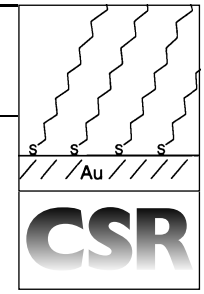
A surface chemistry approach to studying cell adhesion



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Model substrates that present peptide and carbohydrate ligands are becoming important mechanistic tools in cell biology. This review surveys the development of self-assembled monolayers of alkanethiolates on gold as a model substrate for studies of cell adhesion. The review begins with a background that illustrates the opportunity for using tailored substrates in biology and then addresses the characteristics that make monolayers well-suited for these studies. The review concludes with a discussion of recent work that is developing dynamic substrates wherein the activity of immobilized ligands can be modulated in real time.

1 Background

Most mammalian cells are adherent. They must attach to and spread on an underlying matrix in order to carry out normal metabolism, proliferation and differentiation. The biological matrix that serves this role comprises a collection of insoluble proteins and glycoaminoglycans that are collectively referred to as the extracellular matrix (ECM).[1] In addition to maintaining the organization and mechanical properties of tissue, the ECM presents many peptide and carbohydrate ligands that are recognized by cellular receptors. These receptor–ligand interactions are critical to maintaining cell function and enabling cells to respond appropriately to their environments. The primary function of ECM is to mediate the adhesion of cells.[2] Without adhesion, most cells initiate a program of apoptosis that results in their death, while the loss of adhesion-related signal transduction pathways leads to the growth and spreading of cancerous tumors. The study of these and many other interactions between a cell and its matrix is an active area of research in cell biology.

Studies of cell adhesion commonly use glass or polystyrene substrates that are coated with a layer of adsorbed protein. Ligands from ECM that are involved in cell–substrate interactions are often determined by comparing the behaviors of cells on substrates that are coated with distinct domains from a matrix protein, or from mutants of particular domains. The widespread use of these substrates derives in part from the ease with which they can be prepared: the procedure involves applying an aqueous solution of the protein to the culture substrate and allowing the adsorption to proceed over a period of several hours. Protein-coated substrates are also preferred because they retain the range and properties of ligands found in ECM, including the ability to bind other proteins from solution and to be modified by extracellular enzymes. These same substrates, however, have characteristics that limit their utility for mechanistic studies. An understanding of these limitations, which stem from the complex mechanisms by which proteins adsorb to solid surfaces, provides the rationale for employing a surface chemistry approach to developing tailored substrates.

Protein adsorption

There is a vast literature dealing with the adsorption of proteins at solid–liquid interfaces.[3,4] This work has not yet provided a complete molecular level understanding of the adsorption process, but gives several general observations. First, proteins adsorb to essentially all non-natural surfaces. The rate for adsorption is fast—for large proteins it can be near the diffusion limit—and is irreversible on the time scale of routine experiments. Secondly, adsorbed proteins undergo denaturation. The degree of denaturation is difficult to predict since it depends on many variables, including the structures of the protein and surface, and the composition of the solution (pH, ionic strength, temperature). Denaturation also depends on the concentration of protein in solution; on average, lower concentrations are correlated with greater extents of denaturation since the adsorbed protein has a longer time to denature before the surrounding sites are occupied by neighboring proteins.[5] Finally, even under ideal conditions, the layer of protein is typically heterogeneous and the determination of distribution in orientation and structure is essentially impossible.

These characteristics impose many practical limitations on the use of protein-coated substrates in mechanistic cell biology. First, it is difficult to know the density of ligands that are available for binding to cellular receptors—even when the density of adsorbed protein is known precisely—because of the distribution in conformation and orientation of adsorbed protein (Fig. 1). Many studies aimed at investigating the role of ligand density in cell adhesion and migration have improperly assumed a linear correlation between the density of adsorbed protein and

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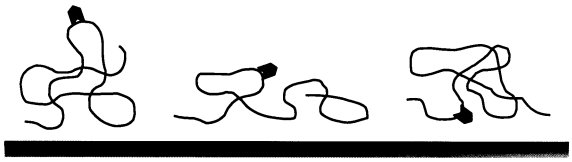

Fig. 1 Substrates that are conditioned for cell culture by allowing protein to adsorb from solution often present proteins in a range of orientations and conformations. Since not all ligands contained within the protein sequence (denoted by the shaded arrow) are available for binding to receptors, it is difficult to characterize or to control the density of a ligand presented to an attached cell.

the concentration of protein used to coat the substrates.[6] The activity of protein-coated substrates can show a dramatic dependence on the choice of substrate. Garcia and coworkers, for example, cultured myoblast cells on two different types of polystyrene substrates. Even though both were coated with comparable densities of fibronectin, cells proliferated on one substrate but differentiated on the other.[7] Adsorbed proteins can also exchange with other proteins present in a contacting solution (or excreted by an attached cell) and lead to changes in the composition of a substrate over time. For this reason it is difficult to control the ligand–receptor interactions between a cell and substrate over longer periods in culture. All of these factors place unavoidable limitations on the interpretation of experiments that use protein-coated substrates. Appropriate model systems that avoid these limitations would be valuable in providing unambiguous information on the role of receptor–ligand interactions that mediate cell adhesion.

