Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Current Opinion in
Colloid & Interface Science

Dynamic substrates for cell biology

Pradeep Bugga and Milan Mrksich

Abstract
The interactions of adherent cells with their insoluble extracellular matrices are complex and challenging to study in the laboratory. Approaches from interface science have been important to preparing models of the biological matrix wherein discrete ligands are immobilized and interact with cellular receptors. A recent theme has been to develop dynamic substrates, where the activities of immobilized ligands can be modulated in real-time during cell culture. This short opinion reviews the strategies to manipulate ligand activity, highlights recent work that has advanced the field and discusses the applications that have been enabled. This work suggests that dynamic substrates will continue to find important uses in basic and applied biointerfaces.

Addresses
Department of Chemistry and Biomedical Engineering, Northwestern University, Evanston, IL, 60208, United States

Corresponding author: Mrksich, Milan (milan.mrksich@northwestern.edu)

Current Opinion in Colloid & Interface Science 2018, **38**:80–87

This review comes from a themed issue on **Biological Colloids and Interfaces**

Edited by **Martin Malmsten** and **Stefan Zauscher**

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.cocis.2018.09.003

1359-0294/© 2018 Elsevier Ltd. All rights reserved.

Keywords
Biointerfaces, Self-assembled monolayer, Cell patterning, Cell migration.

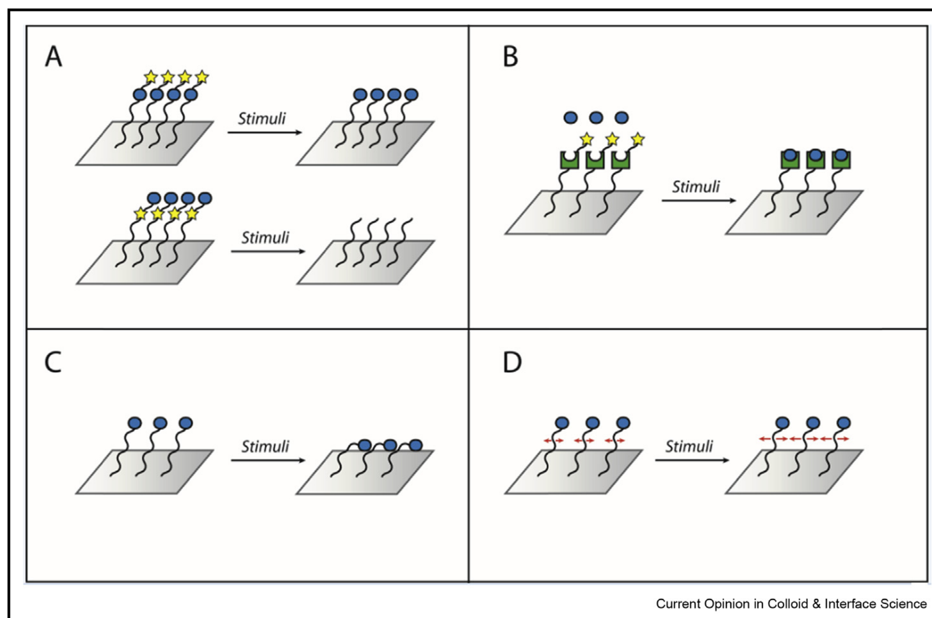
Introduction

Most cells in the body are adherent, and must attach to and interact with a protein matrix in order to survive, proliferate and maintain their normal functions [1–3]. The interactions between the cell and insoluble matrix are mediated by molecular recognition between receptors on the cell-surface and ligands within the matrix. A major theme in bio-interface science has been the design and assembly of model substrates that mimic the protein matrix by presenting ligands that can mediate cell adhesion and isolate signaling activities that are present *in vivo* [4–7]. The first and still majority of model substrates used in cell adhesion are static structures—in that the composition and arrangement of immobilized ligands is not intended to change during cell culture—yet biological matrices are highly dynamic and modulate the display of ligands and mechanical properties in ways that are important to regulating cell function. In this Opinion, we review recent work in developing dynamic substrates that can alter adhesion as well as the ligand-receptor interactions with adherent cells, and we discuss recent applications for these exciting bio-interfaces (see Figure 1).

In tissue, the extracellular matrix is an aggregate of large proteins and glycans—for example, fibronectin, the collagens, and laminin—that present peptide and carbohydrate ligands, that have relevant mechanical properties, and that bind soluble proteins for presentation to the cell [1]. Cells use membrane-bound protein receptors, primarily of the integrin family, to bind peptide motifs in the matrix [8]. The Arg-Gly-Asp (RGD) tripeptide from fibronectin is the best characterized of these ligands and binds to approximately one half of the integrin family of receptors [9]. When bound to the matrix, the receptors organize into clusters known as focal adhesions and which both regulate signaling pathways and integrate the cellular cytoskeleton with the matrix. This peptide has also been the most important for modifying materials to permit cell adhesion, both for fundamental studies of cell adhesion and signaling and also for promoting tissue interactions in clinically relevant biomaterials [10,11].

