Responsive Surfaces for Life Science Applications

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Annu. Rev. Mater. Res. 2012. 42:343–72

First published online as a Review in Advance on April 17, 2012

The *Annual Review of Materials Research* is online at matsci.annualreviews.org

This article's doi: 10.1146/annurev-matsci-070511–155044

1531-7331/12/0804-0343$20.00

Keywords

stimuli-responsive polymers, self-assembled monolayers, protein adsorption, cell adhesion, biosensors, bioseparation membranes

Abstract

This review describes recent developments in stimuli-responsive biointerfaces based on surface-tethered organic molecules, polymer chains, and polymer networks. The existing systems are classified according to the length scale of transformations occurring in the stimuli-responsive material and interactions of the material with the biological environment. In particular, two types of biointerfaces are considered: those whose interactions with proteins and cells can be switched reversibly or one time due to stimuli-triggered changes in molecular conformations or chemical bonds in functional molecules, and those that undergo reversible stimuli-triggered reconstruction at mesoscale due to stimuli-responsive phase behavior. Specific examples of stimuli-responsive surfaces from the recent literature are supplemented with discussion of potential biomedical applications.

INTRODUCTION

The goal of establishing proper interactions between synthetic materials and living systems relates to a number of biomedical applications broadly defined as diagnostics, therapeutics, and surgery. In all these biomedical applications, synthetic materials (e.g., biosensors, implants, catheters, and systems for delivery of drugs and contrast agents) are in contact with blood, living tissues, and various physiological milieus. The synthetic materials, engineered with microscopic precision, interact with life systems at two distinct length scales that are relevant to two major building blocks in living tissues: proteins and nucleic acids (biomolecular level), and protein complexes and cells (mesoscopic scale). Because proteins and DNA carry structural information, they are responsible for specific recognition and catalytic functions, and they are less changeable in time; protein complexes and cells are responsible for signaling, gating, transport, separation, and synthesis processes, so they, in contrast, change and rearrange in time and different environmental conditions. Therefore, there are biomedically relevant needs to establish stimuli-responsive contacts between synthetic materials and living systems for selective delivery and release of chemicals and proteins, selective and sensitive analysis of physiological fluids, and proper stimulation of cells. Those needs drive the engineering of material interfaces with responsive properties that address two length scales of interactions with life systems.

In this review, we present recent achievements in stimuli-responsive surfaces through the prism of two length-scale interactions with proteins and cells. In the first part, we consider surfaces that address switchable and changeable interactions with proteins by stimuli-triggered changes in molecular conformations or chemical bonds in functional molecules at interfaces. In the second part, we review systems that undergo stimuli-triggered reconstruction at mesoscale due to stimuli-sensitive phase behavior at biointerfaces. Both kinds of systems were designed to regulate interactions with biological molecules (first with proteins) and cells. Due to length limitations, we have confined our analysis to experimental studies of responsive surfaces for potential biomedical applications.

SMART SURFACES WITH RESPONSIVE EFFECTS AT THE MOLECULAR LEVEL

Polymeric Materials with Stimuli-Controlled Changes in Surface Polarity or Charge

In this section, we describe stimuli-responsive polymeric materials capable of controlling protein adsorption and cell adhesion due to changes in the polarity and charge of polymeric segments on the material surface.

Thermoresponsive polymeric surfaces. Recent studies indicate that structural characteristics of responsive polymeric surfaces have a strong effect on protein adsorption and cell attachment (1). This suggests a need to identify structure-property relationships that enable the rational design of such materials. The importance of a microstructure is best illustrated for thermoresponsive surfaces by poly(*N*-isopropylacrylamide) (pNIPAm). pNIPAm was the material of choice in the majority of studies because this polymer exhibits a sharp coil-to-globule transition in aqueous solutions at the lower critical solution temperature (LCST) of 32°C, i.e., in a physiologically relevant temperature range. The heating-induced transition from the extended (swollen) state to the collapsed (dehydrated) state implies that inter- and intramolecular hydrogen bonds among the peptide (CONH) groups of the pNIPAm chains become more favorable than intermolecular

hydrogen bonds among the peptide groups and water molecules. Although the pNIPAm collapse leads to a decrease in the conformational entropy of the chains, it also leads to a considerable gain in the configurational entropy associated with the liberation of bound water molecules and a reduction in excluded volume due to the chain collapse. As a result, the coil-to-globule transition can be understood in terms of the enthalpic/entropic balance, where the enthalpic term, favoring hydration, is dominant at temperatures below the LCST, and the entropic term, favoring the compact state, is dominant at temperatures above the LCST. As pNIPAm chains are immobilized on surfaces in the form of polymer brushes or hydrogels, they exhibit collective thermoresponsive behavior, observed in experiments as volumetric changes and switching between hydrophilic and more hydrophobic dehydrated states (2). pNIPAm surfaces resist protein adsorption and hence cell adhesion at temperatures below the LCST when the polymer is swollen. However, protein adsorption and cell adhesion begin to occur at temperatures above the LCST when pNIPAm chains are dehydrated and collapsed.

A number of studies showed that protein adsorption and cell adhesion are affected by structural and physicochemical characteristics of a pNIPAm layer, such as molecular weight and grafting density in the case of pNIPAm grafted layers, and surface energy of the substrate, thickness and cross-linking degree in the case of crosslinked films (3–6). Despite significant progress in our understanding of the behavior of proteins on thermoresponsive pNIPAm surfaces, many unresolved questions remain. In this section, we restrict ourselves to recent studies focusing on relationships between the structural characteristics of pNIPAm layers and attachment of proteins and cells.

Control of protein adsorption. Leckband et al. (7) investigated protein adsorption on pNIPAm brushes of different molecular weights and grafting densities. The brushes were synthesized by surface-initiated atom transfer radical polymerization (SI-ATRP) on silicon (Si) wafers. They employed a very sensitive radioisotope assay technique to determine minute amounts of proteins adsorbed on the brush surface. Below the LCST, pNIPAm brushes resisted adsorption of bovine serum albumin (BSA) with the same efficiency as oligo(ethylene glycol) (OEG) monolayers. In contrast, above the LCST, the amount of adsorbed BSA depended on the grafting density and, to some extent, on the molecular weight. The interactions between proteins and grafted polymer layers were interpreted by three generic modes (as shown in **Figure 1**): (*a*) primary adsorption to the grafting surface, (*b*) ternary adsorption to polymer segments within the brush layer, and (*c*) secondary adsorption at the outer edge of the brush (8, 9). These results demonstrate that densely packed brushes suppress protein adsorption in all three modes, whereas grafted layers with a lower chain density allow protein penetration into the brush. Indeed, larger adsorbed amounts (due to ternary adsorption) were detected above the LCST. Interestingly, the suppression of secondary adsorption in the densely grafted brushes above the LCST implies that the outer edge of the brush remains largely hydrated. The existence of hydrated outer chains is also supported by previous studies involving force measurements and neutron reflectivity (10–12). This example shows that grafting density has a strong effect on the switching properties of grafted pNIPAm layers.

Chen et al. (13) observed brush thickness–dependent protein adsorption behavior for highly dense pNIPAm brushes prepared by SI-ATRP. In a low-thickness regime (<15 nm), pNIPAm brushes were found to be repellent to human serum albumin (HSA) regardless of temperature. In contrast, thicker grafted pNIPAm layers exhibited pronounced thermal sensitivity and size selectivity for protein adsorption. In particular, below the LCST, the adsorption amounts were rather small for HSA and fibrinogen and quite substantial for a smaller protein lysozyme. As the temperature was raised above the LCST, the adsorption of HSA and fibrinogen was dramatically enhanced, whereas relatively small changes in the adsorption level were found for lysozyme. The

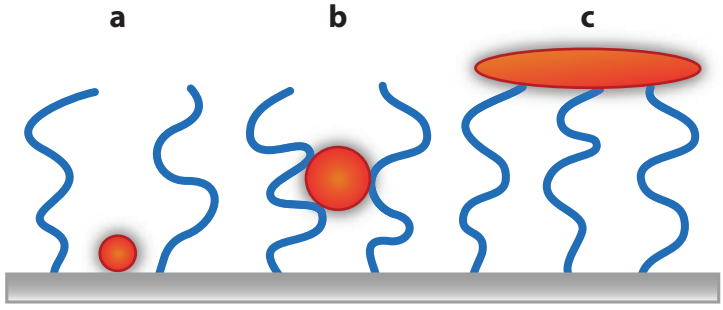

Figure 1

Three regimes of adsorption onto a polymer brush: (*a*) primary adsorption to the grafting surface, (*b*) ternary adsorption within the brush, and (*c*) secondary adsorption at the outer edge of the brush.

observed differences in the adsorption behavior below the LCST point to the penetration of small lysozyme into the swollen brush, although the same brush suppresses the primary and tertiary adsorption of larger proteins. Similar behavior was observed for swollen poly(2-hydroxyethyl methacrylate) brushes (8).

Ratner and coworkers (5) studied adsorption and desorption of three different radiolabeled proteins, namely BSA, antiferritin antibody, and fibrinogen, on plasma-polymerized NIPAm (ppNIPAm) films. They demonstrated that the affinity of the proteins to the crosslinked ppNIPAm films was approximately one order of magnitude higher above the LCST than below this temperature. The adsorption was irreversible for all the proteins except BSA, for which partial detachment was observed at temperatures below the LCST. Furthermore, the activity of antiferritin antibody adsorbed on ppNIPAm was better preserved at temperatures below the critical temperature. The decline of antibody activity above the LCST was attributed to surface-induced changes in the conformation and/or orientation of the antibody. In a different study, Gleason and coworkers (14) employed the quartz crystal microbalance with dissipation monitoring (QCM-D) technique to analyze the kinetics of BSA adsorption on thermoresponsive poly[NIPAm-co-di(ethylene glycol) divinyl ether] crosslinked films prepared via initiated chemical vapor deposition (iCVD) polymerization. Their results demonstrated that nearly a monolayer of albumin was adsorbed on the copolymer surface above the LCST. Below the critical temperature, the interaction of the swollen film with BSA was determined by two distinct processes: the initial adsorption of the protein onto the surface, which was slower than the same process above the LCST and characterized by lower protein-brush interaction strength, followed by protein diffusion into the swollen hydrogel matrix.

