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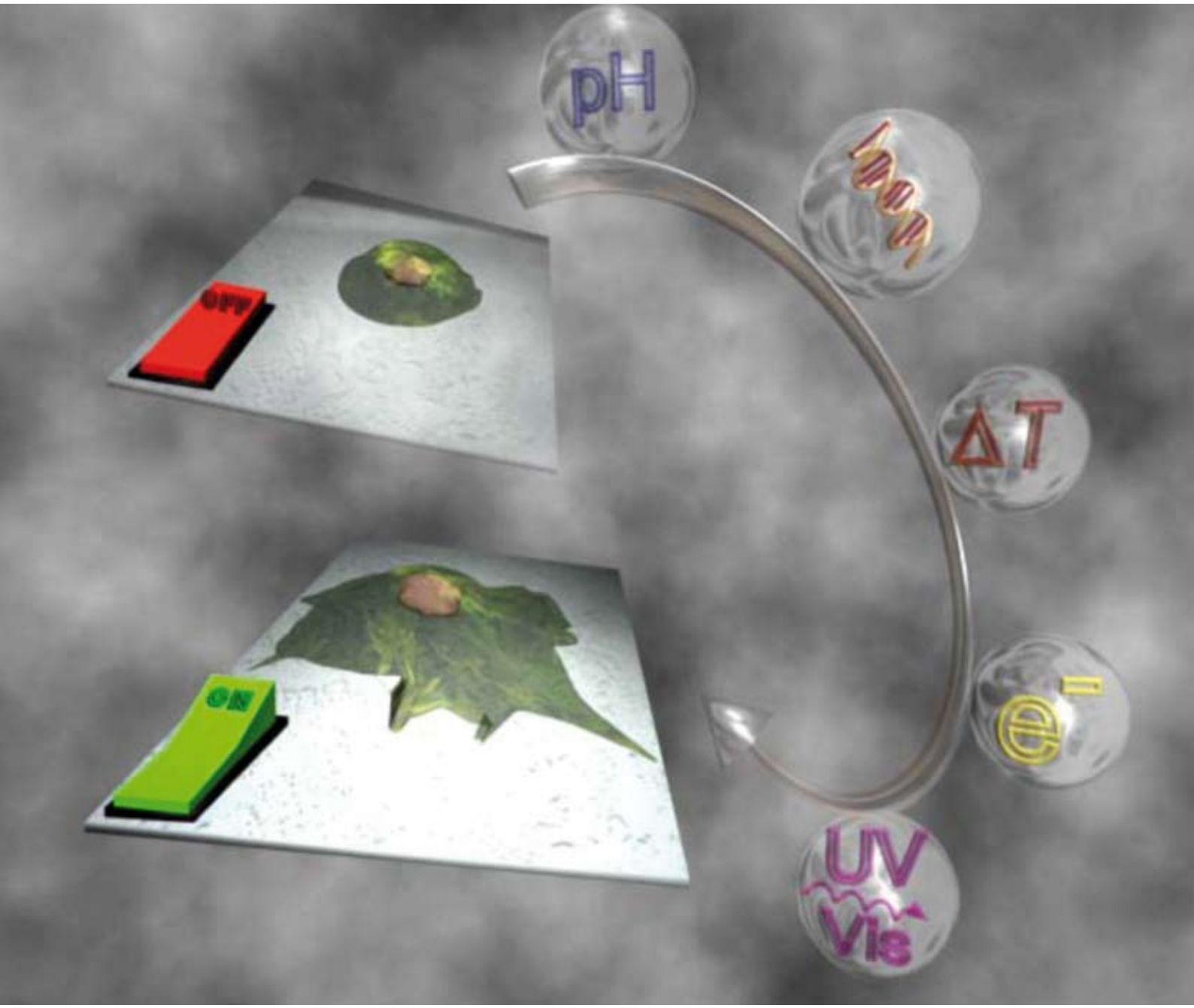