A critical complication in the design and use of any model substrate for cell adhesion is that proteins adsorb non-specifically to essentially all synthetic materials, and this adsorption can block interactions with immobilized ligands and can introduce unintended ligands that facilitate cell adhesion. Hence, it is important to use materials that are modified so that they prevent the non-specific adsorption of protein and attachment of cells, so that all interactions with an adherent cell involve ligands that are immobilized to the substrate. Materials having this property include substrates modified with hydrogels, self-assembled monolayers, and plasma-deposited films that present short oligomers of the ethylene glycol group [12–14]. Many chemistries are available for immobilizing ligands to these substrates. Further, a large effort has developed a suite of methods for patterning ligands to the substrates—with feature sizes ranging from nano to micro—and that can be used to control the shapes, sizes and positions of cells

Figure 1



Distinct strategies for designing dynamic substrates that modulate cell-substrate interactions: A) Uncaging or cleavage of a ligand with a photo/electro-labile group; B) uncaging of a receptor for ligand binding; C) conformational switching of ligand chains; D) modulation of lateral ligand mobility.

as well as the sub-cellular location of cellular attachments to the matrix [15–17].

The first examples of dynamic substrates were reported nearly twenty years ago. Wong, Langer and Ingber cultured aortic endothelial cells on conducting polypyrrole films that could be electrochemically oxidized or reduced, and they showed that cell growth and spreading were different on the two states of the surface [18]. Okano and co-workers developed poly(N-isopropylacrylamide) substrates for cell culture and harnessed the thermal phase transition of this material between states that promoted or prevented cell attachment. They showed that cells could be cultured on the material at incubator temperatures, but released when the substrates were cooled to room temperature [19]. They developed this approach for culturing cells into sheets for tissue engineering applications. To realize molecular-level control over ligand–receptor interactions, our early work developed electroactive self-assembled monolayers of alkanethiolates on gold that allowed the activities of immobilized RGD ligands to be turned on or off in response to applied potentials. In one example, monolayers were patterned with the RGD adhesion ligand, and where the surrounding areas presented a hydroquinone group against an inert background of tri(ethylene glycol) groups. Hence, 3T3 fibroblast cells attached and spread within the patterned regions. Application of an oxidizing potential to the substrate converted the hydroquinone groups to the corresponding benzoquinone groups, which could then undergo a Diels–Alder reaction with a RGD-cyclopentadiene conjugate to immobilize the peptide. This process effectively removed the pattern and allowed the cells to migrate [20]. We also reported strategies for electrochemically releasing ligands from the monolayer and uncaging immobilized ligands [21,22].

These early examples motivated a substantial effort in biointerface science and engineering to develop and apply dynamic substrates in cell biology. Most examples have used applied potentials and light to effect changes in the properties of the substrate, but examples with mechanical and magnetic forces, with enzymes and with solution additives have also been used. In this Opinion, we review these basic strategies, with particular attention to advances reported in the past three years. We end with a discussion of the applications for these dynamic substrates.

Electrochemical modulation

Electrochemical strategies were first used by our group to modulate the structure of bio-interfaces with well-defined molecular control. These strategies were motivated by the use of self-assembled monolayers of alkanethiolates on gold for fundamental studies of electron transfer and showed that redox-active groups that were attached to the monolayers could be efficiently oxidized and reduced through applied potentials [23]. By designing molecular groups that are

stable in one oxidation state but undergo reaction in a second oxidation state, we demonstrated how the activities of immobilized ligands can be modulated. In one example, we prepared monolayers that had the RGD peptide immobilized by way of a benzoquinone ester linker. This linker was stable, but upon reduction, the phenolic group of the hydroquinone reacted with the ester to give a lactone with release of the RGD peptide. We demonstrated that these monolayers could release adherent cells in response to an applied reducing potential [21]. In another example, we exploited the reactivity of aldehyde groups on a surface [24]. We designed an electrolabile protecting group for the aldehyde functionality by forming an acetal with an ortho-hydroxymethyl hydroquinone. Application of a positive potential resulted in uncaging of the aldehyde and allowed the non-specific adhesion of cells. In this way, we could create surfaces that were patterned with RGD and where the alternate regions had the caged aldehyde. Cells would only attach to the former regions and remain there during culture. When an oxidative potential was applied to the substrate cells initiated migration from their initial positions.

Yousaf and co-workers introduced another reaction of benzoquinone groups, termed oxime click chemistry. Ligands functionalized with a hydroxylamine group could be conjugated to the benzoquinone group of a monolayer, but were then released following electrochemical reduction at physiological pH with regeneration of the hydroquinone. Using this strategy, monolayers were prepared that presented both the RGD peptide and a hydroquinone group [25]. In this way, a second ligand could be immobilized—here, the 'synergy' ligand PHSRN from fibronectin—to increase the complexity of the substrate, and still allow release of the ligand at a later time in culture. These surfaces should prove useful for addressing the synergistic/antagonistic effects of the roles multiple ligands play in cellular processes. In another example, these electrically responsive surfaces were used to compare cell migration between static and stimulated conditions [26•]. The observation that cell behavior depended on whether cells were initially confined to a patterned region revealed that cell migration is dependent on history and, in a broader sense, demonstrated the value of these dynamic surfaces for studying how cell-matrix-mediated processes are spatiotemporally regulated.