The above studies demonstrate great potential for thermoresponsive surfaces controlling protein adsorption. However, practical applications of these biointerfaces in the areas of separation and purification of proteins, biosensing, and controlled release will require deeper understanding of the mechanisms of protein-polymer interactions and how such interactions are affected by a material's microstructure. Although the protein adsorption on polymer surfaces was irreversible for most proteins, the adhesion of cells was found to be reversible for many pNIPAm-based materials. The interactions of cells with responsive surfaces are considered in the next section.

Control of cell attachment and cell migration on micropatterned substrates. Thermoresponsive pNIPAm-based materials have been intensively studied for the control of cell attachment (15–17). Because attachment of cells to a material's surface is preceded by adsorption of specific

proteins, such as fibronectin, that create adhesive sites for cell attachment, pNIPAm surfaces in their dehydrated state ($T >$ LCST) facilitate cell adhesion. The same surfaces resist cell attachment below the critical temperature, i.e., when pNIPAm is highly hydrated. An important finding was that cells that were cultured on pNIPAm surfaces above the LCST tended to spontaneously (or after gentle agitation) detach from the surface as the temperature was lowered below the critical temperature of pNIPAm. The ability to harvest living cells under mild (noninvasive) conditions, i.e., without the need for the trypsinization procedure that causes damage to the cell membrane surface and extracellular matrix proteins, has been broadly explored for creating reusable cell culture substrates for tissue engineering. Okano and coworkers (15, 17, 18) pioneered the approach, demonstrating the application of pNIPAm surfaces for cell sheet engineering. In this method, cells are allowed to attach and grow to confluence on the pNIPAm surface above the LCST. The resulting cell sheets are then detached by lowering the temperature below the critical temperature, preserving cell functions and the integrity of extracellular matrix of the cell sheets. Other groups adopted this approach and reported successful growth of cell sheets on well-defined pNIPAm brushes prepared by living radical polymerization and their subsequent release (19–21). Cell sheet engineering has been successfully applied for the regeneration of cornea, skin, liver, and heart tissues (22). This section describes the recent advances in this area.

Recently, Okano and coworkers (23) investigated the effect of the grafting density and molecular weight of pNIPAm brushes on cell behavior. The single-cell studies showed an increasingly slower rate of cell attachment to pNIPAm brushes upon an increase in their molecular weight and chain density. However, different behavior was observed for cell sheets; cells proliferated to confluence within two days on all pNIPAm surfaces above the LCST. The exception was grafted layers with high molecular weight and chain density, which required a long-term incubation to enable cell proliferation. Furthermore, below the LCST, the rate of detachment of confluent cell sheets increased with an increase in chain length and grafting density. No detachment of cell sheets was observed for brushes with short chains and at low grafting density, despite the fact that all the pNIPAm brushes exhibited cell detachment in the single-cell experiments. A previous study by Okano's group (24) showed that cell detachment from pNIPAm surfaces is related to adenosine-5′-triphosphate-dependent cell metabolism and is controlled by cell receptor–mediated signaling and reorganization of the cytoskeleton. These results imply that the efficiency of cell detachment depends not only on the structural characteristics of responsive layers but also on the cellular state at the moment of harvest (e.g., cell age and incubation time).

Okano's group (3, 25) has also investigated cell behavior on crosslinked pNIPAm films immobilized by electron beam irradiation on the hydrophobic surfaces of tissue culture polystyrene (TCPS) and silane-modified glass coverslips (CS). They found that for the pNIPAm films on TCPS, dry film thickness of ∼20 nm was optimal for achieving efficient cell attachment/detachment. The thicker films (>30 nm) exhibited cell-repellent properties above the LCST, whereas the thinner films (<15 nm) impaired cell detachment below the LCST (3). Also, the optimal thickness on TCPS was different from that on CS (∼5 nm). These findings stimulated Okano and his coworkers (25) to investigate pNIPAm films using in situ atomic force microscopy (AFM) and other surface characterization methods. Their analysis showed that the hydration of a polymeric network below the LCST increased with the distance from the substrate (**Figure 2**). The hydrophobic grafting surface caused the dehydration of polymeric segments in the film region adjacent to the substrate, which explained why thin films showed no cell detachment below the critical temperature of pNIPAm. In contrast, in the case of thick pNIPAm films, the outer regions were unaffected by the hydrophobic nature of the substrate and were thus highly hydrated. Such films were cell repellent and exhibited detachment of adhered cells. Interestingly, the outer layer in thick films remained largely hydrated and cell repellent even above the LCST (*right* panel in

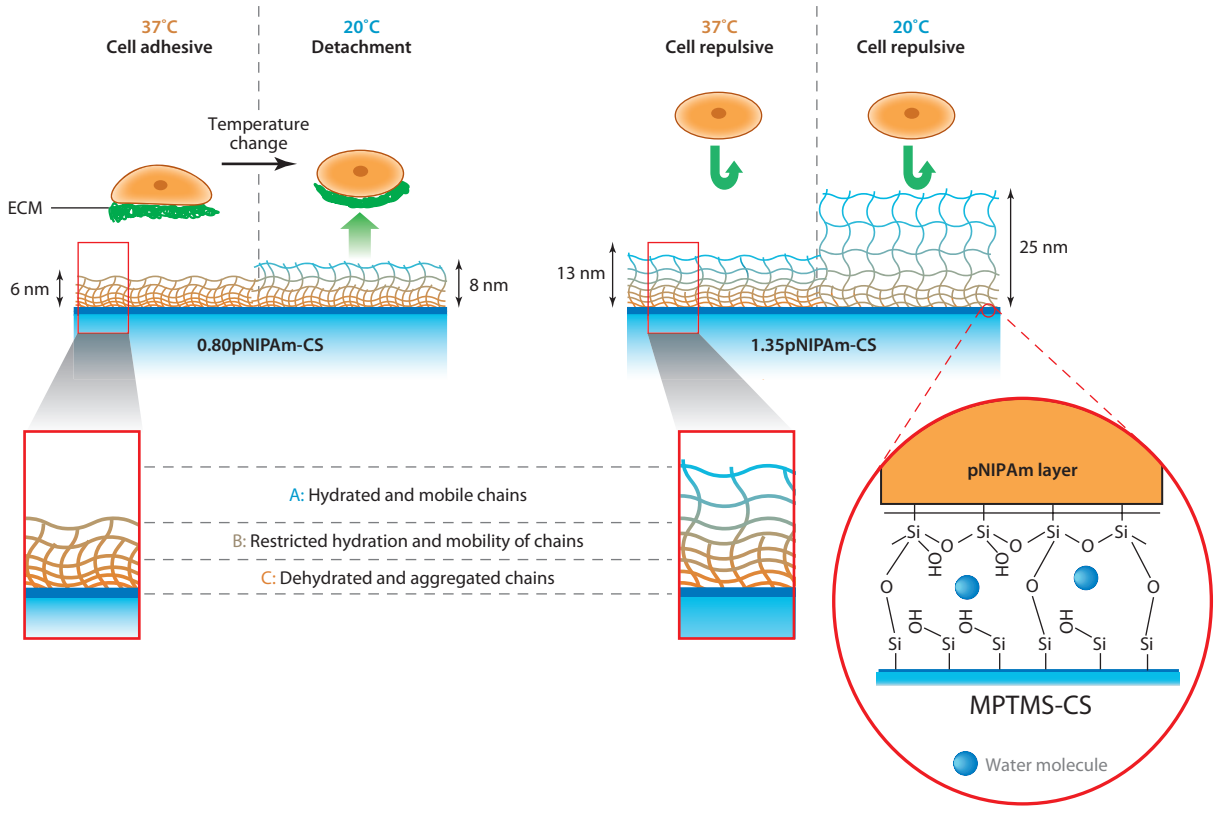

Figure 2

Sketch showing interactions between living cells and thermoresponsive pNIPAm network films of two different thicknesses immobilized onto CS substrates: (*left*) ~6 nm–thick film (above and below the LCST) and (*right*) ~13 nm–thick film (above and below the LCST). The thinner film exhibits switching between cell-adhesive and nonadhesive states, whereas the thicker one is always cell repellent. The hydrophobic grafting surface causes the dehydration of polymeric segments in the film region adjacent to the substrate. Furthermore, the transition to the collapsed state above the LCST starts from the hydrophobic substrate and propagates into the network, leading to a vertical gradient of the hydration degree in the film. It turns out that the outer region of the thicker film is unaffected by the hydrophobic nature of the substrate and remains highly hydrated above the LCST. Reprinted with permission from Reference 25, copyright 2010, Wiley.

Figure 2). Similar behavior was observed for pNIPAm brushes as discussed toward the beginning of this review. The existence of a hydrated outer layer above the LCST indicates that the transition to the collapsed state starts from the hydrophobic substrate and propagates into the network, leading to a vertical gradient of hydration degree in the film. As a result, distinct transition between cell-adhesive and cell-repellent states is possible only for pNIPAm layers of intermediate thicknesses (*left* panel in **Figure 2**). The disparity in optimal thicknesses between the pNIPAm films on TCPS and CS substrates was rationalized by different polarities of the substrates and thus by their ability to impose dehydration on the pNIPAm network above. In a different study, Castner et al. (26, 27) demonstrated temperature-controlled cell attachment/detachment using ~50–80 nm–thick crosslinked ppNIPAm films on TCPS, i.e., films that were substantially thicker than those in Okano's study. The apparent discrepancy between the results of the two groups suggests that other factors, such as layer architecture, must also be taken into account when one is considering cell behavior on pNIPAm surfaces.

The problem of incomplete dehydration of the outer regions of thick pNIPAm layers above the LCST was addressed by Chen et al. (28), who added short hydrophobic fragments to pNIPAm chains to enhance cell attachment. In particular, they synthesized brushes of an asymmetric diblock copolymer, pNIPAm-block-polystyrene (pNIPAm-b-PS), on silicon substrates by SI-ATRP. The resulting brushes with relatively short PS end blocks exhibited low fibrinogen adsorption (<8 ng cm^{-2}) over a broad temperature range. Despite the observed fibrinogen resistance, the pNIPAm-b-PS surface provided cell attachment and promoted cell growth above the LCST. At the same time, a homopolymer pNIPAm brush with similar thickness ($\sim$42 nm) showed very little cell adhesion above the critical temperature, implying that the outermost region of the brush remained largely hydrated. The majority of the cells that adhered to the pNIPAm-b-PS surface above the LCST detached as the temperature was lowered to room temperature. This result points to a delicate balance between hydrophobic and hydrophilic interactions, and the temperature change was sufficient to cause a shift in balance toward a more hydrophilic surface when the hydrophobic PS end blocks were concealed in the swollen pNIPAm brush.

