What can surface chemistry do for cell biology?

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Recent research has enhanced the development of substrates that serve as models of extracellular matrix and their use in studies of cell adhesion and migration. Advances include the development of methods to prepare substrates having ligands immobilized in controlled densities and patterns, and recent work that is developing dynamic substrates which can modulate, in real-time, the activities of ligands. These technologies are providing new opportunities for studies of cell–extracellular-matrix interactions.

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Current Opinion in Chemical Biology 2002, 6:794–797

1367-5931/02/$ – see front matter

Published online 18 October 2002

Abbreviations
ECM extracellular matrix
SAM Self-assembled monolayer

Introduction

Most cells are adherent and remain connected to a protein scaffold during growth, division, differentiation and even death. This scaffold comprises an insoluble aggregate of several large proteins and glycosoaminoglycans — collectively known as the extracellular matrix (ECM) — that provides a three-dimensional environment which organizes cells into tissue [1,2]. The adhesion of cells to the ECM and a host of subsequent signaling events are mediated by specific interactions between cell-surface receptors and ligands of the matrix. The study of these interactions represents an important and active theme in molecular cell biology [3••,4].

The interactions of cells with ECM play important roles in many areas of biology, and several sub-fields have emerged according to the receptor type or cell type that is involved. In all cases, experimental studies usually begin with the aim of identifying matrix-derived ligands and their counterpart cell-surface receptors, and then extend to the characterization of the signal transduction processes that follow these interactions. An emerging theme in matrix biology is addressing roles for dynamic interactions between cells and ECM. The mechanisms and biological roles for cellular remodeling of ECM, however, are still at an early stage of understanding [5].

Experimental studies of ligand–receptor interactions that are involved in cell–ECM interactions are substantially more difficult than are studies of recognition in solution. The traditional approach prepares substrates by treating plastic substrates with a solution of matrix protein, to give a rapid and essentially irreversible adsorption of a single layer of protein. Although this method is experimentally simple and provides good control over the composition of protein on the substrate, it offers poor control over the presentation of active ligands to a cell. The difficulty arises because proteins adsorb to surfaces with a range of orientations and often undergo denaturation at the interface [6]. Both of these factors render a fraction of immobilized ligands inactive for subsequent interactions with cells. A further limitation arises because immobilized proteins can exchange with proteins in a contacting fluid, and therefore introduce additional ligands on the substrate [7].

These limitations have motivated a surface chemistry approach to develop substrates having a defined set of immobilized ligands and which therefore provide complete control over the ligand–receptor interactions [8,9]. Model substrates would avoid many of the ambiguities that are attached to current methods and prove enormously useful for mechanistic studies of ECM in biology. In this short review, I outline recent developments in three key areas of model substrates. The first addresses strategies to immobilize ligands to substrates with control over their activities and densities. The second theme addresses methods to pattern the immobilization of ligands and to use patterned substrates in experimental biology. The third and most recent theme addresses the development of dynamic substrates that can alter, in real-time, the presentation of ligands to a cell and therefore mimic the changes in cell–ECM interactions that govern a wide range of activities.

Immobilizing ligands for biospecific interactions

The development of strategies to tailor substrates with ligands for controlling cell-surface interactions must address two challenges. The first arises from the tendency of proteins to rapidly and non-specifically adsorb to most man-made materials. The unwanted adsorption of protein not only blocks interactions with immobilized ligands, but can also introduce additional ligands that mediate cell interactions (particularly as cells remodel their matrices). It is therefore necessary to identify or develop surfaces that are intrinsically inert — in that they prevent non-specific protein adsorption and therefore cell attachment — and serve as platforms for the immobilization of ligands. Polymeric hydrogels derived from poly(ethylene glycol) are the most common approach, and have been used in a variety of settings to minimize unwanted adsorption [10]. Self-assembled monolayers (SAMs) that are terminated in short oligomers of the ethylene glycol unit have been especially effective at controlling protein adsorption and are now the most important tool for bioanalytical applications [11,12]. Surprisingly, there has been relatively little work to develop new materials that are inert. In one example,

Whitesides and co-workers [13••] surveyed a broad range of functional groups and identified several that are inert under certain conditions. In another example, we found that monolayers presenting the mannitol group are highly effective at preventing protein adsorption, and were stable under conditions of cell culture for one month [14]. Although a mechanistic understanding of the factors that are important to inert surfaces is still lacking, these early reports do suggest that there are still many opportunities for designing and enhancing inert surfaces.