Langer and coworkers developed incompletely formed monolayers, or low-density SAMs (LDSAMs) that present charged tail groups [27]. The low density is achieved by assembly of alkanethiols that have bulky end groups (i.e. trityl groups). These groups are sterically demanding and will limit the density of alkanethiolates within the monolayer—with larger groups giving lower densities—but the groups can then be removed to give monolayers that lack close packing of the alkane chains, and therefore are not constrained to a trans-extended conformation but can sample many conformations. When the chain is terminated in a charged group, application of an applied repulsive potential can either force it into an 'extended' conformation or with the attractive potential, enforce a 'bent' conformation. The wettability of the surface depends on the conformation, with the 'extended' surfaces having a hydrophilic property and the 'bent' having a hydrophobic character. It was also found that the attachment of bacteria to monolayer was dependent on the conformation of alkanethiolates [28,29]. One concern with the low density monolayers is whether the lack of packing of chains gives a less stable layer, or whether lateral mobility of the chains can lead to a surface having patches of well-packed alkanethiolates, which are then unable to undergo electrostatically-driven changes in conformation.

Photochemical modulation

Early photochemical strategies for manipulating the activities of immobilized ligands were based on photoprotected ligands that are inactive, but that can be uncaged with light to yield the active ligand. For example, an early report by Del Campo and coworkers prepared alkylsiloxane monolayers that presented a RGD peptide that was blocked at the aspartate residue with a nitrophenyl protecting group [30]. Without illumination, 3T3 fibroblasts were unable to attach to the substrate. However, irradiation of the substrate with 350 nm light through a mask resulted in spatial deprotection of the RGD peptides and subsequently directed cell attachment to the patterned regions. Nakanishi and coworkers first applied this strategy to demonstrate a dynamic substrate, wherein activation could be performed while cells were already present on the substrate [31]. In this case, however, rather than uncaging RGD, removal of the photoprotecting group resulted in loss of adsorbed albumin followed by adsorption of exogenously added fibronectin. Thus, surfaces were UV irradiated through a photomask to initially pattern the cells followed by a second irradiation in culture to pattern a second cell type. These approaches are general and recently have been applied towards activation of the laminin-based IKVAV peptide and an $\alpha_5\beta_1$ integrin-specific ligand [32•,33].

A limitation in the use of light-controlled strategies for modulating cell adhesion is the potential for cytotoxic effects of UV and near-UV light sources. This concern can be mitigated by using short pulses of light and protecting groups that respond to visible light (i.e. BODIPY-based) or those that undergo two-photon excitation. However, an alternative strategy employs lanthanide-doped upconversion nanoparticles (UCNPs), wherein multiple photons at near-IR wavelengths can be absorbed and re-emitted *locally* at shorter wavelength UV light, hence enabling the uses of traditional photoprotecting groups with milder light treatments [34,35•]. The Qu lab first demonstrated the use of these strategy for controlling cell adhesion by

immobilizing nanoparticles on quartz substrates and covalently attaching photocleavable linkers terminated in RGD. Light with a wavelength of 980 nm could be used to uncage the peptides; the greater tissue penetration with longer wavelengths allowed *in vivo* applications of this method.

Additionally, though photocleavable methods are irreversible and allow one-time switching of adhesion states, photoisomerization approaches enable dynamic surfaces that can be reversibly modulated multiple times. In these approaches, the structural changes triggered in a chromophore lead to an alteration of the accessibility of a terminal ligand (i.e. RGD) for cell attachment. The two most commonly used isomerization groups are the azobenzenes, which undergo isomeric changes in double bond configuration in the presence of UV light, and the spiropyrans, which undergo a ring-opening at 350 nm to the merocyanine form and ring-closing at 560 nm. The spiropyran group can be directly used as a cell adhesion “ligand” as the spiropyran form displays a stronger interaction with fibronectin than does the merocyanine form [35•]. With the azobenzenes, the group can either be used as a linker terminated in RGD [36] or as a terminal group itself for host-guest interactions with functionalized cyclodextrins [37–40]. In the former case, Selhuber-Unkel and co-workers altered the photophysical properties of the azobenzene via “push–pull” substitution with an electron-withdrawing and electron-donating group [41•]. The generated species displayed a rapid *cis -trans* back-reaction (10^2–10^5 Hz) that enabled studies of integrin-mediated cellular responses to oscillatory “tickling” forces at previously inaccessible time scales, though the incomplete photoisomerization of the azobenzene chromophore is a limitation.

The photochemical strategies for modulating the activities of immobilized ligands offer many benefits over other strategies. First, the literature on photocaged molecules is extensive—with a significant amount directed towards uncaging ligands in cell culture—and offers a wide choice of protecting groups for different functional groups. Second, the photodeprotection reactions often proceed rapidly—in seconds when using laser sources on a microscope—and in high yield. Third, these strategies give excellent spatio-temporal control in activating immobilized ligands, making it straightforward to control activation at μm length scales. The potential concerns are that the photoprotecting groups can sometimes cause significant non-specific adsorption of protein and therefore cell attachment, and care must be taken to avoid unintentional exposure to light before or during the cell culture.