Living tissues consist of multiple types of cells with a spatially ordered structure; thus, recent efforts have been directed toward the development of various cell patterning methods, which are overviewed in References 17 and 29. In a recent example of a cell patterning approach by Okano et al. (30), patterned cell sheets with a well-defined structure were produced using stripe-like micropatterns consisting of pNIPAm domains and pNIPAm-b-poly-*N*-acryloylmorpholine (PAcMo) domains. The micropatterns were fabricated using a combination of reversible addition-fragmentation chain transfer–mediated block copolymerization and photolithography. Because PAcMo is a hydrophilic polymer with cell-repellent properties, cells seeded onto the patterned surfaces adhered exclusively to the pNIPAm domains above the LCST. The patterned cell sheets with a well-defined structure were harvested from the pNIPAm surfaces by reducing the temperature below the LCST. Similar approaches were used for the fabrication of cocultured cell sheets containing different types of cells and tissue-like multilayered cell constructs using a dual-patterned polymeric surface (31, 32).

Polyelectrolyte surfaces. Polyelectrolyte layers represent a second common example of responsive surfaces for controlling protein adsorption and cell adhesion (33–37). The mechanism of protein adsorption on polyelectrolyte surfaces is rather complex (38–43), as demonstrated by the fact that negatively charged polyelectrolyte brushes, such as poly(acrylic acid) (PAA) and poly(styrene sulfate) (PSS) brushes, can strongly bind even negatively charged proteins at low ionic strength, whereas the same brushes exhibit virtually no protein adsorption at high ionic strength. Previous studies established that patches of positive charge on the protein surface play an important role in this phenomenon. Because the protein adsorption is accompanied by release of counterions, the resulting significant entropic gain prevails over electrostatic repulsion between the similarly charged proteins and brush surface and is thought to be the main driving force of the process. Furthermore, the secondary structure of proteins adsorbed in polyelectrolyte brushes was nearly undistorted and the proteins' activity was largely preserved (38, 41).

Positively charged poly[2-(dimethylamino)ethyl methacrylate] (PDMAEMA) brushes synthesized by SI-ATRP have demonstrated high adsorption capacity and rapid adsorption kinetics for net negatively charged BSA (44). BSA uptake was proportional to the amount of grafted PDMAEMA per unit area and approached the aqueous solubility limit for brushes at high grafting densities. Changes in pH and ionic strength were used to cause desorption of the proteins from the brush due to suppression of electrostatic interactions between the proteins and the brush. Adsorption on the PDMAEMA brushes was charge selective, as concluded from the zero adsorption of net positively charged enzyme lysozyme. In a different study, the combination

of poly(methacrylic acid) brushes and Si nanowire arrays allowed the creation of very efficient pH-controlled systems for protein adsorption and release (45). An 80-fold increase in the amount of adsorbed lysozyme was reported for such nanostructured surfaces compared with plane geometry. Releasing lysozyme from the system by increasing the solution pH was found to preserve the protein's initial activity.

Organic and Polymeric Materials with Signal-Triggered Transformation of Functional Groups or Deprotection of Ligands

In addition to pNIPAm surfaces, other smart polymeric or organic monolayer surfaces enabling growth and release of cells in response to temperature (46), light (47–54), electric potential (55–61), specific ion (62), and other signals have been developed. Some of those systems enable signal-triggered exposure of functional groups for the control of cell attachment. Examples of such systems are given in this and subsequent sections. We distinguish irreversible (one-way) and reversible (potentially reusable) systems.

One-way transition systems. Ulijn et al. (63) developed enzyme-responsive organic monolayers for controlling cell attachment. In their approach, cell-adhesive arginine–glycine–aspartic acid (RGD) moieties tethered to the monolayer were initially blocked by fluorenylmethoxycarbonyl groups, leading to no cell attachment. The subsequent deprotection of the RGD ligands by enzymatic hydrolysis exposed them to the surface, enabling cell attachment. Thus, the enzymatic signal was used to activate the surface.

Electroactive self-assembled monolayers (SAMs) for manipulating cell adhesion were explored by Mrksich and coworkers (55–57). In particular, they developed responsive SAMs with electroactive hydroquinone groups. By application of an electric potential, the nonadhesive hydroquinone groups were converted into quinone groups, which were then used for selective immobilization of RGD peptides via a Diels-Alder reaction (55, 56). In an alternative approach, RGD peptides were coupled to the monolayer surface via *O*-silyl hydroquinone ether moieties (57). The electroactive moieties allowed selective release of the primary RGD ligand from the surface by an electric signal, exposing benzoquinone groups to the surface. These groups were used to immobilize a secondary RGD ligand via a Diels-Alder reaction. The proposed approach for the modulation of ligand activity was used to pattern cells and activate cell migration.

SAMs that switch from a nonadhesive state to a cell-adhesive state by UV exposure have been developed by several groups. Mrksich's group (48) reported photoactive monolayers in which hydroquinone moieties were deprotected by exposure to UV light, thus enabling the selective immobilization of cell-adhesive ligands. In another example, SAMs with photocleavable 2-nitrobenzyl groups were used by Maeda et al. (47, 51) to generate photoactive surfaces for spatiotemporal control of cell adhesion. Such a photoactive SAM with hydrophobic terminal groups was covered with BSA, which rendered the surface with non–cell adhesive properties. The surface was then exposed to UV light through a photomask, resulting in cleavage of 2-nitrobenzyl groups and desorption of BSA from the surface of the irradiated regions. The subsequent adsorption of fibronectin on the unprotected surface made the irradiated regions adhesive to cells, allowing the production of cell patterns. Ohmuro-Matsuyama et al. (52) developed responsive interfaces in which RGD peptides immobilized to a culture dish via poly(ethylene glycol) (PEG) tethers were terminated (caged) with photoactive 2-nitrobenzyl groups. Photo-triggered cleavage of the photoactive group converted the initially nonadhesive surface to a cell-adhesive one due to the surface exposure of the RGD peptides.

Kanamori et al. (64) developed an in situ cell micropatterning method that allowed stepwise cell patterning using photoresponsive surfaces. In this approach, shown in **Figure 3*a***, cell-repellent

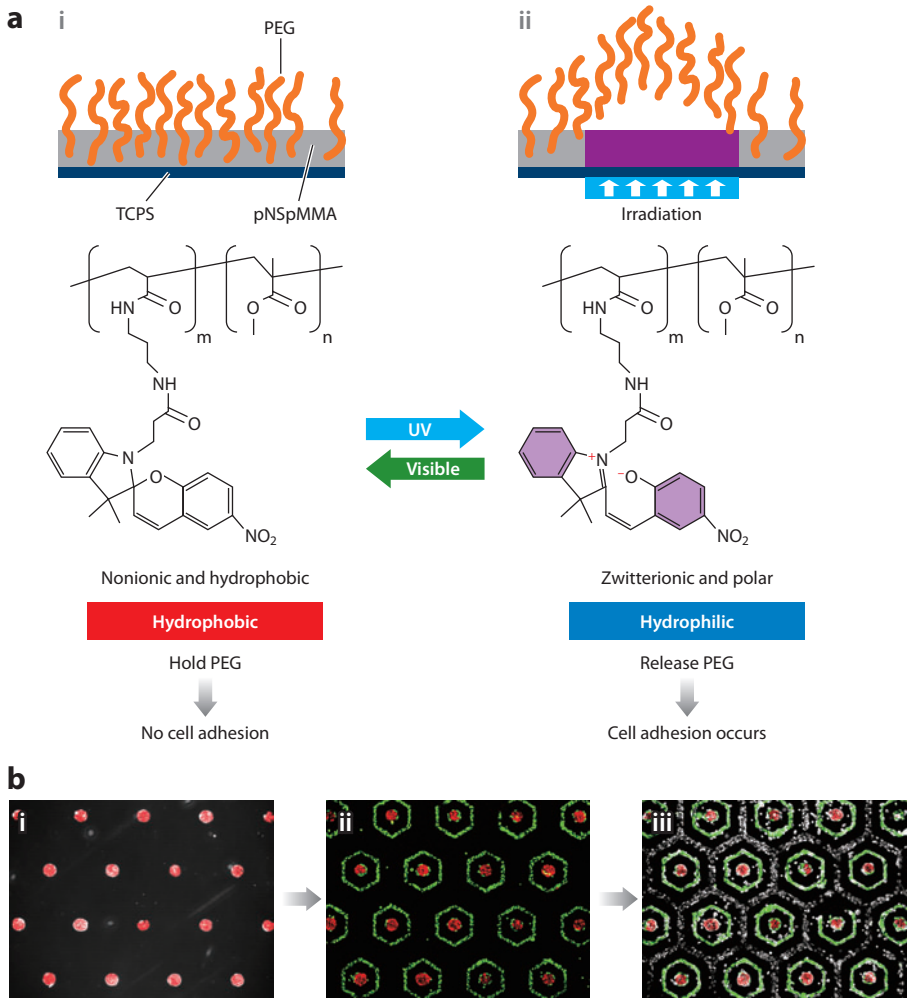

Figure 3

(*a*) Schematic of an in situ cell micropatterning system. (*i*) Cell-repellent PEG chains shield a photoresponsive pNSpMMA surface. (*ii*) UV irradiation causes chromophore isomerization and release of the PEG chains from the surface. The irradiated surface regions become hydrophobic after irradiation, enabling cell adhesion. (*b*) Three consecutive patterning steps using different types of cells as observed with a confocal laser scanning microscope: (*i*) a dot pattern of cells of the first type (stained with *red dye*), (*ii*) a hexagonal pattern of cells of the second type (stained with *green dye*), and (*iii*) a honeycomb pattern of cells of the third type (unstained). Reprinted with permission from Reference 64, copyright 2009, Wiley.