Model substrates

Model substrates should have several characteristics if they are to be important in mechanistic cell biology. The substrate should present structurally defined ligands (or proteins) in a homogenous environment at the interface and permit the density of each ligand to be controlled independently. The substrate must resist the non-specific adsorption of protein, so that the immobilized ligands are not obstructed when proteins adsorb, and so the composition of ligands does not change during the course of an experiment. The model substrate should be compatible with the conditions of attached cell culture and with routine methods for the characterization of cells. Finally, methods should be available that can pattern the immobilization of ligands in specific regions or in gradients.

Many classes of substrates have been used as models for ECM, including polymeric resins, single crystalline solids, plasma-treated plastics, supported lipids, and self-assembled monolayers. This short review will focus exclusively on self-assembled monolayers (SAMs) formed by the adsorption of alkanethiols to gold. These substrates are structurally well-defined and allow wide flexibility in attaching and patterning ligands. Many reports over the last five year period have demonstrated that this class of model surfaces has the characteristics required to be broadly useful in mechanistic cell biology.

Self-assembled monolayers

Self-assembled monolayers of alkanethiolates on gold form spontaneously upon immersion of a gold-coated substrate in a solution of alkanethiols. The structure of these monolayers is now well established (Fig. 2).[8,9] The sulfur atoms coordinate to the gold(111) surface to give a densely packed and ordered array of long chain molecules. The properties of the monolayer depend on the functional groups that are exposed at the surface. The primary advantage with this class of model substrate is that a variety of functional groups and molecules can be introduced—either before or after the monolayer is formed—through straightforward synthetic procedures. Monolayers prepared on glass slides coated with a 10 nm film of gold are transparent and compatible with optical and fluorescence microscopies used to characterize cell behavior. These thin films are also electrically conductive and permit the use of electrochemical strategies to modulate the activity of immobilized ligands.

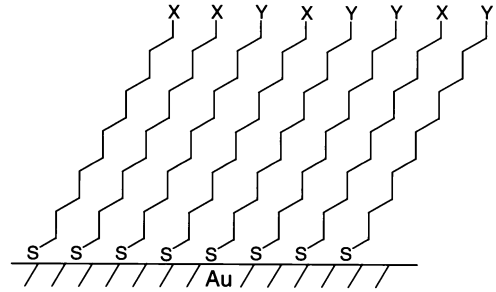

Fig. 2 Representation of the structure of a self-assembled monolayer of alkanethiolates on gold. The sulfur atoms coordinate to the gold and the *trans*-extended alkyl chains present the terminal groups (X,Y) at the interface. The composition of groups presented at the surface, and the densities of these groups, can be controlled by adjusting the ratio of alkanethiols in the solution from which the monolayer assembles.

2 Key features of SAMs

This section describes three characteristics of SAMs that make them well-suited for mechanistic studies in biology. First, monolayers prepared from alkanethiols terminated in oligo(ethylene glycol) groups are inert to the adsorption of protein. Second, the association of proteins with monolayers presenting ligands can be studied using surface plasmon resonance spectroscopy. Finally, simple methods are available to pattern the formation of monolayers for directing the attachment of cells to particular regions of the substrate.

Inert monolayers

It is essential that model substrates prevent the non-specific adsorption of protein because the tendency of proteins to adsorb to non-natural materials would limit the utility of SAMs that present discrete ligands. Not only would the adsorbed protein obstruct the immobilized ligands, but it could also introduce additional ligands on the substrate. Prime and Whitesides found that monolayers prepared from alkanethiols terminated in short oligomers of the ethylene glycol group ($HS(CH_2)_{11}(OCH_2CH_2)_nOH$; n = 3–7) were highly effective at resisting the adsorption of protein.[10] These monolayers appear to provide a general solution to controlling non-specific adsorption; they prevent the adsorption of proteins having a range of MW and pI, and under a wide range of solution compositions.[11,12] The mechanisms underlying this resistance are not completely clear. Recent studies suggest that the structure of the glycol groups—they can assume either helical or *trans*-extended conformations—and the degree of solvation of the chains are important. Indeed, the characterization of factors that correlate with protein resistance, and the development of other inert surface chemistries (particularly since PEG has the limitation that it oxidizes over time) remain important areas of research in surface chemistry.