Solution additives for modulation

The development of zwitterionic surfaces as a leading class of inert substrates has led to the strategy of manipulating interfacial charge to control cell adhesion. The anti-fouling property of these surfaces is based on having strong solvation about each ionic center, but maintaining an overall net neutral charge. Hence, strategies that screen the charges or remove the zwitterionic character (through changes in pH) have been recently used to modulate cell adhesion. In one example, the Yang group developed polyzwitterionic brushes with imidazolium and sulfonate components (polyVBIPS) that were anti-fouling at high ionic strengths and adhesive at low ionic strengths [42]. With this “anti-polyelectrolyte effect,” the presence of salt disrupts the inter/intra-chain electrostatic interactions of the polymer brushes in the collapsed conformation to yield a more extended (and inert) state. In another example, the Jiang lab employed a zwitterionic polymer brush containing a tertiary amine and carboxylic acid groups and could cycle between charge-neutral states that are cell adhesive (pH 4–8) and charged states that promote adhesion ($pH < 4$ or $pH > 8$) [43••]. Importantly, the cationic and anionic adhesive regimes could support adsorption of both oppositely charged proteins and be cycled for sequential adsorption and release. We note that polyelectrolyte gels also exhibit pH and ionic strength-dependent properties but are less commonly employed for dynamically controlling cell adhesion.

Magnetic modulation

The dynamic strategies described above give examples of the modulation of ligand activity. However, interfacial interactions in biology between cellular receptors and surfaces are often multivalent and can be dependent on the lateral organization or clustering of a ligand. Indeed, extensive studies with supported lipid bilayer substrates (SLBs) that are functionalized with adhesion ligands have allowed the modulation of lateral organization of ligands for cell adhesion. Recently, Bian and coworkers pioneered the development of RGD-presenting surfaces whose two-dimensional mobility could be tuned in response to an applied magnetic field [44,45••]. The ligands were conjugated to magnetic nanoparticles, which in turn were bound to a silica surface by way of a flexible linker. The ligand mobility could then be modulated with a magnetic field oscillating at varying frequencies. Importantly, this work found that stem cell adhesion, spreading, and differentiation were dependent on the frequency of the field oscillation, with greater spreading observed at lower frequencies. This observation aligns with our current understanding of integrin-mediated events that require sufficient time and pre-organization for focal adhesion maturation and traction force sensing. In addition, considering magnetic fields are routinely used for patient imaging and can penetrate tissue, this stimulus bears a real potential for the modulation of cell adhesion *in vivo*. Indeed, the Bian group reported the successful modulation of adhesion

and polarization with magnetically responsive substrates implanted in mice [45••,46].

Enzymatic modulation

The *in vivo* properties of extracellular matrix are often modulated through the action of enzymes on the protein scaffold. For example, metalloproteases digest the insoluble matrix and allow for its remodeling and they are also harnessed by cancer cells to permit their migration and metastasis. Lysine 6-oxidase converts lysine side chain amines in collagen and elastin to reactive aldehydes that facilitate cross-linking and fibril stabilization. Recently, citrullination (with protein arginine deaminase enzymes) has been found to modify cell adhesion by way of modification of arginine residues in integrin binding sites [47]. In pioneering work, Hubbell and coworkers developed hydrogel matrices that included a RGD peptide for cell adhesion and a second peptide cross-linker that was a substrate for the metalloprotease MMP-1 [48]. In this way, the biomimetic matrix could be used to study the protease-mediated migration of cells. Ulijn and coworkers reported a related strategy, wherein an immobilized RGD peptide was inactive towards cell adhesion because it was terminated in a bulky FMOC-Phe group [49]. Treatment with a protease released this group and afforded a functional RGD ligand that could then mediate cell adhesion. Ulijn and Dalby went on to apply this strategy to control the differentiation of mesenchymal stem cells [50•]. They could switch on enhanced adhesion and spreading by proteolytically uncaging RGD peptides to increase the affinity of the peptides. The resulting cells had a more contractile cytoskeleton and an increased preference for osteogenesis.

These enzyme-directed strategies are akin to the use of light to remove photoprotecting groups, but have the benefits that they do not require light sources and they also use specific enzyme-substrate pairs that can give multiple orthogonally controlled routes to modulate the biological properties of a synthetic matrix, though they do not offer the spatial resolution that is possible with focused light. Finally, another exciting example showed that cells could be engineered with designed receptors that presented an enzyme that could act on an immobilized substrate. We reported an example wherein the enzyme cutinase could hydrolyze a hydroquinone ester, and yield the hydroquinone, which could then be detected using cyclic voltammetry, demonstrating a bioelectronic interface [51].