PEG chains were immobilized on the surface of a photoresponsive polymer, poly(nitrospyropyrane methyl methacrylate) (pNSpMMA). UV exposure via a photomask caused chromophore isomerization and release of the PEG chains from the surface of irradiated regions. The surface of the exposed regions turned hydrophilic due to the zwitterionic form of the spiropyran moiety. However, the surface gradually returned to the hydrophobic state after the UV light was turned off, creating favorable conditions for cell adhesion to the exposed regions. Three consecutive patterning steps using different types of cells were demonstrated (**Figure 3*b***), and cells were found to tolerate the patterning procedure well. In a different example, Ober et al. (65) studied

polyelectrolyte brushes with side groups containing photocleavable cationic moieties. The cleavage of these moieties upon UV irradiation led to the transformation of the cationic side groups into anionic ones. Flood exposure or masked exposure of the cationic brush to UV light resulted in the reversal of the brush charge or to a patterned cationic/anionic binary brush, respectively. The oppositely charged binary brush was used to prepare dual-protein micropatterns by electrostatic adsorption.

Reversible systems. Yousaf et al. (61) created electroactive quinine-terminated monolayers to reversibly capture and release ligands for protein and cell adhesion. In this system, the hydroquinone groups were electrochemically oxidized to quinines, resulting in the selective immobilization of oxyamine-tagged ligands via oxime conjugation. This enabled the ligand-mediated capture of proteins and cells. Furthermore, the oxime moieties were electrochemically reduced under physiological conditions (phosphate buffer solution, pH 7), causing the detachment of ligands from the surface and hence the release of proteins and cells. Importantly, it was possible to regenerate the monolayer to the hydroquinone form for subsequent immobilization and release. In a recent study, the same group demonstrated a switchable surface for the control of cell adhesion that could change cell affinity by altering the molecular structure of arginine–glycine–aspartic acid–serine (RGDS) peptides from cyclic to linear through a redox-responsive linkage (66).

Low-Density Monolayers with Signal-Controlled Conformation of Functionalized Molecules

For tissue engineering and protein separation applications, fabrication of biointerfaces that can reversibly expose and conceal bioadhesive sites would be beneficial. Such reusable systems have been recently demonstrated for low-density organic monolayers and polymer brushes.

Low-density organic monolayers were initially introduced as interfaces whose wettability can be controlled by an electric signal (67, 68). The reversible switching in wettability was due to electric field–induced transition between bent and straight conformational states in surface-immobilized flexible molecules that are capped with charged groups. Sufficient spacing between the molecules is necessary to enable such a transition. It can be achieved for mixed monolayers in which flexible stimuli-responsive chains are coimmobilized with much shorter molecules that dilute (increase an average distance between) the responsive chains tethered to the surface. In some cases, low-density molecular layers can be obtained by controlling the chemisorption process and ceasing it before a complete monolayer is formed. Because most SAM-forming compounds tend to crystallize, forming island-like 2D crystalline structures of densely packed molecules, alternative approaches have been developed, in which a complete monolayer is assembled from specially designed organic molecules bearing bulky cleavable side groups or from molecular complexes. In the former case, cleavage of the side groups provides the space needed for conformational transitions. In the latter case, the nonreactive, space-filling component of the complex is extracted from the assembled monolayer with the same outcome.

Kong et al. (69) extended the functionality of such low-density monolayers to control protein adsorption. They fabricated acid- and amino-terminated monolayers by assembling a preformed inclusion complex, cyclodextrin (CD)-wrapped alkanethiolate, on a gold substrate, followed by unwrapping the space-filling CD from the monolayers. The application of an electric potential allowed the transition between bent and straight conformational states in the surface-tethered molecules through electrostatic interactions between the ionized terminal groups and the charged gold substrate. As a result, these monolayers allowed electric field–triggered exposure and concealment of bioadhesive sites (charged groups interacting with oppositely charged proteins, in this

case), leading to the reversible and selective adsorption and release of proteins in response to an external signal. Furthermore, smart microfluidic chips for separation of two proteins with different isoelectric points in a mixture were demonstrated. When an electric potential was applied, one protein in the mixture was adsorbed on the chip surface while the other protein was allowed to flow away. The adsorbed protein was released from the surface in the next step by switching the polarity of the applied potential (70).

Mendes et al. (71) developed electroactive monolayers consisting of positively charged oligolysine peptides tethered to a gold electrode surface and end functionalized with specific biomolecular ligands (biotin). In the system, as shown in **Figure 4*a***, the peptide chains exposed or concealed the biotin sites in response to applied electric potential, dramatically altering the binding activity of biotin to neutravidin (**Figure 4*b***).

Organic and Polymeric Materials with Signal-Controlled Exposure/Concealment of Ligands

This section describes responsive surfaces in which specific ligands for biomolecular recognition are exposed to the surface or concealed inside the responsive layer on demand. We classify the existing systems as one-component and multicomponent systems.

One-component systems. In one-component systems, the availability of bioadhesive sites on the surface is controlled by an optical signal. Thus, Kessler et al. (50) prepared photoactive surfaces consisting of RGD-containing peptides anchored to the substrate surface via photoswitchable azobenzene-containing spacers (50). The photoactive surfaces enabled the reversible modulation of cell adhesion by irradiation with visible and UV light. In particular, the photoactive surface was cell adhesive with azobenzene units in the *trans*-conformational state. In contrast, a lower cell adhesion was observed in the *cis*-conformational state owing to greater proximity of RGD peptides to the substrate (shorter spacers made it more difficult for cells to access RGD sequences).

In a different example, cell-adhesive RGDS peptides were immobilized onto grafted poly(NIPAm-co-2-carboxyisopropylacrylamide) (pNIPAm-co-CIPAM) layers using the reaction between amino groups of the peptide and carboxyl groups of the copolymer (72). The pNIPAm-co-CIPAM surface with RGDS ligands facilitated the attachment and spreading of cells even in the absence of serum via affinity interactions between the exposed ligands and the integrin receptors of the cells above the LCST. The cells were spontaneously detached from the surfaces by decreasing the temperature below the LCST, as shown in **Figure 5**. Moreover, cell attachment and spreading were enhanced by the coimmobilization of RGDS and proline-histidine-serine-arginine-asparagine sequences onto the pNIPAm-co-CIPAM surface due to the synergistic role of the binding to integrin receptors (73).

Multicomponent systems. This subgroup of materials is typically represented by binary systems with two basic designs. In the first design, ligands are attached to the surface via stimuli-responsive flexible spacers capable of changing their conformation between the extended and compact states. The ligands are presented to the surface in the extended conformational state of the spacers. As the spacers shrink in response to an external signal, the ligands hide within the chains of a second nonresponsive component whose function is to suppress protein adsorption. In the second design, ligands are immobilized on the surface via spacers of intermediate length. These functionalized molecules are mixed with chains of a second, stimuli-responsive component. When the responsive component is switched to the compact conformational state, the spacer length is sufficient to bring

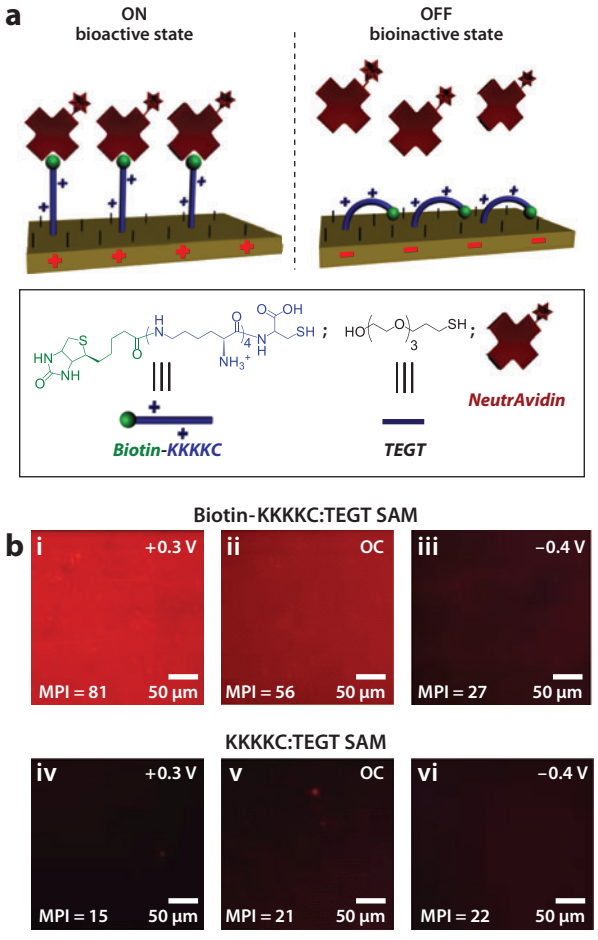

Figure 4

(*a*) Schematic showing a mixed SAM containing biotinylated oligolysine peptides (biotin-Lys-Lys-Lys-Lys-Cys, denoted as biotin-KKKKC) tethered to a gold electrode surface and OEG chains [tri(ethylene glycol)-terminated thiol, denoted as TEGT] and its reversible switching between bioactive and bioinactive states in response to applied electric potential. The positively charged side amino groups of the oligolysine peptides allow bending of biotin-KKKKC molecules toward the negatively charged electrode and concealment of the biotin end groups. (*b*) Fluorescence microscope images of mixed SAMs with end-functionalized biotinyl peptide chains (*i–iii*) and with nonfunctionalized peptide chains (*iv–vi*) when treated with fluorescent-tagged neutravidin while applying a voltage of +0.3 V (*i*,*iv*), under open-circuit (OC) conditions (*ii*,*v*), and while applying a voltage of −0.4 V (*iii*,*vi*). MPI denotes the mean pixel intensity. Reprinted with permission from Reference 71, copyright 2010, Wiley.

the ligands to the surface. Conversely, in the extended state, the ligands become hidden within the chains of the responsive component. Here we present several examples of such reversible systems.