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Stimuli-responsive surfaces for bio-applications

Paula M Mendes

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The development of surfaces that have switchable properties, also known as smart surfaces, have been actively pursued in the past few years. The recent surge of interest in these switchable systems stems from the widespread number of applications to many areas in science and technology ranging from environmental cleanup to data storage, micro- and nanofluidic devices. Moreover, the ability to modulate biomolecule activity, protein immobilisation, and cell adhesion at the liquid–solid interface is important in a variety of biological and medical applications, including biofouling, chromatography, cell culture, regenerative medicine and tissue engineering. Different materials have been exploited to induce such changes in surface biological properties that are mostly based on self-assembled monolayers or polymer films. This *critical review* focuses on the recent progress in the preparation of these switchable surfaces, and highlights their applications in biological environments. The review is organized according to the external stimuli used to manipulate the properties of the substrate—chemical/biochemical, thermal, electric and optical stimuli. Current and future challenges in the field of smart biological surfaces are addressed (189 references).

1. Introduction

Surfaces with stimuli-responsive properties, also known as smart surfaces, have attracted substantial research interest in the past few years.[1–5] This recent surge of interest in surfaces that can be changed or tuned in an accurate and predictable manner by using an external stimulus stems from the widespread number of applications to many areas in science and technology ranging from environmental cleanup to data storage.[1–5] Smart surfaces with stimuli-responsive changes in wettability—hydrophilic or hydrophobic—are of interest for the development of, for instance, micro- and nanofluidic devices, self-cleaning and anti-fog surfaces and sensor devices.[6,7] In addition to demonstrating photochemical,[8–18] electrical,[19–23] solvent,[24–27] temperature,[28–30] and pH[31] control over surface wettability, recent work[8,15] has shown that stimulus driven molecular motions of surface-bound molecules can be used to drive droplets of various liquids across these surfaces in a controlled manner.

Another important research area under investigation is the development of substrates that dynamically regulate biological functions in response to applied stimuli, thereby mimicking the dynamic properties of biological systems. Surfaces that can modulate biomolecule activity,[32–38] protein immobilisation,[39–41] and cell adhesion and migration[33–37,42–46] at the liquid–solid interface can be tremendously useful in diverse biological and medical applications. For instance, dynamic, synthetic substrates that can control the presentation of regulatory signals to a cell provide unprecedented opportunities in studies of cell behaviour. Cells in tissues adhere to and interact with their extracellular environment *via* specialised cell–cell and cell–extracellular matrix (ECM) contacts.[47] The ECM, which changes its structure and composition with time, is a highly hydrated network hosting three major components: fibrous elements (*e.g.* collagens, elastin and reticulin), space filling molecules (*e.g.* glycosaminoglycans covalently linked to proteins in the form of proteoglycans) and adhesive glycoproteins (*e.g.* fibronectin, vitronectin and laminin).[47] The ECM proteins interact with cells *via* a class

Department of Chemical Engineering, University of Birmingham, Birmingham, UK B15 2TT. E-mail: p.m.mendes@bham.ac.uk; Fax: +44 (0) 121 414 5324; Tel: +44 (0) 121 414 5343

Paula M Mendes

Paula M Mendes received her MSc (1997) and PhD (2002) degrees in Chemical Engineering from the Faculty of Engineering, University of Porto, Portugal. She undertook post-doctoral research firstly (2002–04) in the Research group of Professor Jon Preece, School of Chemistry, University of Birmingham, UK, and subsequently (2004–06) at the Department of Chemistry and Biochemistry, University of California, Los Angeles, USA in the research laboratories of Professor Sir Fraser Stoddart. In October 2006, she became a University Academic Fellow in the Department of Chemical Engineering, University of Birmingham, UK. The research in her group lies in the interdisciplinary area of nanoscale science, and currently focuses on the development of biomimetic materials for studies of cell behaviour, and bio- and nanomaterials with applications in medical imaging and catalysis.