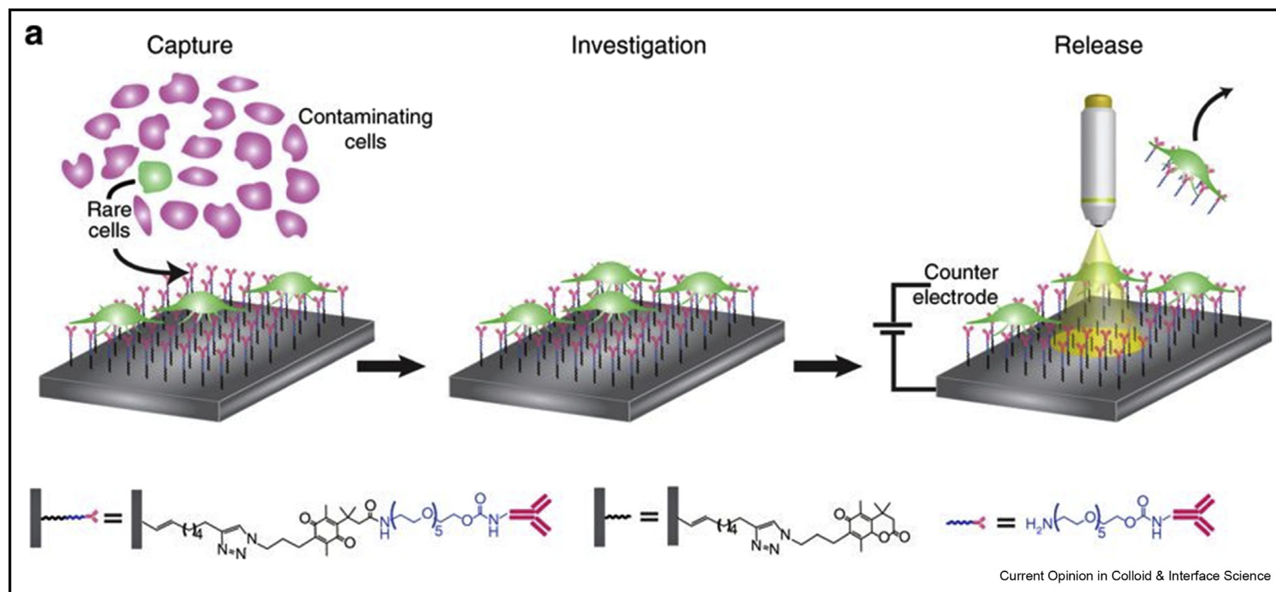
We have organized the discussion above by the modulation strategy, but were not explicit as to which strategies are most relevant for the different surface chemistries (principally, those based on self-assembled monolayers and on polymers). Electrochemical strategies require a conductive surface to transduce applied electrical potentials and thus are best suited for manipulating ligand activities present on SAMs. Thermal methods are based on phase transitions in the surface layer and therefore are most relevant to polymeric and hydrogel layers. The use of light and solution additives (including pH, metal ions, enzymes and chemical reagents) are applicable to the broad range of chemistries discussed here. Magnetic strategies, in contrast, necessitate paramagnetic species — iron nanoparticles, for example — and this requirement will limit the types of chemistries that can be used. Finally, we note that there has been relatively little work in extending these strategies to three-dimensional culture systems, though the most important strategies have relied on photochemical manipulation [52].

Applications

One notable application for the dynamic substrates is directed towards methods for single cell analysis. Here, surfaces are designed to initially allow the adhesion and culture of cells, but that can then be stimulated to release cells. As noted earlier, early work by Okano's group had developed poly(N-isopropylacrylamide) gels as such a substrate [19]. When the gel composition is tuned to undergo the phase transition just below 37 C (which is typical in growth incubators), then cells can be cultured but will detach when the substrate is removed from the incubator and allowed to cool to room temperature. These materials have been developed for tissue engineering applications, for growing and recovering interconnected sheets of cells. Wang and coworkers have extended this approach by modifying silicon nanopillar substrates with this polymeric coating but that is further modified with an antibody that binds epithelial cell adhesion molecule, a receptor that is overexpressed on certain cancer cells [53]. In this way, the surfaces are more selective in allowing a target cell population to attach. As before, the cultured cells can be released from the substrate upon cooling to 20 C, and the surface undergoes reversible phase transitions to allow repeated attachment and release of cells. In another example, these authors prepared acrylamide gels that were functionalized with phenylboronic acid groups for engagement of carbohydrates on the cell surface, and demonstrated reversible cell attachment that was regulated by pH and soluble glucose [54].

One limitation of the thermally responsive gels is that it is difficult to selectively release an individual cell from a culture, since thermal conductivity of the substrate does not allow spatial localization of the phase transition at small ($\sim$100 μm) length scales. Photochemically-active substrates can be used for this purpose, where focused light can trigger the modification of a substrate and release an individual cell, but carries the limitation that the cultures cannot be analyzed by fluorescent

Figure 2



A design for using a combination of applied potential and light to stimulate the release of cells from the irradiated regions [55].

microscopy prior to release of the cells, since the surfaces (by design) are not stable to the light. Hence, these strategies would make it difficult to monitor cells in culture using microscopy, where cells having a relevant phenotype can be identified and selected for release. Gooding and coworkers recently described an innovative light-activated electrochemical method for releasing individual cells [55••] (See Figure 2). This work used p-type silicon surfaces that presented an anti-EpCAM antibody by way of a benzoquinone-based linker that undergoes cleavage when reduced to the corresponding hydroquinone. Cells having the EpCAM receptor on their surfaces could attach and spread on the substrate. Application of an electrical potential does not lead to reduction of the benzoquinone because of poor conductivity of the silicon substrate, but illumination of the substrate promotes electrons to the conducting band and allows for redox reactions at the interface, and release of cells only in the illuminated region. This paper demonstrated that a population of lung tumor cells could be treated with the drug doxorubicin and that only those cells that displayed efficient uptake of the drug could be identified and released to permit gene expression profiling of those cells. This example represents a compelling application for dynamic substrates and will likely find wide use.