Jiang and coworkers (54) reported mixed monolayers of OEG chains, and chains bearing azobenzene moieties and terminated with cell-adhesive RGD-containing peptides were proposed. Such photoactive surfaces allowed the reversible photoswitchable control of cell adhesion due to exposure of the peptides to the surface in the azobenzene moieties' *trans*-conformational state and concealment of the peptides in the OEG layer in the *cis* state. In another example of control of

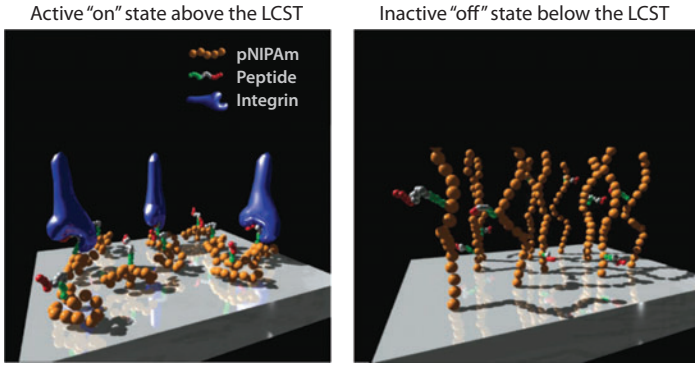

Figure 5

Systematic illustrations of thermoresponsive surfaces with switchable affinity interactions to cells. (*Left*) Above the LCST, the pNIPAm-co-CIPAM surface with RGDS facilitates the spreading of cells via affinity interactions between the exposed RGDS ligands and the integrin receptors. (*Right*) The cells are spontaneously detached from the surfaces by decreasing the temperature below the LCST. Reprinted with permission from Reference 73, copyright 2008, Wiley.

biomolecular binding events, Bulmus et al. (74) used mixed monolayers of thermoresponsive OEG and biotinylated disulfide on a gold substrate (see **Figure 6**). The adsorption of streptavidin on such monolayers increased ~29-fold when the temperature was increased from below to above the LCST of OEG. The increase in adsorption of streptavidin at high temperatures was attributed to the increased availability of biotin molecules exposed to the surface due to the collapse of neighboring OEG chains.

In many studies, stimuli-responsive pNIPAm brushes were employed to control biomolecular binding events. In particular, affinity chromatographic systems for protein separation based on stimuli-responsive polymers grafted on the surface of beads were reviewed by Okano et al. (75) and Mattiasson et al. (76). In one example of such systems, pNIPAm brushes were immobilized on the surface of polymer beads along with cibacron blue, an albumin ligand (77). The latter was immobilized to the surface via flexible spacers of an appropriate length. Above the LCST, when pNIPAm chains were in the collapsed state, the ligand was exposed to the surface, and significant amounts of albumin were adsorbed. As the temperature decreased below the LCST, the proteins tended to desorb due to the kicking-off effect of the swelling pNIPAm brush. In another example of affinity chromatography, thermoresponsive coatings consisting of NIPAm copolymers with an affinity ligand, *Ricinus communis* agglutinin (RCA_{120}), and lactose were synthesized on a bead surface (78). The target, asialotransferrin, was captured below the LCST, whereas most of this glycoprotein was eluted above the critical temperature because the surface-immobilized lactose displaced the ligand-bound glycoprotein molecules due to the close proximity of immobilized ligands and lactose in the collapsed pNIPAm brushes. Therefore, these experimental chromatographic systems allowed control of the binding affinity between ligands and specific proteins due to thermally triggered conformational changes in pNIPAm brushes.

In a more recent study, Diez et al. (79) demonstrated a biomimetic interface that controls adsorption and motility of microtubules using thermoresponsive pNIPAm brushes and kinesin molecules incorporated between pNIPAm chains (**Figure 7**). The reversible switching of microtubule motility was realized with this artificial system: Microtubules landed and glided freely on the kinesin molecules above the LCST, i.e., when the pNIPAm brush was collapsed, and were released from the surface, not being able to bind to the kinesin heads, below the critical temperature, i.e., when the brush was swollen.

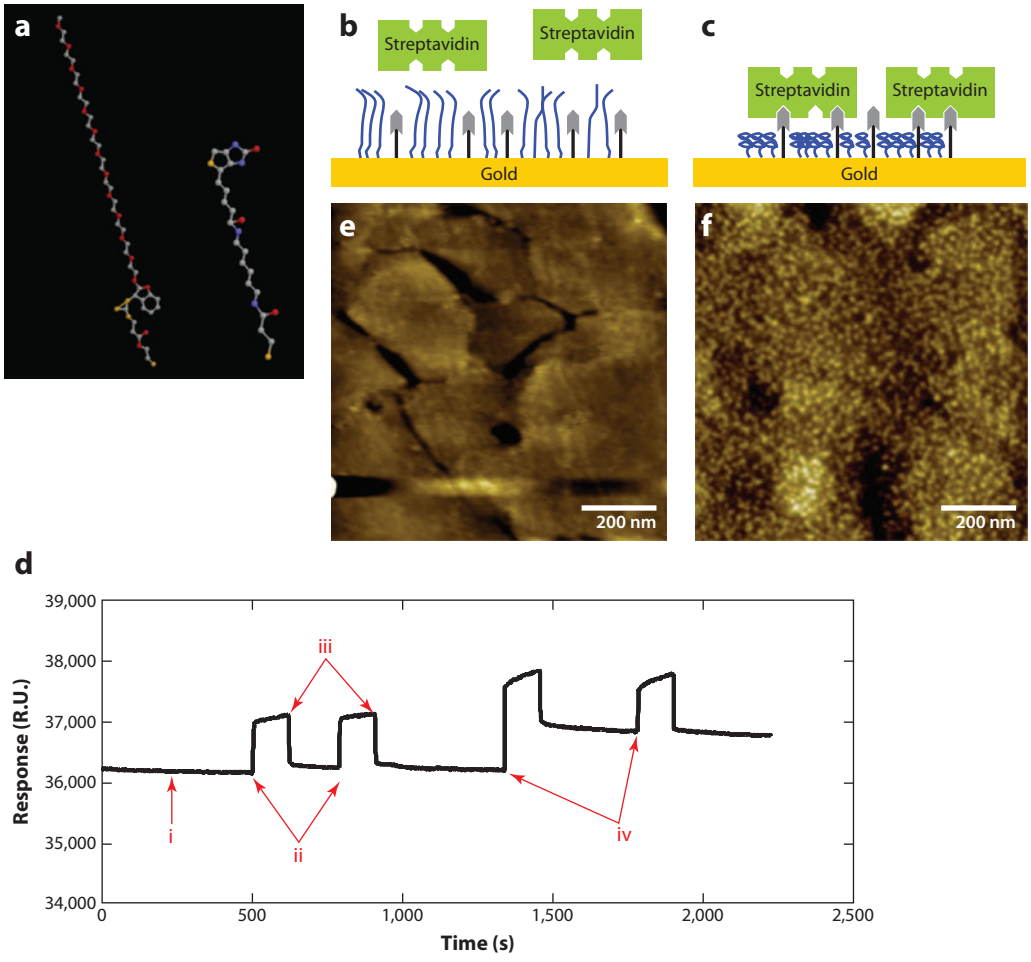

Figure 6

(*a*) Chemical structures of SAM components: 12-unit OEG with a pyridyldisulfide end group (*left*) and biotinylated disulfide (termed Ez-link, *right*). (*b*,*c*) Schematic showing a mixed OEG-Ez-link monolayer in which relatively long OEG chains are (*b*) swollen below the LCST, hiding the biotin functionality in the layer, and (*c*) collapsed above the LCST, exposing biotin to the surface and allowing streptavidin binding. (*d*) Data of surface plasmon resonance (SPR) measurements of streptavidin adsorption below and above the LCST. The arrows show injections of (*i*) water, (*ii*,*iii*) streptavidin solution and water, respectively, below the LCST, and (*iv*) streptavidin solution followed by water rinse above the LCST. (*e*,*f*) In situ AFM images of the surface after injection of a streptavidin solution (*e*) below and (*f*) above the LCST. Reprinted with permission from Reference 74, copyright 2008, ACS.

SMART SURFACES WITH RESPONSIVE EFFECTS AT MESOSCALE

In this section, we consider polymeric systems whose functionality relies on volumetric swelling and microphase transitions of surface-confined polymers. This category includes hydrogel thin films and polymer brushes with stimuli-induced changes occurring at mesoscale.

Versatile stimuli-responsive interfaces with complex phase-segregation behavior were demonstrated for mixed polymer brushes composed of two or more polymeric components (80–82). The phase segregation between incompatible polymers constituting the brush caused their lateral and vertical rearrangement so that polymer chain fragments of one of the polymers or both

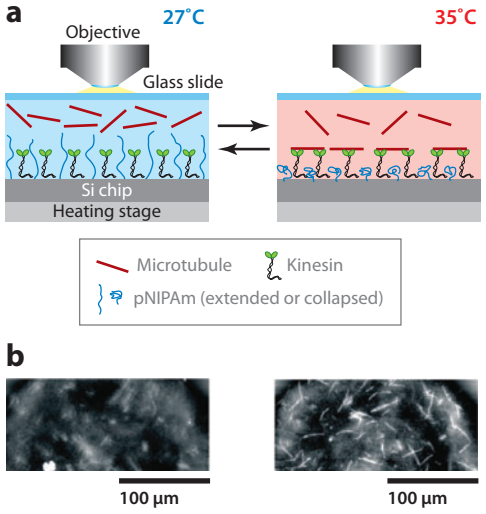

Figure 7

(*a*) Schematic showing binding and gliding motility of microtubules on a thermoresponsive surface consisting of grafted pNIPAm chains and adsorbed kinesin. Reversible thermal switching of the pNIPAm chains between the extended and collapsed conformational states allowed for the modulation of microtubule binding affinity to kinesin. (*b*) Fluorescence micrographs of dye-labeled microtubules on the thermoresponsive surface. No bound microtubules were observed below the LCST (*left*), whereas gliding microtubules could be seen above the critical temperature (*right*). Reprinted with permission from Reference 79, copyright 2006, ACS.

polymers populated the brush-medium interface, depending on environmental conditions. This chain arrangement could be reversibly switched or tuned by changing the quality of a solvent or by other external factors, resulting in the surface being enriched with certain polymeric fragments as well as in different surface morphologies. These molecular changes translated into changes of macroscopic surface properties, such as wetting, adhesion, and surface charge, and led to the formation of geometric and chemical nanoscale patterns. The swelling and collapse of polymeric chains in response to external stimuli changed the permeability of homopolymer and mixed-brush layers to ions, and this property could be explored for the creation of electrochemical sensors and biosensors (83–94). In the next subsection, we provide several recent examples of the use of mixed polymer brushes for the regulation of protein adsorption.