The second challenge in applying model substrates to cell biology stems from the non-uniform activities often displayed by immobilized ligands [15••]. In some cases, the variability results from the use of non-regioselective methods to immobilize ligands. In many other cases, however, it is due to the range of microenvironments in which ligands are found. When using polymer gels as the substrate, for example, a fraction of the ligands will be displayed at the surface, and hence may have a higher affinity for a receptor than do ligands which are internal to the gel. The use of substrates that present ligands having non-uniform activity makes it difficult to quantitatively interpret results from experiments. SAMs of alkanethiolates currently offer the best option for controlling the presentation of ligands. These substrates are structurally well-defined and offer wide synthetic flexibility in tailoring the environments about the ligands. In one example, we prepared a series of substrates that presented the peptide Arg–Gly–Asp — a ligand for the integrin receptors that mediate cell adhesion — at a uniform density but on linkers having varying length from the substrate [16]. We found that the binding affinity of peptide for receptor did decrease as the peptide was made less accessible, and that even modest changes in affinity resulted in substantial effects on cell adhesion and spreading. In biochip applications, we have demonstrated that the uniform activity of ligands attached to SAMs permits quantitative assays of enzymatic and protein binding activities [17••,18]. The issue of quantitative control over ligand activity will increase in importance for mechanistic studies of cell adhesion.

Controlling density

Strategies that permit rigorous control over the density of immobilized ligands have a special relevance in cell biology, where many signaling events stem from clustering of receptors at the cell surface. Unlike soluble signaling molecules — which mediate their effects through monomeric or dimeric membrane-bound receptors — immobilized ligands often signal by clustering multiple receptors (and, often, multiple receptor types). Several activities have been found to display a strong dependence on ligand density. In one example, Kahne and co-workers studied the binding of lectins to monolayers presenting carbohydrate ligands and found that the binding specificity of *Bauhinia purpurea* lectin switched from one carbohydrate ligand to another as the density was increased over a 10-fold range. The authors suggested that a secondary interaction between carbohydrates and the lectin promoted the switch in specificity [19]. An example that illustrates the complexities that can underlie cell-surface signaling was provided by Gestwicki and Kiessling [20] in a study of bacterial chemotaxis. This group used polyvalent ligands — which have analogy to clustered ligands presented on a surface — to illustrate that multiple receptors are involved in regulating the response of cells to gradients of signaling molecules.

A related goal is the development of methods that can prepare substrates wherein ligands are immobilized in a gradient. Many cellular activities in developmental and regenerative biology are directed by gradients of signaling molecules in the proximal matrix. Mechanistic studies of these problems have not been practical, but significant recent work is providing new tools for generating stable gradients over the length scale of individual cells. Whitesides and co-workers, for example, demonstrated the use of laminar flow in microfluidic devices to systematically vary the concentration of a protein in a fluid to give gradients. In one example, this group characterized the migration of neutrophils in gradients of interleukin-8 [21]. In a different approach, Distefano and co-workers used a photochemical method to immobilize cell-adhesive peptides in a gradient. Although this example did not use inert surfaces, analogous methods will provide well-controlled routes to a variety of immobilized gradients [22].

Patterning cells

Several methods have been described over the past 10 years for patterning the immobilization of ligands or proteins, and consequently the attachment of cells [23]. Each of these methods combines a means of imposing a pattern on the substrate with a means of modifying surface properties so that one region of the substrate promotes cell attachment and the other region prevents adhesion. The past two years have seen a substantial increase in programs that use patterned substrates in cell biology. Healy and co-workers [24] used patterned substrates to investigate gene expression in the differentiation of bone cells. Nelson and Chen [25] used patterned substrates to confine pairs of contacting cells and demonstrated a novel proliferative signal that stems from a direct cell–cell contact. These approaches are certain to grow in importance and will ultimately find common use in the biological sciences.