In another strategy, Yeo and coworkers prepared low density monolayers that included three different chains terminated in acidic and basic functional groups as well as an uncharged chain. This monolayer could be switched between three states—zwitterionic, anionic or cationic—in response to applied potentials [56•]. Interestingly, each state had a distinct property; the zwitterionic state resisted bacterial attachment; the anionic state maintained attachment; and the cationic state presented quaternary ammonium salts that have bactericidal properties.

These examples have demonstrated the enabling applications of dynamic substrates, particularly for controlling cell adhesion, proliferation and growth. These properties can be quite useful in bioanalytical systems to analyze and manipulate cells. Yet, the field still has not addressed problems in signaling, where the introduction or removal of a ligand at the cell-ECM interface results in activation of specific signal transduction pathways. We believe the prospects for such applications are exciting and that the dynamic substrates can emerge as a powerful approach in cell biology. But these studies are more challenging and will require the collaboration of cell biologists in designing studies and interpreting data.

The past decade has seen a substantial increase in the development and application of strategies to dynamically regulate ligand-receptor interactions at the cell-matrix interface. The range of methods, and the further opportunities they motivate for molecular engineering of the interface, now represent a frontier area in biointerface science. Future work will certainly see growth in the application of these dynamic substrates to a range of studies in cell signaling and as enabling components of cell-based assays for drug discovery and diagnostics.

Conflict of interest statement

Nothing declared.

Acknowledgement

We are grateful for support of our work by the National Cancer Institute of the National Institutes of Health (U54CA199091) and the Air Force Office of Scientific Research (AFOSR FA9550-16-1-0150).

References

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Frantz C, Stewart KM, Weaver VM: **The extracellular matrix at a glance**. *J Cell Sci* 2010, **123**:4195.
2. Hynes RO: **Extracellular matrix: not just pretty fibrils**. *Science* 2009, **326**:1216–1219.
3. Bonnans C, Chou J, Werb Z: **Remodelling the extracellular matrix in development and disease**. *Nat Rev Mol Cell Biol* 2014, **15**:786.
4. Mrksich M: **Using self-assembled monolayers to model the extracellular matrix**. *Acta Biomater* 2009, **5**:832–841.
5. Gooding JJ, Parker SG, Lu Y, Gaus K: **Molecularly engineered surfaces for cell biology: from static to dynamic surfaces**. *Langmuir* 2014, **30**:3290–3302.
6. Kyburz KA, Anseth KS: **Synthetic mimics of the extracellular matrix: how simple is complex enough?** *Ann Biomed Eng* 2015, **43**:489–500.
7. Kocer G, Jonkheijm P: **About chemical strategies to fabricate cell-instructive biointerfaces with static and dynamic complexity**. *Adv Healthc Mater* 2018, **0**:e1701192.
8. Campbell ID, Humphries MJ: **Integrin structure, activation, and interactions**. *Cold Spring Harb Perspect Biol* 2011, **3**:a004994.
9. Ruoslahti E: **RGD and other recognition sequences for integrins**. *Annu Rev Cell Dev Biol* 1996, **12**:697–715.
10. Bellis SL: **Advantages of RGD peptides for directing cell association with biomaterials**. *Biomaterials* 2011, **32**: 4205–4210.
11. Hersel U, Dahmen C, Kessler H: **RGD modified polymers: biomaterials for stimulated cell adhesion and beyond**. *Biomaterials* 2003, **24**:4385–4415.
12. Chen S, Li L, Zhao C, Zheng J: **Surface hydration: principles and applications toward low-fouling/nonfouling biomaterials**. *Polymer* 2010, **51**:5283–5293.
13. Banerjee I, Pangule RC, Kane RS: **Antifouling coatings: recent developments in the design of surfaces that prevent fouling by proteins, bacteria, and marine organisms**. *Adv Mater* 2010, **23**:690–718.
14. Löpez GP, Ratner BD, Tidwell CD, Haycox CL, Rapoza RJ, Horbett TA: **Glow discharge plasma deposition of tetraethylene glycol dimethyl ether for fouling-resistant biomaterial surfaces**. *J Biomed Mater Res* 1992, **26**:415–439.
15. Théry M: **Micropatterning as a tool to decipher cell morphogenesis and functions**. *J Cell Sci* 2010, **123**:4201.
16. Yap FL, Zhang Y: **Protein and cell micropatterning and its integration with micro/nanoparticles assembly**. *Biosens Bioelectron* 2007, **22**:775–788.
17. Arnold M, Cavalcanti-Adam EA, Glass R, Blümmel J, Eck W, Kantlehner M, Kessler H, Spatz JP: **Activation of integrin function by nanopatterned adhesive interfaces**. *ChemPhysChem* 2004, **5**:383–388.
18. Wong JY, Langer R, Ingber DE: **Electrically conducting polymers can noninvasively control the shape and growth of mammalian cells**. *Proc Natl Acad Sci USA* 1994, **91**: 3201–3204.
19. Okano T, Yamada N, Okuhara M, Sakai H, Sakurai Y: **Mechanism of cell detachment from temperature-modulated, hydrophilic-hydrophobic polymer surfaces**. *Biomaterials* 1995, **16**:297–303.
20. Yousaf MN, Houseman BT, Mrksich M: **Turning on cell migration with electroactive substrates**. *Angew Chem Int Ed* 2001, **40**:1093–1096.
21. Yeo W-S, Mrksich M: **Electroactive self-assembled monolayers that permit orthogonal control over the adhesion of cells to patterned substrates**. *Langmuir* 2006, **22**: 10816–10820.
22. Yeo W-S, Hodneland CD, Mrksich M: **Electroactive monolayer substrates that selectively release adherent cells**. *ChemBioChem* 2001, **2**:590–593.
23. Chidsey CED: **Free energy and temperature dependence of electron transfer at the metal-electrolyte interface**. *Science* 1991, **251**:919.
24. Yeo WS, Mrksich M: **Electroactive substrates that reveal aldehyde groups for bio-immobilization**. *Adv Mater* 2004, **16**: 1352–1356.
25. Pulsipher A, Park S, Dutta D, Luo W, Yousaf MN: **In situ modulation of cell behavior via smart dual-ligand surfaces**. *Langmuir* 2014, **30**:13656–13666.
26. • Lee E-j, Luo W, Chan EWL, Yousaf MN: **A molecular smart surface for spatio-temporal studies of cell mobility**. *PLoS One* 2015, **10**:e0118126.