of cell membrane-spanning receptors called integrins.[48] As such, integrins transmit information across the cell's plasma membrane and are critical regulators of diverse cellular functions, including cell adhesion and migration.[48–50] Cell–ECM interactions are very complex and no complete molecular-level understanding exists to date. Cell biologists have long realised that understanding cell behaviour within their complex physiological microenvironments requires systematically studying cells within the context of specific model microenvironments. These ECM model systems should significantly reduce its complexity, but at same time mimic to a certain degree the *in vivo* situation. Thus, surfaces equipped with molecular cues mimicking certain aspects of structure or function of natural ECM will offer new opportunities for mechanistic studies of the pathways by which cells sense, integrate and respond to changes in their environments. Apart from being important for fundamental cellular studies, smart ECM models will also have an impact in the field of tissue engineering and medicine regeneration.[51] For instance, placed at the site of a tissue defect *in vivo*, such smart ECM materials could actively and temporarily participate in the regeneration process by providing a platform on which cell-triggered remodeling could occur.

Biosensors, which transduce a bio-recognition event into measurable electronic or opto-electronic signals, have a crucial role in a wide range of applications, including clinical diagnosis, environmental monitoring, forensic analysis and anti-terrorism. The ability to modulate biomolecule activity on surfaces can be tremendously useful as a way to develop reagentless, sensitive, re-usable, and real-time biosensors.[52–58] Such smart surfaces also hold the potential to enable development of highly functional microfluidic, bioanalysis, and bioseparation systems.[59–62] The rapid development of biotechnology and the pharmaceutical potential of recombinant proteins in the treatment of various human diseases are fuelling the demand for more reliable and efficient protein separation and purification methods.

This review highlights the recent accomplishments made in the development of smart biological surfaces (Fig. 1) and their relevance for bio-applications. Recent advances in surface science and polymer technology have led to the fabrication of a variety of smart biological surface designs in which control of surface properties is induced by diverse external stimulus, such as chemical/biochemical, thermal, electric and optical stimulus. These smart designs are mostly based on stimuli-responsive materials forming self-assembled monolayers (SAMs) and polymer films or on utilizing the SAMs and the polymer films as platforms for linking the stimuli-responsive material.[63] This critical review begins with a brief, but essential, introduction to both classes of surface modifiers and their peculiar properties. From there on, the next four sections will be devoted to recent advancements in designing and fabricating SAM- and polymer-based surfaces that can change their biological properties through either chemical/biochemical, thermal, electric or optical stimulus. A brief look at the current status and the future outlook of the field concludes this review.

2. SAMs

SAMs, which form spontaneously by the adsorption of an active surfactant onto a solid surface, possess important properties of self-organisation and adaptability to a number of technologically relevant surface substrates. Molecular structures forming SAMs can be divided into three features: (i) a headgroup which strongly binds to the substrate; (ii) an endgroup that allows the introduction of a variety of organic functionalities (*e.g.* acids, esters, amides and alcohols) to be incorporated into monomolecular films; (iii) and a spacer unit that connects the headgroup and endgroup and strongly affects the intermolecular separation, molecular orientation and the degree of order in the film. As such the properties of a SAM (thickness, structure, surface energy, stability) can be easily controlled and specific functionalities can also be introduced into the building blocks. SAMs of thiol headgroup derivatives on gold[64] and silane headgroup derivatives on silicon dioxide (SiO_2)[65] are examples of two widely used systems to modify the surface properties of metallic and inorganic substrates (Fig. 2). In comparison to thiols, silanes that contain reactive functional groups are experimentally more difficult to form. In part, this difficulty arises from the water that is required for the formation of a siloxane-anchored SAM, which also promotes the formation of oligomeric siloxanes. These oligomeric siloxanes then physisorb to the