The confinement of polymeric materials to the surface has a profound effect on their swelling properties. It is appropriate to compare the swelling of a bulk gel and a gel thin film immobilized on a solid support. The swelling of the isotropic bulk gel takes place uniformly in all directions, whereas in the case of the gel thin film, the lateral (in-plane) swelling is restricted by the attachment (chemical or physical) of the gel to the substrate surface. As a result, the thin film can expand only in the direction normal to the substrate plane, thus exhibiting anisotropic swelling behavior. There are several main consequences of the surface confinement for gel thin films. First, the swelling degree, defined as the relative change in volume of a film as it progresses from the shrunken to the swollen state, is smaller for thin films compared with bulk gels. At the same time, the linear expansion of the gel thin film exceeds that of its bulk counterpart, assuming the changes are in one dimension only. The latter property is important in polymeric systems that regulate mass transport across porous filtration membranes, discussed hereafter in this section. Second, the lateral confinement may affect the conditions (temperature, pH, etc.) at which the

swelling-shrinking transition takes place and may even limit the ability of the film to deswell. These factors should be taken into account when one is designing thin-film hydrogel devices. Third, an osmotic pressure in a swollen gel imposes a strong biaxial compressive stress on the film in the lateral dimension. The mechanical stress is sufficient to cause the deformation of flexible components of micro-electro-mechanical system devices (e.g., cantilevers and membranes) coated on one side with the gel film. This approach was used to create highly sensitive biosensors. The same mechanical stress may cause elastic creasing instabilities on the free surface of a film prepared on a rigid support (95). The resulting well-defined topographic structures have been used for biological patterning. Films with prefabricated topographic patterns, in particular those with an array of continuous circular pores in the gel layer, use the free space provided by the pores for swelling. Planar compressive forces in the film cause the gel to expand toward the pore interior, thus causing pore contraction. This distinctive property of the patterned gel films has been explored for the regulation of mass transport.

Hereafter we review some recent examples of polymeric systems based on the aforementioned principles.

Mixed Polymer Brushes

Stamm et al. (96) developed switchable mixed-polymer-brush interfaces consisting of two oppositely charged weak polyelectrolytes, poly(2-vinyl pyridine) (P2VP) and PAA. The P2VP-PAA brushes exhibited amphiphilic behavior; an isoelectric point of the brush was 4.9 (97). The surface charge, surface tension, and extent of swelling of these brush layers could be varied as a function of the pH or salt concentration of a surrounding aqueous solution. Furthermore, in a high–salt concentration regime, protein adsorption on the surface was similar to that on hydrophobic surfaces, whereas at low ionic strength, the system's adsorption properties were determined by the delicate pH-dependent balance of electrostatic and steric repulsive interactions (opposing protein adsorption) and a strong entropic component related largely to counterion release occurring as proteins penetrated the brush (favoring adsorption). In a related study, binary P2VP-PAA brushes with composition gradients were investigated as a convenient platform for creating concentration gradients of adsorbed proteins (98). Such brushes showed a range of useful pH-controlled surface properties, including reversible tuning and switching on/off of protein adsorption and switching the direction of an adsorption gradient.

Mixed polymer brushes that change their surface properties in response to several environmental stimuli have also been developed. For example, binary brushes consisting of thermoresponsive polymer pNIPAm and a pH- and salt-sensitive polyelectrolyte, PAA (99) or P2VP (6), showed complex responsive behavior. These surfaces were examined for the regulation of protein adsorption in response to temperature changes. Protein adsorption on mixed brushes was enhanced compared with one-component pNIPAm brushes, indicating a significant influence on the adsorption process by the polyelectrolyte component. The adsorption amount could be controlled by temperature due to the thermoresponsive pNIPAm component.

Tuning of strength and a range of hydrophobic interactions were demonstrated with specially engineered multicomponent brushes (100). These responsive brush interfaces allowed the manipulation of interactions with proteins in an aqueous environment. To create such interfaces, a mixed polymer brush consisting of a homopolymer, PEG, and an amphiphilic diblock copolymer, PAA-b-PS, was used (**Figure 8*a***). The grafted PEG component formed a hydrophilic protein-repellent surface, whereas the grafted PAA-b-PS copolymer self-assembled into a micellar structure, in which a nanometer-sized hydrophobic PS domain was linked to the grafting surface via multiple stimuli-responsive PAA legs. At specified conditions (pH, ionic strength of media, and presence of

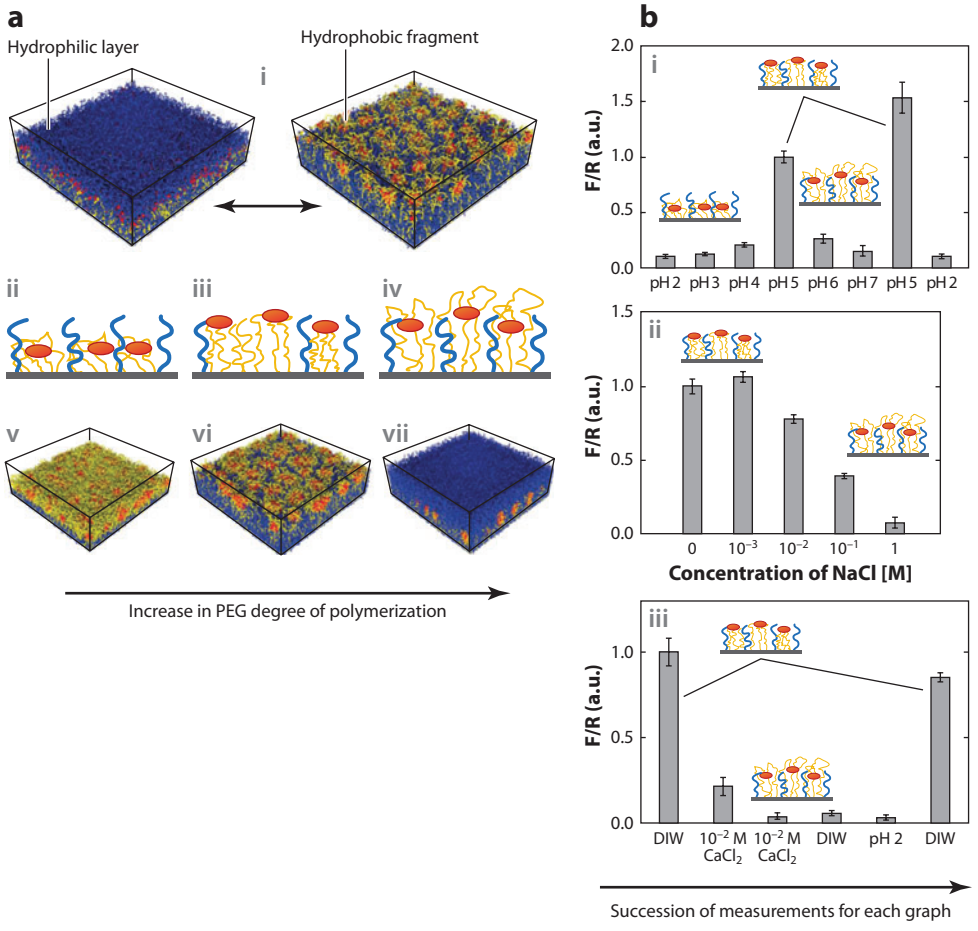

Figure 8

(*a*) (*i*) Responsive hydrophilic polymer layer with incorporated hydrophobic fragments. Results of single-chain-in-mean-field (SCMF) simulations for a PEG/PAA-b-PS mixed brush placed in a solvent (*left*) that is good for PEG (*blue*) and bad for PS (*red*) and PAA (*yellow*), and a solvent (*right*) that is good for PEG and PAA, but bad for PS. (*ii–iv*) Schematic representation of the three different states that a mixed polymer brush exhibits upon increase in pH and change of ionic strength: (*ii*) low pH, PAA chains are collapsed; (*iii*) pK region, PAA chains start to stretch; and (*iv*) high pH or high ionic strength, PAA chains are highly extended. (*v–vii*) Configuration snapshots of SCMF simulations for a PEG/PAA-b-PS mixed brush in a good solvent for PAA, where the chain length ratio between the diblock, PAA-b-PS, and the homopolymer, PEG, is varied: (*v*) 8, (*vi*) 3, and (*vii*) 2. The ratio of grafting densities of PEG/PAA-b-PS is 3. (*b*) Adhesion forces between the hydrophobic glass bead and the surface of the mixed brush versus (*i*) pH, (*ii*) concentration of NaCl at pH 5, and (*iii*) presence of Ca^{2+} ions at pH 5. Reprinted with permission from Reference 100, copyright 2010, Wiley.

calcium ions), the hydrophobic PS domains were brought inside the hydrophilic PEG layer by the collapsed PAA legs. When conditions were changed, these domains were pushed out to the layer surface, due to PAA swelling. Reversible switching in the interfacial position of the hydrophobic fragments in response to environmental signals was used to turn protein adsorption on and off. Furthermore, the strength and range of hydrophobic interactions could be finely tuned by altering the distance of the hydrophobic domain from the solid substrate.

Surface-Creasing Instabilities for Reversible Biochemical Patterning and Adhesives

Hayward et al. (101) used the creasing phenomenon in hydrogel thin films to create reversible biochemical patterns. They proposed that the surface of a temperature-responsive pNIPAm-based hydrogel thin film can be patterned with biological ligands using a two-step modification procedure. In the first step, the free surface of a swollen hydrogel film is protected with a biocompatible polymer layer. In the second step, when the gel film is caused to shrink and unfold by a temperature trigger, the surface regions that were hidden inside tight folds, and thus unprotected by the initial polymer layer, are made accessible for modification with another polymer-bearing ligand group. Functionalized this way, the coatings can reversibly hide and display the biochemical pattern in response to temperature changes near the transition range of pNIPAm. The authors demonstrated that such dynamic structures could perform a number of tasks, such as dynamic selective capture, sequestration and release of micrometer-sized objects, tunable activity of surface-immobilized enzymes, and reversible encapsulation of cells. Importantly, the positions of creases in the gel film could be preprogrammed by a lithographically fabricated topographic pattern on the solid substrate, thus offering precise control over the placement of functional groups on the gel surface.

Elastic creasing instabilities in polymer thin films have been found to be useful for creating reusable smart adhesives whose adhesion to surfaces are enhanced compared with flat surfaces owing to the mechanism of contact splitting (an increase in total contact perimeter rather than an increase in contact area) (102). The splitting enhancement, well known from studies of sticky feet of geckos and certain insects, was greatest when the characteristic wavelength of creases was smallest, which could be tuned by changing film properties. Similar adhesives made of a hydrogel can change their wrinkle morphology upon swelling in water (103). When such an adhesive was in contact with a hydrated, compliant material, the contact area increased with contact time due to wrinkling of the adhesive that adsorbed water from the underlying material. This was accompanied by embossing the crease relief into the material. Enhanced adhesion was observed upon debonding, which was explained by local pinning of crack propagation by surface creases. This adhesion enhancement mechanism is promising for the creation of biomedical tape-based adhesives for soft tissues.