Surface plasmon resonance spectroscopy

The characterization of the interactions (or lack thereof) of proteins with surfaces requires sensitive analytical tools that can

measure the rates and amounts of association. Surface plasmon resonance (SPR) spectroscopy is inherently well suited for these measurements, since it is an optical technique that measures changes in the refractive index of a solution near a thin gold film.[13] Because SPR is an *in situ* technique, it provides kinetic data (with a resolution of 1 Hz) and can measure changes in the amount of adsorbed protein as the composition of the contacting fluid is changed. This technique also has excellent sensitivity; it is capable of detecting adsorbed protein at a density of 5 pg mm^{-2}. A commercial instrument for performing SPR is available from Biacore which makes this technique accessible to most research groups. The instrument can accept substrates other than those sold by the manufacturer and it offers excellent microfluidic manipulation under the control of a straightforward software interface. The use of SPR to measure the association of proteins with immobilized ligands will be described in a later section.

Patterned substrates

By patterning substrates into regions that alternately promote or prevent the adsorption of protein, the attachment and spreading of cells can be controlled. Several methods have been used to pattern substrates for cell attachment. They differ in the choice of methods to pattern the surface chemistry (usually on a scale of tens of microns) and of methods to modify the surface properties to either permit or prevent protein adsorption.[14] A strategy based on contact printing of SAMs is now the method of choice for patterning cells (Fig. 3).[15,16] This method uses an elastomeric stamp to print a pattern of alkanethiolates on a gold film. The stamp is often prepared by casting polydimethylsiloxane against a photolithographic master, and is 'inked' by wetting the face with a cotton swab. The method works best when the ink is a methyl-terminated alkanethiol. After the printing step, the substrate can be immersed in a solution of a second alkanethiol to define the surface properties in the remaining regions of gold. When the second alkanethiol is an oligo(ethylene glycol) terminated thiol, the resulting substrates will permit cells to attach only to the printed regions. Cells attach best to the patterned substrates after they have been immersed in a solution of ECM protein to introduce matrix on the printed regions. Fig. 3 shows a micrograph of capillary endothelial cells attached to a patterned substrate. This method has been instrumental in studies of the relationship between cell survival and morphology.[17,18] This method is also finding use in drug discovery and diagnostic assays to position cells in predetermined locations on chip substrates.

3 Biospecific recognition of protein

The attachment of low molecular weight ligands to SAMs terminated in oligo(ethylene glycol) groups gives substrates with which proteins can associate biospecifically—by way of receptor–ligand interactions—but that prevent the nonspecific association of other proteins. The following section describes examples that illustrate the association of proteins with immobilized ligands, the action of enzymes on immobilized molecules and the attachment of cells to substrates presenting peptides found in ECM.

Biospecific recognition

An early example of biospecific recognition investigated the association of the protein carbonic anhydrase with a mixed SAM presenting benzenesulfonamide ligands and glycol groups.[19] SPR was used to show that the protein bound to ligands on the monolayer and that the association was reversible

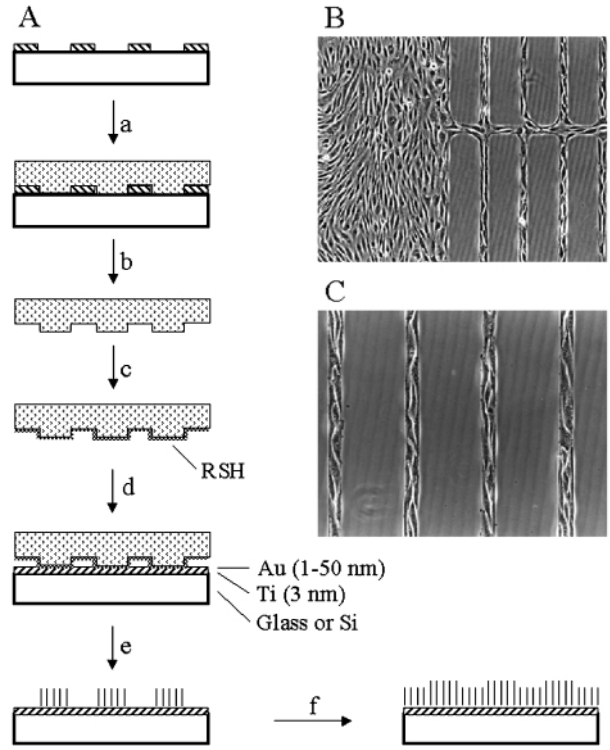

Fig. 3 (A) Microcontact printing is a convenient and simple method for patterning monolayers. The method begins with a master mold used to cast an elastomeric stamp made of polydimethylsiloxane (PDMS, a). The stamp is removed from the master (b), inked with a solution of alkanethiol (c) and brought into contact with a gold-coated substrate (d). The stamp is left in place for thirty seconds and on removal gives a substrate having a pattern of monolayer only in the regions of contact between the stamp and substrate (e). Immersion of the substrate in a solution of a second alkanethiol results in the formation of a different monolayer in the remaining regions of gold (f). (B) An optical micrograph of capillary endothelial cells attached to a monolayer that was patterned into regions terminated in methyl groups (to which cells attached) and hexa(ethylene glycol) groups (which prevented the attachment of cells). (C) A micrograph at higher resolution shows the confinement of cell spreading to the pattern of underlying monolayer.