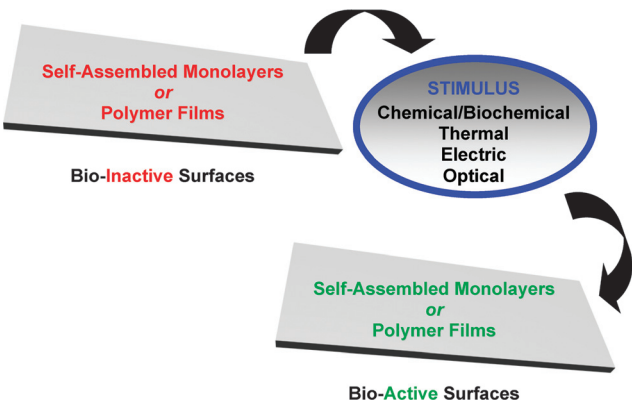

Fig. 1 Schematic depicting the range of stimuli that can be used to modulate the bioactivity of surfaces based on self-assembled monolayers or thin polymer films.

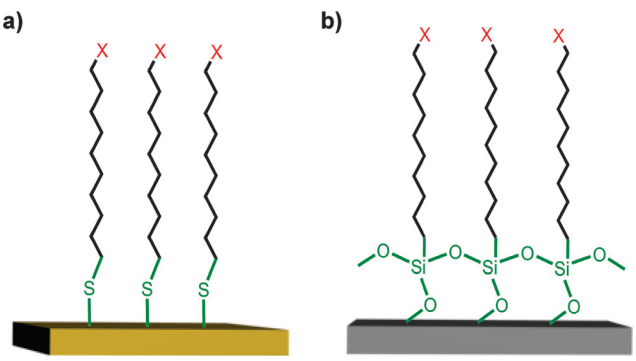

Fig. 2 Representation of the structures of SAMs of (a) alkanethiolates on gold and (b) alkanesilanes on silicon oxide. X, terminal functional group.

surface, forming a multilayer structure.[65] Although the preparation of silane-based SAMs requires a distinctly more elaborate treatment, these SAMs are significantly more chemically and thermally stable than thiols on gold surfaces.

Adsorption of silane derivatives on oxide surfaces and thiol derivatives on gold have both been successfully used for producing chemically well-defined biointerfaces. SAMs have been exploited[66–71] to provide the surfaces not only with surfaces that resist the adsorption of biological materials, but also with active groups that specifically bind targeted biomolecules on surfaces for studies of biospecific interactions such as DNA hybridization, antibody–antigen, ligand–receptor and protein–protein recognition interactions. Many different classes of biomolecules such as DNA, antibodies, enzymes, growth factors, peptide fragments and proteins have been immobilised on surfaces.[66–71] For instance, proteins have been immobilised onto SAM surfaces by either adsorption of the proteins,[70] molecular recognition between the proteins and immobilised ligands[72] or covalent coupling to the SAM surface.[67,69] Oligo(ethylene glycol) (OEG)-based SAMs have also been widely used for many biological applications because of their capacity to resist protein and cell adhesion.[70] Until recently, SAM-based biointerfaces have been fabricated based on the static character of the monolayers. In other words, SAMs perform their predetermined roles once they are formed on the surfaces. Dynamic or switchable SAMs have now been developed, which offer superior properties to the SAM-based biointerfaces as discussed in the following sections. The switching of a SAM-modified surface between different states is normally based on chemical and/or conformational changes in response to the external stimuli.