Microcantilever-Based Biosensors

Since Peppas and coworkers (104) published their results on an ultrasensitive pH sensor based on the combination of flexible Si microcantilevers and pH-responsive hydrogels immobilized on one of the cantilever's sides, this sensing scheme has been explored by many research groups. Buchapudi et al. (105) offer an up-to-date overview of microcantilever biosensors for detection of biomolecules and microorganisms. Although the bulk of studies have been done using SAMs as sensing layers, the use of responsive polymer films often results in higher sensitivity toward targeted species. High flexibility of micromachined cantilevers allows the efficient transduction of even minute changes in the swelling-induced compressive stress of surface-grafted hydrogel and brush layers into measurable bending of a cantilever. An optical, laser-based reflection system (such as the one used in atomic force microscopy) is used for monitoring the bending response. In this section, we restrict ourselves to a recent example of a glucose biosensor in which a copolymer brush with a block bearing phenylboronic acid (PBA) ligands was used as a glucose sensing layer (106). PBA is known to bind diols through the formation of reversible boronate ester bonds. Such brushes exhibited a large, reversible swelling response to glucose at physiologically relevant

concentrations, which translated into reliably measured bending of microcantilevers. The response was substantially higher than that for cantilevers modified with PBA-conjugated SAMs.

Tunable Filtration Membranes

The coupling of responsive polymer layers with porous polymer and inorganic membranes results in composite membranes whose permeability for solvents and solutes can be tuned by external stimuli. This combination is of particular interest for biomedical and biotechnological applications, in particular in separation of valuable proteins, membrane chromatography, and biocatalysis. In the case of bioseparation, the most common design is an asymmetric one that combines a relatively thick (typically in the range of tens to hundreds of micrometers) micro- or macroporous membrane (polymer or inorganic) and a thin (typically <1 μm) layer of a responsive polymer immobilized on one of the membrane's sides. The former serves as a mechanically stable support, whereas the latter forms a selective layer (often referred to as "skin") that defines the rejection properties of the resulting composite membrane. The porosity and thickness of the skin layer have to be maximized and minimized, respectively, to achieve high permeation rates for filtrated molecules. Detailed discussion of stimuli-responsive membranes and their potential applications is provided in several up-to-date reviews (107–109). Here we restrict ourselves to a number of recent examples of tunable membranes specifically designed for the concentration and fractionation of biomacromolecules as well as the capture of viruses and other colloidal impurities in protein purification processes. In these applications, pore size distribution and charge become crucial characteristics. In particular, nearly monodisperse pores are required to achieve sharp cut-off values and hence enable the efficient separation of different solutes on the basis of their size or molecular weight (size-selective filtration). The separation efficiency can be enhanced if solutes to be separated are charged differently (charge-selective filtration). For membranes whose pore walls bear weak acid or base groups, the solute permeation can be controlled or tuned by the pH and the ionic strength of a solution.

Peinemann et al. (110, 111) reported an elegant approach for the fabrication of pH-responsive asymmetric membranes with a skin layer consisting of uniform nanochannels (<10 nm in diameter and 400 nm in length) with narrow size distribution and at exceptionally high packing density ($>2 \cdot 10^{14}$ pores m^{-2}). The conventional method of membrane fabrication by phase inversion was used in combination with block copolymer self-organization to produce the well-ordered, self-supporting membranes. No postmodification was required in this approach. Water flux through the membrane nanochannels could be reversibly tuned by more than two orders of magnitude by changing the solution pH owing to a pH-sensitive brush of P2VP block decorating the channel walls—a property that may be explored for drug delivery devices. The pH control could be used to tune membrane cut-off values for macromolecules in a broad range of molecular weights. Similar approaches for the fabrication of monodisperse membranes have also been demonstrated for different block copolymers by other groups (112, 113). Blending a block copolymer with a homopolymer [poly(4-vinyl pyridine) (P4VP) in one study] that selectively partitioned into the pore-forming P4VP domain of the phase-segregated copolymer allowed the production of membranes with larger pore sizes, covering the range between 16 and 30 nm (112). In another example, double stimuli-responsive monodisperse membranes with a pore size of <100 nm and tunable cut-off values were prepared using a diblock copolymer with a temperature- and pH-sensitive PDMAEMA block (113). These materials may find applications for capturing viruses, bacteria, and colloidal particles.

Furthermore, commercial membranes whose pore walls are modified with a stimuli-responsive poly(*N*-vinyl lactam) hydrogel have been demonstrated to be suitable for protein purification and

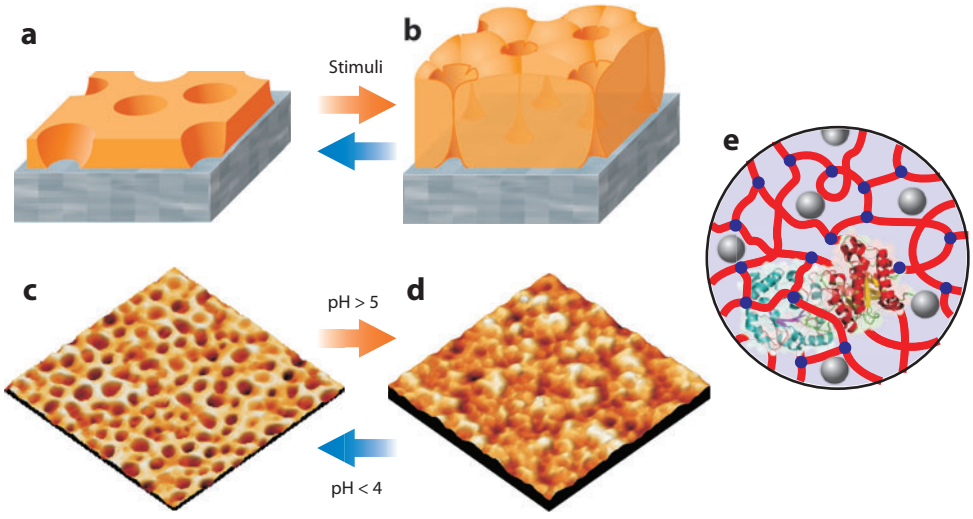

Figure 9

(*a*,*b*) Sketch showing a stimuli-responsive thin gel membrane whose pore size can be tuned in response to external stimuli: (*a*) the shrunken state and (*b*) the swollen state of a thin gel membrane. (*c*,*d*) AFM topography images of the ionically crosslinked alginate membrane in water (*c*) at pH < 4 and (*d*) at pH > 5. (*e*) The hydrogel network can serve as a container for incorporation of enzymes and as a reactor for synthesis of inorganic nanoparticles.

multicomponent protein separation (membrane chromatography) (114–116). In the presence of lyotropic salts, this initially hydrophilic hydrogel underwent hydrophobic collapse. The addition of the salts decreased the LCST of poly(*N*-vinyl lactam). The hydrophilic-hydrophobic transition allowed the selective capture of antibodies from the cell culture milieu (114, 115). The transition was reversible, enabling the recovery of the captured proteins during the elution step (no salt was present). Because proteins adsorbed on the pore walls of the membranes, their specific surface had to be maximized to achieve high recovery efficiency. In this example, the membrane operation relied not on volumetric changes, resulting in regulation of pore size, but on changes in the surface polarity, leading to regulation of the affinity of proteins to the pore walls. In this regard, the system belongs to the group of materials considered above in the section addressing polymeric materials with stimuli-controlled changes in surface polarity or charge. The fractionation of proteins in binary mixtures using poly(*N*-vinyl lactam) hydrogel membranes has also been demonstrated (116). In one such case, salt was added at different concentrations to cause the partial collapse of the hydrogel layer onto the pore walls. The corresponding changes in pore size allowed tuning of the sieving coefficients for several proteins with different hydrodynamic radii.

An alternative approach to membranes with tunable pore size involves responsive gel thin films with continuous circular pores (**Figure 9*a***) that are immobilized as skin layers onto mechanically stable macroporous supports (117–119). The operation of these hybrid membranes differs from the ones described above with regard to the way the pore is regulated. As the surface-attached porous gel layer swells, it expands both in the vertical direction and toward the pore interior, causing pores to shrink (**Figure 9*b***). The characteristic size of shrunken pores depends on the extent of swelling of the polymer network and can be regulated from partially to completely closed pores. The shrunken pores adopt a distinctive bottleneck shape (**Figure 9*b***), with the narrowest

part defining the rejection properties of the membrane. Thin-film design provides the membranes with a low hydrodynamic resistance. The ability to tune pore dimensions and membrane porosity may be used to create separation membranes, whose sieving and mass-transport properties can be tailored specifically to a given task (e.g., separation of protein mixtures or control of drug release).

Two different kinds of membranes have been demonstrated based on the above-described concept: chemically crosslinked P2VP membranes (118–121) and ionically crosslinked alginate membranes (117). These thin-film membranes were prepared through the liquid-liquid phase separation process in solutions containing a membrane-forming polymer (P2VP or alginate) and a pore-forming additive (1,4-diodobutane in the case of P2VP and gelatin in the case of alginate). The subsequent crosslinking and extraction of additive resulted in porous gel membranes. Both kinds of membranes showed reversible changes in pore size and porosity in response to changes in the solution pH (**Figure 9*c*** and ***d***); this property was used to regulate permeability of water through the membranes (117–119). Furthermore, pores of P2VP gel membranes shrank upon addition of certain low-molar-mass hydrophobic chemicals (e.g., cholesterol), whose molecules were able to penetrate into the network and form hydrogen bonds with pyridine groups of P2VP (120). In this case, pore size depended on the concentration of the chemisorbed chemical. The gel body of the membrane was used as a chemical reactor to synthesize inorganic particles (gold and silver) and to immobilize functional molecules (enzymes) on the gel surface using reactive groups of the network (**Figure 9*e***) (117, 121). These modification possibilities considerably extend the functional properties of the gel membranes beyond the regulation of mass transport. Antimicrobial, biocatalytic (117), and sensing (using plasmonic effects in noble metal nanoparticles) (121) properties were demonstrated for these membranes. The membranes made of alginate also had important advantages of biocompatibility and antifouling properties (117).