when buffer was flowed over the substrate. The maximum density of protein that associated with the monolayer depended on the density of ligand in the monolayer. The association of protein was biospecific: it was inhibited by the addition of a soluble benzenesulfonamide to the solution and the substrates prevented the non-specific adsorption of several other proteins. Because these experiments provided rate constants for both association and dissociation of the protein, equilibrium binding constants could be determined and compared with binding of soluble ligand. In this example, the association constant for binding of protein to immobilized ligand was smaller by a factor of five relative to that for binding a soluble ligand. This same strategy has been used successfully to prepare monolayers for the recognition of immobilized D-Ala-D-Ala by vancomycin,[20] of Ni(II) by His-tagged proteins[21] and of biotin by streptavidin.[22,23] In a related example, Lowe and coworkers have immobilized protein immunoglobulins to monolayers to create immunosensors.[24] These examples establish a general strategy for preparing monolayers with which proteins can specifically associate, and serve as the basis for preparing model substrates for cell adhesion discussed below.

Enzymatic modification of substrate

Monolayers can also be used to investigate the processes by which cells modify the ECM with which they interact. One example for which we have designed model substrates comes from the migration of cells on surfaces coated with the ECM protein laminin. The migration is facilitated by the interaction of the cell surface enzyme galactosyltransferase (GalTase) with

the carbohydrate *N*-acetylglucosamine of the matrix, but it is not known whether the enzyme serves only as a receptor for the carbohydrate or whether the modification of the substrate is important. Because lectin proteins are available that specifically bind either GlcNAc or LacNAc (the product of the enzymatic reaction), SPR could be used to probe the presence of the carbohydrates on the surface and characterize the enzymatic glycosylation (Fig. 4).[25] The *BS-II* lectin bound specifically to a monolayer presenting GlcNAc groups, while the *E. cristagalli* lectin (which is specific for the disaccharide) displayed no binding. After the substrate was treated with GalTase and UDP-Gal, SPR showed that only the *E. cristagalli* bound to the substrates, and that all of the carbohydrate had been enzymatically glycosylated. This work also found that the yield of the glycosylation—which is a measure of the biological activity of the immobilized carbohydrate—depended on the density at which GlcNAc was immobilized. The amount of incorporation of galactose increased linearly with the density of carbohydrate up to a density of 70%, and then decreased sharply for higher densities (Fig. 4C). This observation demonstrates that the activity of an immobilized ligand can be compromised when it is immobilized at high densities, and is an important consideration in designing model substrates.

Cell adhesion

The adhesion of most cells to ECM is mediated by a class of cell surface receptors known as the integrins.[26] Integrins comprise a family of about twenty heterodimeric proteins that are involved in regulating many aspects of cell behavior, including adhesion, migration, proliferation and differentiation. Studies that address the role of distinct integrin receptors are complicated because most cells present several types of integrins on their surfaces and because the ECM contains many ligands for the integrins (including several ligands that have not yet been identified). The use of model substrates that present only a single ligand (or a small group of ligands) would be valuable for interpreting the roles of these interactions. This strategy has been most successful for investigating the tripeptide Arg-Gly-Asp, which is found in fibronectin and is a ligand for several integrin receptors. Much previous work has shown that derivatizing materials with this peptide enhances the attachment and growth of cells.[27,28] Many of these early substrates have the limitation that they do not simultaneously prevent the adsorption of protein. Hence they do not permit unambiguous control over the interactions between cell and substrate that influence cell behavior and highlight the need for surface chemistries that permit biospecific association of protein but prevent non-specific adsorption of proteins.

Monolayers that present peptide ligands mixed with oligo(ethylene glycol) groups meet these requirements. We studied the adhesion of Swiss 3T3 cells on monolayers prepared from a mixture of alkanethiols terminated in the peptide Gly-Arg-Gly-Asp-Ser and the tri(ethylene glycol) group.[29,30] For monolayers presenting peptide at densities of 0.01 to 1.0% (relative to total alkanethiol) cells attached and spread efficiently (Fig. 5). Immunostaining showed that the adherent cells assembled normal focal adhesion complexes—a cluster of integrin receptors that forms strong attachments to the substrate and initiates adhesion signals—and actin stress filaments. For monolayers presenting ligand at lower densities, attachment was less efficient and the attached cells spread to a lesser degree. Many reports that addressed the role of ligand density in cell adhesion found similar trends in adhesion and spreading with density, but the absolute densities that were required for attachment or spreading varied. We showed that the environment in which the peptide is presented is also an important factor in adhesion. Cells attached and spread efficiently on monolayers that presented the peptide at a density of 0.5% mixed with tri(ethylene glycol) groups; when the peptide was presented at the same density but mixed with hexa(ethylene