3. Polymer films

Polymer films can be prepared on substrate surfaces using several deposition techniques with different complexity and applicability. Methods such as spin coating, chemical vapour deposition, laser ablation, plasma deposition, and chemical or electrochemical reactions have been widely applied to the fabrication of thin polymer films.[73–76] The choice of deposition technique depends upon the physicochemical properties of the polymer material, the film quality requirements, and the substrate being coated. One of the simplest techniques of applying thin films onto substrates is spin coating.[73] However, there are some technical challenges related with spin coating, including large wastage of material and difficulties involved in creating thin films with polymers exhibiting low solubility. Electropolymerization is another widely used method for film deposition due its capability for growing polymer films *via* monomer oxidation.[75,76] The electrochemical polymerization is performed in an electrochemical cell, whose liquid electrolyte contains the monomer under polymerization. Owing to the insolubility of several conducting polymers, this fabrication procedure is widely applicable to many representative materials of the conducting polymer family. Nevertheless, insulating polymer films have also been successfully electrochemically synthesised.[77] The synthesis of conductive or insulated films can be either carried out on metallic substrates or carbon electrode materials. No polymeric films can be obtained on insulating surfaces by this polymerization technique. The final properties of the electropolymerized polymer film are sensitive to the different deposition conditions, including supporting electrolyte, temperature, electrical stimulus (galvanostatic, potentiostatic or potentiodynamic), and electrode material.[75,76]

Surface-tethered polymers, known as polymer brushes, have recently emerged as an extremely versatile approach to create polymer films in a robust and controlled way.[78,79] These materials are broadly relevant to a variety of modern technologies, ranging from biotechnology to advanced micro- and nanoelectronics.[80] Polymer brushes can be defined as long-chain polymer molecules that are attached with one or with a few anchor sites to a surface.[78] The tethering should be sufficiently dense so that the polymer chains can adopt a defined, stretched chain conformation, which significantly differs from the random-walk conformation of free polymer chains in solution, or in conventional solution casted polymer coatings. The tethering of the polymer to the surface is generally performed either through physical adsorption or covalent attachment.[80] Covalent attachment is often preferred due to the inherent resistance to degradation by temperature and solvents.[80] Two primary covalent attachment techniques, *i.e.* "grafting-to" and "grafting-from", have been reported to create polymer brushes (Fig. 3). In the "grafting-to" technique, a pre-formed end-functionalised polymer reacts from solution onto a suitable substrate surface to form a tethered polymer brush. In the "grafting-from" method, also called the surface-initiated polymerization method,[81] monomers are polymerised from surface-anchored initiators generally immobilised by the SAM technique.[63,81–83] The "grafting-from" process has the advantage that high grafting densities can be reached because the "grafting-to" process eventually suffers from serious steric hindrance by surrounding bonded chains.[80] Polymer brushes resulting from the "grafting-from" technique have been prepared *via* a number of polymerization mechanisms, including free-radical, cationic, anionic, atom-transfer free-radical (ATRP), ring opening metathesis polymerization (ROMP), reversible addition–fragmentation transfer (RAFT),

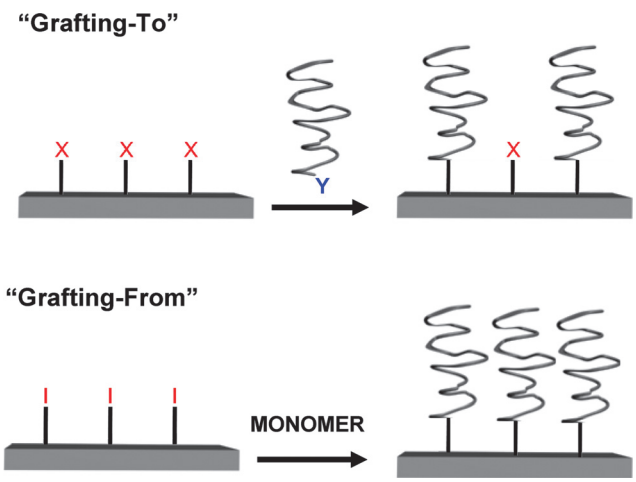

Fig. 3 Scheme of the synthesis of polymer brushes *via* "grafting-to" and "grafting-from" methods. X, end-functionalised surface; Y, end-functionalised polymer; I, initiator.

and nitroxide-mediated radical polymerization (NMRP). Detailed descriptions of these polymerization techniques can be found in two excellent text books, Advincula *et al.*[80] and Jordan.[81] Surface-initiated polymerization techniques are now routinely used with a very wide range of monomers to generate functional polymer brushes with a high degree of control over the thickness, composition, chain architecture and grafting density of the brush. While SAMs offer ease of preparation and versatile surface chemistry, polymer brushes can be produced by surface-initiated polymerization techniques with a better control over surface coverage, thickness and composition.