Hydroquinone-based dynamic surfaces were used to study electrochemically-initiated cell migration as a function of initial adhesion parameters.

27. Lahann J, Mitragotri S, Tran T-N, Kaido H, Sundaram J, Choi IS, Hoffer S, Somorjai GA, Langer R: **A reversibly switching surface**. *Science* 2003, **299**:371.
28. Pranzetti A, Mieszkin S, Iqbal P, Rawson FJ, Callow ME, Callow JA, Koelsch P, Preece JA, Mendes PM: **An electrically reversible switchable surface to control and study early bacterial adhesion dynamics in real-time**. *Adv Mater* 2013, **25**: 2181–2185.
29. Ng CC, Magenau A, Ngalim SH, Ciampi S, Chockalingham M, Harper JB, Gaus K, Gooding JJ: **Using an electrical potential to reversibly switch surfaces between two states for dynamically controlling cell adhesion**. *Angew Chem Int Ed* 2012, **51**: 7706–7710.
30. Petersen S, Alonso José M, Specht A, Duodu P, Goeldner M, del Campo A: **Phototriggering of cell adhesion by caged cyclic RGD peptides**. *Angew Chem Int Ed* 2008, **47**:3192–3195.
31. Kikuchi Y, Nakanishi J, Shimizu T, Nakayama H, Inoue S, Yamaguchi K, Iwai H, Yoshida Y, Horiike Y, Takarada T, Maeda M: **Arraying heterotypic single cells on photoactivatable cell-culturing substrates**. *Langmuir* 2008, **24**:13084–13095.
32. • Farrukh A, Fan W, Zhao S, Salierno M, Paez JI, Del Campo A: **Photoactivatable adhesive ligands for light-guided neuronal growth**. *Chembiochem* 2018, **19**:1271–1279.

A leading example of the application of dynamic substrates in neuronal cell biology.

33. Nair Roshna V, Farrukh A, del Campo A: **A photoactivatable $\alpha5\beta1$-specific integrin ligand**. *ChemBioChem* 2018, **19**: 1280–1287.
34. Li W, Wang J, Ren J, Qu X: **Near-infrared upconversion controls photocaged cell adhesion**. *J Am Chem Soc* 2014, **136**: 2248–2251.
35. • Li W, Chen Z, Zhou L, Li Z, Ren J, Qu X: **Noninvasive and reversible cell adhesion and detachment via single-wavelength near-infrared laser mediated photoisomerization**. *J Am Chem Soc* 2015, **137**:8199–8205.

A spiropyran-functionalized UCNP (upconversion nanoparticle) surface can be switched repeatedly using low energy photons that are not cytotoxic.

36. Auernheimer J, Dahmen C, Hersel U, Bausch A, Kessler H: **Photoswitched cell adhesion on surfaces with RGD peptides**. *J Am Chem Soc* 2005, **127**:16107–16110.

37. Ren T, Ni Y, Du W, Yu S, Mao Z, Gao C: **Dual responsive surfaces based on host–guest interaction for dynamic mediation of cell–substrate interaction and cell migration**. *Adv Mater Interface* 2016, **4**:1500865.

38. Bian Q, Wang W, Wang S, Wang G: **Light-triggered specific cancer cell release from cyclodextrin/azobenzene and aptamer-modified substrate**. *ACS Appl Mater Interfaces* 2016, **8**:27360–27367.

39. Shen Q, Liu L, Zhang W: **Fabrication of a photocontrolled surface with switchable wettability based on host–guest inclusion complexation and protein resistance**. *Langmuir* 2014, **30**:9361–9369.