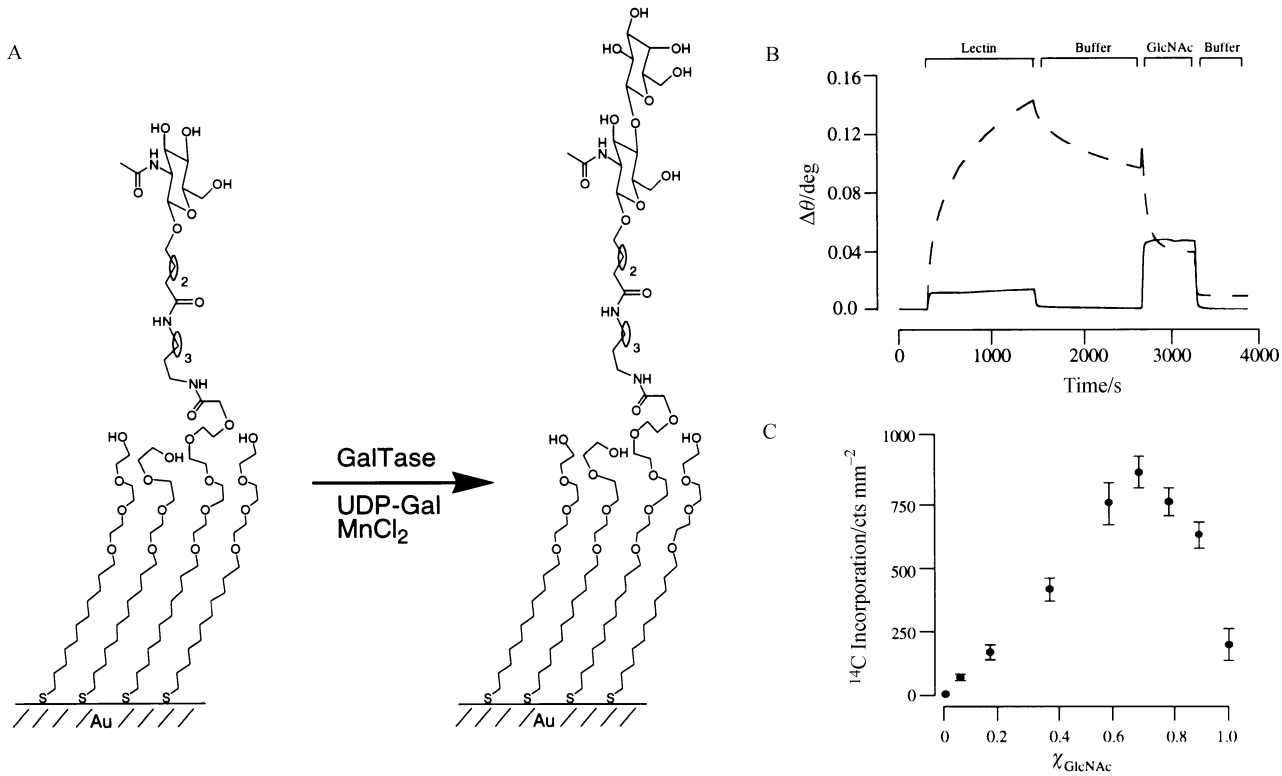

Fig. 4 (A) Model substrate that presents the carbohydrate *N*-acetylglucosamine (GlcNAc) for mechanistic studies of the galactosyltransferase (GalTase) mediated glycosylation to give the disaccharide (LacNAc). (B) Surface plasmon resonance spectroscopy was used in combination with carbohydrate-binding lectins to determine the amount of each carbohydrate on the monolayer. The data show that the *BS-II* lectin (dash line) but not the *E. cristagalli* lectin (solid line) associated with monolayers presenting GlcNAc groups. (C) An investigation of the amount of glycosylation (determined by using a radiolabeled UDP-Gal) for monolayers presenting GlcNAc at different densities showed that at high densities steric crowding of the carbohydrates inhibited enzyme–substrate interactions.

glycol) groups, fewer cells attached and those that did remained in a rounded morphology. For all these experiments, cells that were cultured on the substrates for 24 hours could be detached by the addition of a soluble Arg-Gly-Asp peptide, demonstrating that the interactions of cells with the substrates were mediated entirely by the peptide ligand.