Responsive and not responsive polymer films have been readily used as effective methods to fabricate biointerfaces.[63,84] Stimuli-responsive polymer films have been designed by using a variety of approaches, including reversible photoisomerisation reactions, reversible swelling/collapsing of grafted polymers, and phase separation in mixed grafted brushes or diblock copolymers.[5,85] These surfaces are capable of responding to very subtle changes in the surrounding environment such as light, temperature, salt concentration and pH.[86] The macroscopic responses are caused by the reorganization of the internal or external surface structure of the deposited polymer layers.

4. Chemically or biochemically-controlled switchable surfaces

4.1. SAMs

Chemically- and biochemically-responsive surfaces offer intriguing possibilities for the development of novel biological sensors, smart bioadhesive surfaces and delivery systems with controlled release capabilities. Indeed, smart surfaces that respond to specific chemical and biological species have been the basis for the fabrication of highly sensitive, reagentless, re-usable biosensors. One recent development is the electrochemical DNA (E-DNA) sensor,[52–55] which is the electrochemical equivalent of an optical molecular beacon[87]—oligonucleotide probes that become fluorescent upon hybridization with target DNA molecules. The detection method in the E-DNA sensor is based on the alteration of the electron-transfer dynamics as a consequence of a structural rearrangement induced by target hybridization. An E-DNA sensor is comprised of a surface-confined DNA stem-loop labeled with an electroactive reporter as the hybridization sensing element (Fig. 4a).[52] In the absence of a target, the stem-loop holds the redox moiety (*e.g.* ferrocene, Fc) in proximity to the electrode, producing a large Faradaic current. Upon hybridization with the complementary nucleic acid, the stem-loop is disrupted in favour of the thermodynamically more stable, rigid rod-like target–sensor duplex, increasing significantly the distance between the redox moiety and the electrode. This behaviour produces a readily measurable reduction in current that can be related to the presence and concentration of the target sequence. Studies on the effect of probe DNA surface density have demonstrated[54] that the highest signal suppression is obtained at the highest probe densities, despite the fact that hybridization efficiency may be reduced as probe density increases.[88–92] It was suggested[54] that poorer signal suppression at lower-density sensors was due to collisions of the hybridized probe DNA with the electrode surface, leading to the transfer of electrons. In contrast, the steric bulk of the densely packed probe layers prevent these collisions after target hybridization.