40. Wei T, Zhan W, Yu Q, Chen H: **Smart biointerface with photoswitched functions between bactericidal activity and bacteria-releasing ability**. *ACS Appl Mater Interfaces* 2017, **9**: 25767–25774.

41. • Kadem Laith F, Suana KG, Holz M, Wang W, Westerhaus H, Herges R, Selhuber-Unkel C: **High-frequency mechanostimulation of cell adhesion**. *Angew Chem Int Ed* 2016, **56**:225–229.
An unprecedented demonstration of reversible modulation to rapidly regulate engagement of adhesion receptors.

42. Chen H, Yang J, Xiao S, Hu R, Bhaway SM, Vogt BD, Zhang M, Chen Q, Ma J, Chang Y, Li L, Zheng J: **Salt-responsive polyzwitterionic materials for surface regeneration between switchable fouling and antifouling properties**. *Acta Biomater* 2016, **40**:62–69.

43. •• Sundaram HS, Ella-Menye J-R, Brault ND, Shao Q, Jiang S: **Reversibly switchable polymer with cationic/zwitterionic/anionic behavior through synergistic protonation and deprotonation**. *Chem Sci* 2014, **5**:200–205.
An innovative yet simple route to regulate protein/cell interactions with a polymeric material.

44. Wong DSH, Li J, Yan X, Wang B, Li R, Zhang L, Bian L: **Magnetically tuning tether mobility of integrin ligand regulates adhesion, spreading, and differentiation of stem cells**. *Nano Lett* 2017, **17**:1685–1695.

45. •• Kang H, Wong DSH, Yan X, Jung HJ, Kim S, Lin S, Wei K, Li G, Dravid VP, Bian L: **Remote control of multimodal nanoscale ligand oscillations regulates stem cell adhesion and differentiation**. *ACS Nano* 2017, **11**:9636–9649.
A surprising study that reveals the extent to which even subtle manipulation of ligand-receptor interactions can have on stem cell differentiation.

46. Kang H, Kim S, Wong DSH, Jung HJ, Lin S, Zou K, Li R, Li G, Dravid VP, Bian L: **Remote manipulation of ligand nano-oscillations regulates adhesion and polarization of macrophages in vivo**. *Nano Lett* 2017, **17**:6415–6427.

47. Sipilä KH, Ranga V, Rappu P, Mali M, Pirilä L, Heino I, Jokinen J, Käpylä J, Johnson MS, Heino J: **Joint inflammation related citrullination of functional arginines in extracellular proteins**. *Sci Rep* 2017, **7**:8246.

48. Lutolf MP, Lauer-Fields JL, Schmoekel HG, Metters AT, Weber FE, Fields GB, Hubbell JA: **Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics**. *Proc Natl Acad Sci* 2003, **100**:5413.

49. Todd SJ, Farrar D, Gough JE, Ulijn RV: **Enzyme-triggered cell attachment to hydrogel surfaces**. *Soft Matter* 2007, **3**: 547–550.

50. • Roberts JN, Sahoo JK, McNamara LE, Burgess KV, Yang J, Alakpa EV, Anderson HJ, Hay J, Turner L-A, Yarwood SJ, Zelzer M, Oreffo ROC, Ulijn RV, Dalby MJ: **Dynamic surfaces for the study of mesenchymal stem cell growth through adhesion regulation**. *ACS Nano* 2016, **10**:6667–6679.
An important example of applying dynamic substrates in stem cell biology.

51. Collier JH, Mrksich M: **Engineering a biospecific communication pathway between cells and electrodes**. *Proc Natl Acad Sci USA* 2006, **103**:2021.

52. Kloxin AM, Kasko AM, Salinas CN, Anseth KS: **Photodegradable hydrogels for dynamic tuning of physical and chemical properties**. *Science* 2009, **324**:59–63.

53. Liu H, Liu X, Meng J, Zhang P, Yang G, Su B, Sun K, Chen L, Han D, Wang S, Jiang L: **Hydrophobic interaction-mediated capture and release of cancer cells on thermoresponsive nanostructured surfaces**. *Adv Mater* 2012, **25**:922–927.

54. Liu H, Li Y, Sun K, Fan J, Zhang P, Meng J, Wang S, Jiang L: **Dual-responsive surfaces modified with phenylboronic acid-containing polymer brush to reversibly capture and release cancer cells**. *J Am Chem Soc* 2013, **135**:7603–7609.

55. •• Parker SG, Yang Y, Ciampi S, Gupta B, Kimpton K, Mansfeld FM, Kavallaris M, Gaus K, Gooding JJ: **A photoelectrochemical platform for the capture and release of rare single cells**. *Nat Commun* 2018, **9**:2288.
An ingenious strategy to use light irradiation to direct applied potentials for spatial modulation of a dynamic substrate at high resolution.

56. • Choi I, Lee J, Kim W, Kang H, Bae SW, Chang R, Kim S, Yeo W-S: **On-demand modulation of bacterial cell fates on multifunctional dynamic substrates**. *ACS Appl Mater Interfaces* 2018, **10**:4324–4332.
An impressive demonstration of electrically responsive substrates based on low-density SAMs manipulating the adhesion of mycoplasma contaminants from mammalian cell cultures.