These early studies validate the use of SAMs that present ligands for mechanistic studies of adhesion-dependent cell behavior. With these monolayers, the interactions between a substrate and cell can be defined completely and maintained over days in culture. This property will be important for determining the influences that other ligands in ECM have on attached cells. A current example comes from studies of the spreading of baby hamster kidney cells on fibronectin.[31] By comparing adhesion of cells on substrates coated with fragments and mutants of fibronectin, it was found that Arg-Gly-Asp alone is insufficient for complete spreading of cells, but that the presence of a second peptide, Pro-His-Ser-Arg-Asn, in an adjacent domain permits cells to attach and spread on the substrate. The use of protein-coated substrates makes it difficult to make quantitative comparisons of the adhesions on the two substrates (for the reasons indicated in Fig. 1). This problem highlights the opportunity to use model substrates that present peptides at controlled densities for mechanistic studies of adhesion. Indeed, many other peptide ligands have been suggested to play a role in adhesion, and the monolayers described here would be valuable in determining the properties of these candidate ligands. In addition to characterizing the properties of candidate ligands, SAMs may find use in rapidly identifying new ligands. This combinatorial screen could be performed with substrates that are patterned into hundreds of regions, each of which presents a single peptide ligand at a constant density.

4 Dynamic substrates

This section discusses the development of dynamic substrates, wherein the activity of immobilized ligands can be modulated in real time. Such substrates would provide new opportunities for mechanistic studies of the pathways by which cells respond to changes in their environments. Dynamic changes in ECM influence cell behavior in many important contexts, including migration and differentiation of cells during development and the metastasis of tumor cells. But studies of these processes are difficult due to the lack of methods that can unambiguously change the properties of ECM underlying a cell.

The development of dynamic substrates begins with the design of chemical strategies that can alter the composition of active ligands on a substrate. These changes can be caused by isomerization of an immobilized ligand between a structure that is active and one that is inactive. Alternatively, a ligand can be 'turned on' by activating the substrate for immobilization of

