on cell culture substrates. Hepatocytes attached to the substrates normally at 37°C; when the culture was chilled to 4°C, the cells detached from the substrate. The features of SAMs described in this review make them well-suited model surfaces for studies in biology that require substrates with tailored properties.

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