Plasmonic Biosensors

Reversible swelling-shrinking transitions in polyelectrolyte brushes and hydrogel thin films have been used for the microactuating mechanism of noble metal (gold or silver) nanoparticles embedded in the layer. The nanoparticles deposited on the top of the polyelectrolyte brush could be actuated by stretching and shrinking of polyelectrolyte chains caused by pH changes, so that the average distance between the nanoparticles and the grafting surface increases when the brush is ionized and swollen, and decreases when the brush collapses to the grafting surface. If noble metal clusters (termed nanoislands) are deposited on the solid substrate–polyelectrolyte brush interface (grafting surface), the microactuation results in a pH-induced change in the average distance between the noble metal nanoparticles embedded in the brush and the nanoislands deposited on the grafting surface. These changes in electromagnetic interactions (known as plasmon coupling) between the nanoparticles and nanoislands result in a pronounced optical response by the thin film (a 50 nm shift in the absorption maximum in the UV-visible spectrum). This approach, termed in the literature noble metal nanoparticle–enhanced transmission localized surface plasmon resonance (T-LSPR) spectroscopy, has been used to develop nanosensors that monitor pH changes (122), the concentration of cholesterol (123), and the concentration of glucose using the enzymatic reaction of glucose oxidase (GOx) (124, 125).

In one of the relevant examples, the plasmon-sensing platform, shown in **Figure 10*a***, consisted of a transparent glass support decorated with silver nanoislands, a 20–25 nm–thick, pH-responsive hydrogel film, and silver nanoparticles encapsulated within the film (124). In that study, a hydrogel material composed of an ionically crosslinked alginate-gelatin complex, which shows a swelling transition in the pH range in which most enzymes remain catalytically active, was used. The

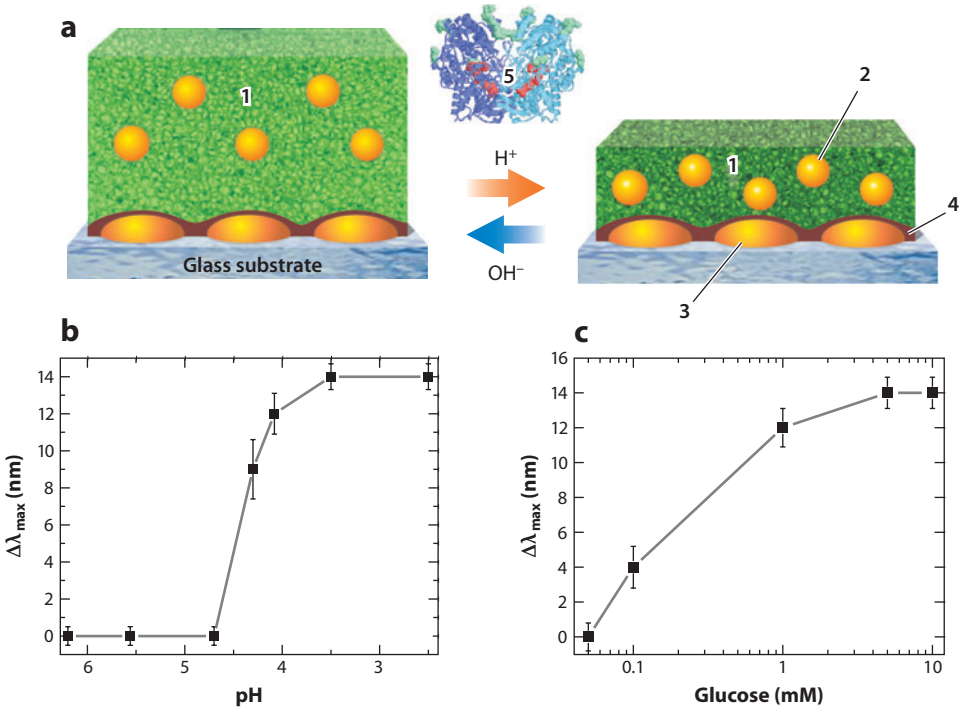

Figure 10

(*a*) Enzymatic sensor consisting of (*1*) an ultrathin alginate–gelatin gel film (*2*) with silver nanoparticles (*3*) immobilized on silver nanoislands (*4*) via an adhesive polymer layer. The gel film undergoes a reversible swelling in response to pH changes caused by the biocatalytic reaction of GOx (*5*) and glucose. (*b*) Shift of the absorbance maximum ($\Delta\lambda_{max}$) as a function of pH; the solution contained 0.3 M NaCl to suppress swelling. (*c*) Shift of $\Delta\lambda_{max}$ at $\lambda = 470$ nm as a function of the glucose concentration in a glucose solution containing 15 units of GOx and 0.3 M NaCl. Reprinted with permission from Reference 124, copyright 2010, Wiley.

alginate-gelatin gel films served as chemical reactors for the synthesis of silver nanoparticles (with an average size of ~11 nm). The swelling-shrinking transition in this film was driven by a pH change that was produced by biocatalytic oxidation of glucose with GOx. The hydrogel-mediated changes in the interparticle spacing altered the strength of the plasmon coupling and hence caused the modification of the absorption spectrum (**Figure 10*b*,*c***). In addition, the surface of the sensing hydrogel film was found to resist protein adsorption. In another example, gold nanoislands on a glass support and gold nanoparticles were integrated via a pH-responsive PDMAEMA brush to create an enzymatic biosensor operating in near-physiological pH range (125). The material of the sensing layer was chosen because PDMAEMA is known to undergo the coil-to-globule transition at pH values close to neutral pH.

These examples demonstrate that the combination of a biocatalytic process, a pH-responsive polymer ultrathin film, and noble metal nanoparticle–enhanced T-LSPR spectroscopy was successfully used for the development of a biosensor platform.

CONCLUSION AND FUTURE PROSPECTS

This review covers 5–7 recent years of research in the challenging area of responsive and switchable biointerfaces for biomedical applications. Starting from conventional antifouling surface coatings,

a number of teams developed surfaces with stimuli-responsive functions when various functional groups (nonspecific or specific) were used for engineering surface response and interactions with life systems. In general, hydrophilic surfaces that are nonreactive to proteins or cells are alternated by adding functional molecules that can undergo conformational or phase transition in such a way that they expose demanded molecular fragments or even phases at the interface upon external signal. In many cases, hydrophobic fragments, electrically charged groups, or polypeptide chains were used for the stimuli-triggered exposure at interface. In this way, nonspecific hydrophobic and electrostatic interactions or specific antibody-antigen interactions were switched on to bind proteins and cells by external signals. This interaction could be irreversible or reversible, if a proper balance between attractive interactions and steric repulsion was achieved in the latter case.

The platform for a reconfigurable biointerface that combines three major components—antifouling coatings, domains that can be named stimuli-responsive delivery vehicles, and biofunctional cargo—seems to be the most functional example of a stimuli-responsive biointerface. In this platform, the initially bioinert surface carries bioactive functional groups that are hidden by the antifouling coatings. Upon external signal, this biointerface releases the functional groups when external stimuli cause the delivery domains to expose the functional cargo to the interface.

Combining surface modification methods with lithography, 2D spatially arranged stimuli-responsive biointerfaces have been fabricated. This recent approach attempts to address issues of a complex structure of cell and tissues.

Because the range of physical parameters for proper function of biological systems is quite narrow, the task of engineering stimuli-responsive biointerfaces includes the added challenge of designing reconfigurable biocompatible materials that undergo demanded changes within these constraints. There are a limited number of basic platforms for such responsive systems, including temperature-dependent phase transitions (e.g., pNIPAm) in a physiological range of temperatures, photochemical isomerization or cleavage of chemical bonds, pH- and counterion-responsive phase behavior of polyelectrolytes, electrochemical cleavage of chemical bonds, electrochemical generation of local (at the electrode surface) changes in concentration of ions (pH, polyvalent metal ions, and organic ions), and magnetic actuations of magnetic structures. The most studied are pNIPAm-based systems; to date there is little research on the other systems. All the systems listed above have limitations. There is no universal platform; hence, selection of platform will depend on specific applications.

The applications of responsive interfaces have addressed two major building blocks: proteins and cells. The majority of studies focus on switching on and off protein adsorption/desorption and cell attachment/detachment. The successful development of stimuli-responsive surfaces has produced a number of new applications that use switchable surfaces in combination with other important properties of the building blocks such as signaling (stem cell stimulation), biocatalytic (switchable heterogeneous catalysis at biointerface), electrochemical (electrochemical biosensors), bioelectrochemical (biofuel cells), optical (plasmonic biosensors), mechanical (microactuators, buckling, folding interfaces), and sieving (selective membranes, drug delivery capsules) properties, to name a few.

It is likely that future developments will be based on multicomponent and multifunctional, hierarchically organized biointerfaces that combine properties of functional materials and devices. Many important applications may be developed on the basis of implementation of biomimetic concepts into responsive surfaces in the near future. We foresee development of smart surfaces that can, for example, recognize specific biological signals, selectively separate biological molecules, release functional groups or proteins, and change mechanical properties.

SUMMARY POINTS

1. Smart biointerfaces that (*a*) switch their surface properties due to stimuli-triggered changes in molecular conformations or chemical bonds in functional molecules and (*b*) undergo reversible stimuli-triggered reconstruction at mesoscale due to the stimuli-responsive phase behavior are considered.
2. A very narrow parameter window for proper function of biological systems implies heavy restrictions on materials and designs of stimuli-responsive biointerfaces.
3. There is no universal platform; hence, selection of biointerface design will depend on specific applications.
4. A complex structure of tissues can be mimicked in 2D spatially arranged biointerfaces fabricated by the combination of surface modification techniques and lithography.
5. An attractive platform for a reconfigurable biointerface combines three major components: antifouling coatings, biofunctional cargo, and stimuli-responsive domains that deliver the cargo to the surface or hide it inside the antifouling coatings on demand.
6. The successful development of stimuli-responsive biointerfaces is bringing a number of new applications in the areas of stem cell stimulation, biocatalysis, biosensors, microactuators, selective membranes, drug delivery capsules, and many others.
7. Future developments will be based on multicomponent and multifunctional, hierarchically organized biointerfaces that combine properties of functional materials and devices.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of the National Science Foundation (NSF) via grants CBET-0966526 and DMR-1107786.

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Materials Research

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