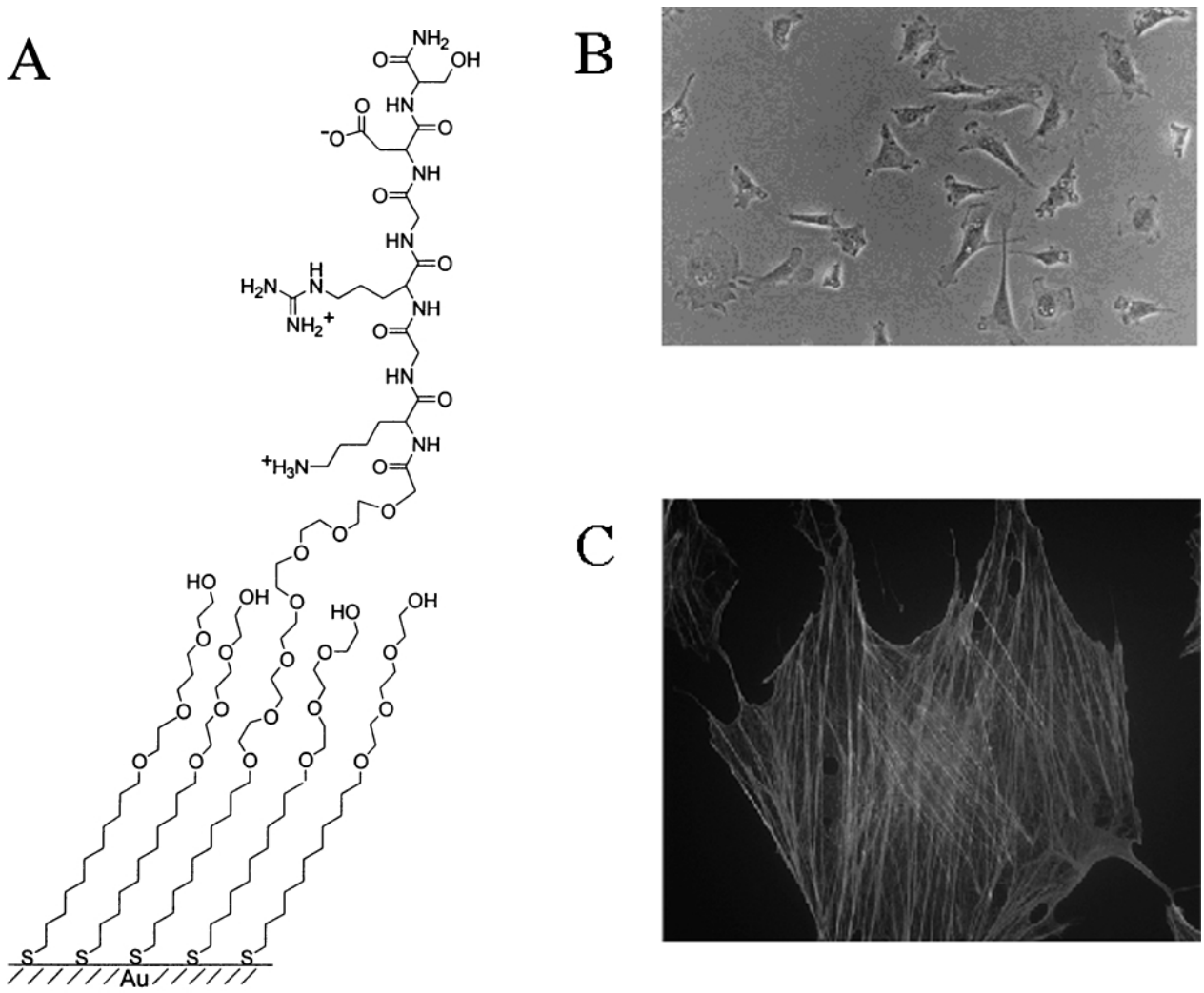

Fig. 5 (A) Structure of a monolayer that presents the peptide Gly-Arg-Gly-Asp-Ser mixed with tri(ethylene glycol) groups. (B) Optical micrograph of 3T3 fibroblasts attached to a monolayer wherein 0.5% of the alkanethiolates present the peptide ligand. (C) Fluorescent micrograph of a cell that was adherent on these monolayers for five hours, fixed and stained with phalloidin–rhodamine to visualize actin stress filaments. The cells assembled stress fibers that were characteristic of those found in cells adhered to fibronectin.

ligand from solution and, by analogy, 'turned off' by releasing it from the substrate. Each of these strategies relies on a chemical reaction at the interface. Because the substrates must be modulated in real time, the reactions should be initiated by non-invasive means, using either photochemical or electrochemical stimuli.

Substrates that immobilize ligands

We developed an electroactive substrate that can turn on the selective immobilization of ligands. The design takes advantage of the ability to carry out electrochemical conversions of molecules attached to the monolayers by applying electrical potentials to the underlying gold. The strategy is based on the Diels–Alder reaction of a substituted cyclopentadiene with benzoquinone attached to the SAM.[32] The reaction of these two molecules is rapid and selective and gives a covalent attachment of ligand. The reaction proceeds with well-behaved second order kinetics and therefore gives excellent control over the density of immobilized ligand, even at low densities where direct characterization is not feasible. The reactivity of the monolayer can be turned off by electrochemical reduction that converts the quinone to the corresponding hydroquinone, which is unreactive towards the diene. Because the oxidation of hydroquinone is reversible over many cycles, it is possible to repeatedly turn the reactivity of the monolayer on and off. We have used this chemistry to demonstrate the immobilization of biotin for recognition by the protein streptavidin and of the peptide RGD for the attachment of cells (Fig. 6).[32]

Substrates that release groups

A similar strategy can be employed to design redox active groups that can selectively release attached ligands from a monolayer. The alkanethiol requires a molecule that undergoes oxidation (or reduction) to give an intermediate that then rearranges to release a substituent that tethers the ligand. We demonstrated one strategy that used a catechol orthoformate group as the electroactive linker that tethered the ligand to the SAM.[33] Electrochemical oxidation of this group occurred at a potential of 850 mV (*versus* Ag/AgCl reference) and gave the corresponding orthoquinone with concomitant hydrolysis, and release, of the formate substituent. The conversion was essentially complete on a single cyclic voltammetric scan showing that it was rapid and efficient. This reaction, however, was not suited for the design of culture substrates that could release ligands because application of potentials greater than 600 mV (or greater than −750 mV for reductions) compromises the ability of glycol-terminated monolayers to prevent protein adsorption. Clearly, the development of reactive groups for incorporation into dynamic SAMs must meet several requirements to be competent for studies involving attached cell culture.

Substrates that isomerize ligands

Willner and coworkers developed a class of photoactive substrates that modulate the recognition of a dinitrophenyl antigen by an IgG antibody.[34] These workers immobilized a dinitrophenyl spiropyran on a gold film. When the phenol oxygen was bound in the spiropyran, the aromatic antigen was not available to bind antibody. Illumination of the spiropyran with light at 370 nm resulted in the isomerization of an olefin and ring opening of the pyran to reveal the dinitrophenyl group which was recognized by an antibody. Subsequent illumination of this substrate at 500 nm returned the phenol to the spiropyran and eliminated binding of the antigen. Because this photoisomerization is reversible, the activity of the ligand could be alternately turned on and off many times.

These three examples represent the level of control that can be applied to the preparation of active substrates. Each of these substrates was *designed* from first principles to exhibit the particular property. The combination of physical organic principles and synthetic chemistry makes possible the molecular engineering of substrates that have a range of other properties.