A recent theme in patterning has been the development of methods that can control the positions of ligands at a submicron resolution. Substrates having ligands patterned at the nanoscale would enable studies of a whole class of mechanistic issues related to receptor clustering. Focal adhesions, for example, assemble when ligated integrin receptors cluster into large aggregates within the membrane [26]. The clustered receptors recruit the assembly of a large complex that regulates signaling pathways and provides the anchoring points for the cellular cytoskeleton. Because the sizes of focal adhesions range from 50 nm to 500 nm, existing patterning methods could not be used to explore

the relationships of focal adhesion size and distribution on cell adhesion. Griffith and Mayes recently prepared substrates by self-assembly of diblock copolymers wherein one block was derivatized with the RGD peptide. The resulting substrates presented RGD in an ordered array and were used to show that cell adhesion and signaling did indeed depend on sizes of the nanopatterns and the density of peptide within those patterns [27]. This method takes advantage of pattern formation to prepare nanometre-scale features on the substrate, but it does not permit flexibility in defining non-periodic patterns. To address this need, we applied dip-pen nanolithography to directly pattern the formation of monolayers into nanometre-scale islands that adsorb protein and surrounding areas that are strictly inert [28]. This method effectively extends patterning to a scale of 30 nm. There is still a need, however, for patterning methods that offer better control in defining the ligands and controlling their densities.

Dynamic substrates

The interface between a cell and the protein matrix environment is highly dynamic. Cells release metalloproteases that degrade the matrix and continuously excrete newly synthesized proteins to remodel the matrix. Signaling proteins can selectively bind to matrix proteins, thereby presenting new ligands that interact with cells [2]. The mechanical stretching of matrix can unfold proteins and present hidden ligands to a cell [29]. In total, these activities make the ECM an important site of cell regulation and not just a static scaffold for organizing cells into tissue. Yet, efforts to study the roles for dynamic cell–ECM interactions are still in need of new experimental tools. Very recent work is addressing the development of dynamic substrates that allow the character of the matrix to be switched in real-time.

Modulating protein adsorption

One strategy takes advantage of thermally induced phase transitions in certain polymers. Okano and co-workers, for example, prepared substrates that were patterned with poly(*N*-isopropyl acrylamide). At 37°C, this polymer is in a collapsed, solvent-excluded structure that is conducive to cell culture, but on cooling below 32°C, the polymer undergoes a phase transition to yield a swollen structure that is inert to cell adhesion. Hence, removal of a cell culture from an incubator results in release of cells only from the patterned regions, which can then be seeded with a second cell type to generate patterned cocultures [30••]. The development of methods that can pattern co-cultures will prove very significant to studies of heterotypic cell–cell interactions. Chilkoti [31] is developing a class of elastin-like polypeptides that undergo similar phase transitions and may offer new opportunities for creating dynamic substrates.

Modulating ligand activity

My group has developed strategies for preparing substrates wherein the activities of discrete ligands can be modulated. This work uses SAMs presenting electroactive moieties that are designed to activate or inactivate immobilized ligands when electrical potentials are applied. One example used a Diels–Alder reaction between an immobilized benzoquinone group and a cyclopentadiene-ligand conjugate in solution to turn on the migration of patterned cells [32••]. This active substrate has also been applied to a method for patterning the attachment of multiple cell types (by sequentially turning on regions of the substrate for attachment of each cell type) [33]. A second example employed substrates that presented ligands by way of an electroactive linker that is selectively cleaved on application of electrical potentials. This strategy was used to selectively release fibroblast cells from a patterned substrate [34].

Biochemically responsive substrates

Hubbell has pioneered a class of polymeric materials that respond to cellular processes for remodeling the ECM [35••]. One example uses fibrin-based matrices that are modified with peptide ligands which mediate cell adhesion and with peptide substrates for cellular proteases. In this way, the release of proteases by resident cells results in remodeling of the substrate. These strategies have been extended to introduce binding sites for growth factors and other signaling molecules within the synthetic matrix. These strategies are significant because they increase the complexity of model substrates and allow a sophisticated interplay of cellular and matrix processes.

Conclusions

The intrinsic complexity of ECM makes studies of cell adhesion, migration and other processes difficult. A portfolio of methods from surface chemistry are well-suited to designing model substrates wherein the ligand–receptor interactions between cell and substrate are well-defined. Several recent examples illustrate the importance of model substrates in biology and the development and application of surface chemistries is certain to accelerate. In particular, I believe that model substrates — and especially the dynamic substrates — will be central to addressing the roles for focal adhesions and the dynamic processes by which focal adhesions assemble and evolve [36,37].

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