5 Conclusions and outlook

This review illustrates the motivation and rationale for using self-assembled monolayers as model substrates for studies in experimental cell biology. SAMs have several characteristics that make them practical and important tools for a broad range of studies. They are easily prepared in ordinary laboratories from alkanethiols and gold-coated substrates. The peptide-terminated alkanethiols are now accessible by a straightforward solid-phase synthesis[29] but the gold-coated substrates still require access to metal evaporators. The most important characteristic of these monolayers is the availability of surfaces

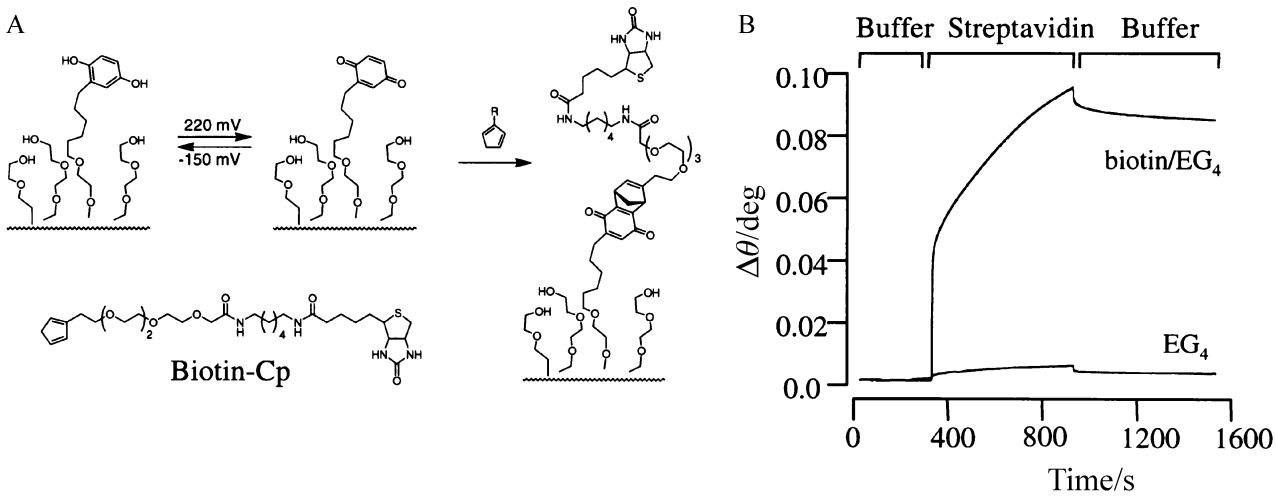

Fig. 6 Illustration of a dynamic substrate that can turn on the immobilization of a biotin ligand and permit the protein streptavidin to associate with the monolayer. (A) Reversible electrochemical oxidation of a monolayer that presents hydroquinone mixed with oligo(ethylene glycol) groups affords a monolayer that presents the quinone group. The quinone in turn reacts with a conjugate of cyclopentadiene and biotin (Biotin–Cp) to give a covalent Diels–Alder adduct. (B) SPR showed that streptavidin associated with a monolayer that had been activated in this way (curve for biotin/EG_4) but not to monolayers that presented only oligo(ethylene glycol) groups (EG_4) or to monolayers that had not been activated by conversion to the quinone prior to treatment with the conjugate.

that are inert to the adsorption of protein because they provide a route to installing biospecific interactions at the interface. These monolayers also have the stability and properties required to be used in cell culture. Microcontact printing provides a reliable and simple method for patterning substrates to control the shapes, sizes and positions of adherent cells. The ability to assemble monolayers from alkanethiols terminated in a wide variety of groups and to modify the groups after the monolayers have assembled makes possible the design of monolayers having tailored properties, including those that can modulate the activities of ligands that interact with receptors of an attached cell.

The examples described in this review illustrate early ways in which SAMs are proving useful as model substrates in cell biology. This approach will be valuable for countless other studies that elucidate the interactions of cellular receptors with extracellular matrix that underlie cell proliferation, migration and differentiation. The use of patterned substrates—including those that position several different cell types on a common substrate—will provide new opportunities to study the mechanisms by which cells influence one another, including for example, in the course of development and formation of tissues and organs. New methods that can immobilize ligands in gradients will be valuable for studies of cell polarization and migration.[35]

These characteristics that make SAMs of alkanethiolates on gold excellent substrates for mechanistic cell biology also make them important in biotechnology. Assays for screening drug candidates in discovery programs and for analyzing samples in medical diagnostics are increasingly using engineered cells as the sensing element.[36,37] These efforts have emphasized the need to control the surface chemistry to ensure the viability of cells and the reproducibility of data generated in the assays. With the miniaturization of the assay formats there is a need to pattern the locations of cells to prevent them from migrating and proliferating on the substrate. The engineering community has a growing interest in microfabricated devices that join cellular components with sophisticated integrated circuits that are now available. These devices—which are collectively referred to as bio-microelectromechanical systems—will require active surface chemistries that can interface the biological functions of a cell with electrical processes in the device and may find use as new sensors and as implantable neural prostheses.

Each of these research areas is at an early stage of development and is certain to witness significant growth during the next decade. Surface chemists will play an important role in this emerging field by providing the molecular level engineering to design the substrates, developing synthetic approaches to build the substrates and applying a range of analytical and physical methods to characterize the structures and properties of the substrates. The combined efforts of surface chemists, cell biologists and engineers will surely make exciting contributions to fundamental biology and to biotechnology.

6 Acknowledgements

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