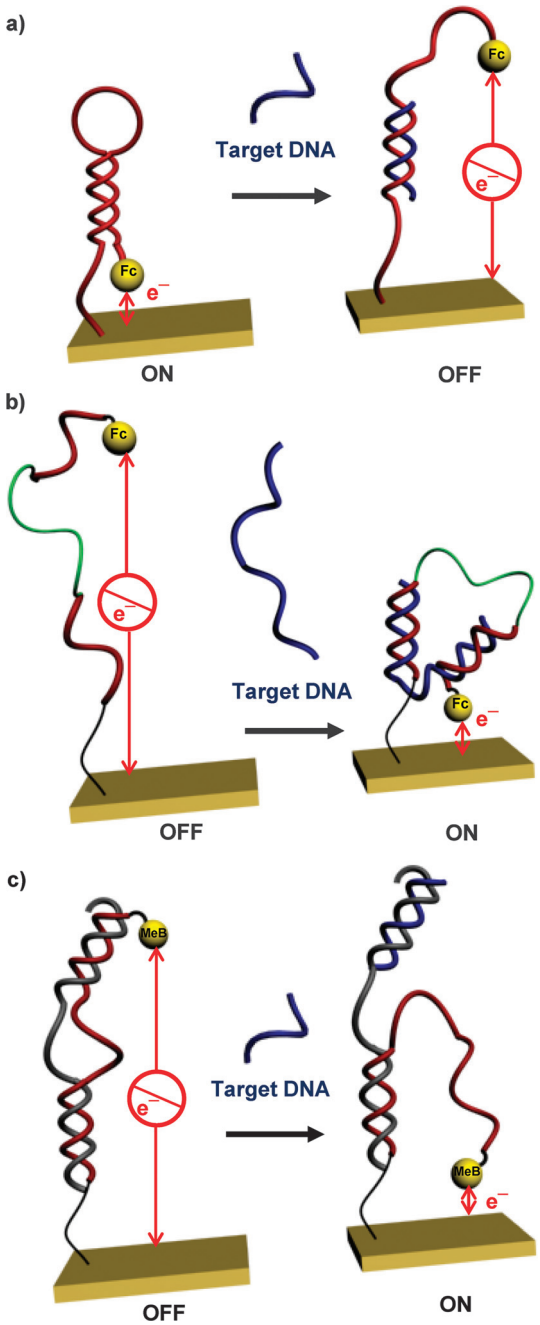

Fig. 4 (a) Signal-off E-DNA sensor based on a surface-confined stem-loop oligonucleotide that holds the ferrocene (Fc) group into close proximity with the gold electrode surface, thus allowing facile electron transfer from the redox group to the electrode. On hybridization with the target sequence, the distance between the Fc group and the electrode is altered, decreasing the electron transfer efficiency.[52] (b) and (c) Signal-on E-DNA sensors, wherein a large detection signal arises upon hybridization with target DNA.[57,58]

Although this original E-DNA sensor did not require the addition of exogenous reagents to generate a signal, the

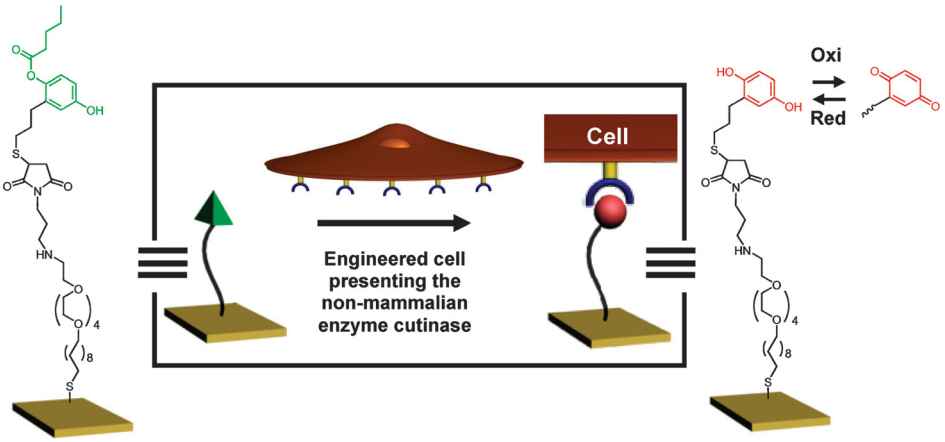

Fig. 5 Enzymatic action from an engineered cell presenting the non-mammalian enzyme cutinase switches a non-electroactive hydroxyphenyl ester-terminated SAM to an electroactive hydroquinone-terminated SAM, which can be reversibly oxidised to give the corresponding benzoquinone. This redox cycle can be monitored and quantified by cyclic voltammetry.[99]

detection method recorded target DNA binding as an "off signal", thus limiting the sensitivity of the device ($\approx$ 10 pM).[52] The gain of signal-off sensors is restricted because the target can suppress no more than 100% of the original signal. More recently, this sensing method has been successfully implemented to generate an electrochemical signal upon recognition of the target DNA, *i.e.* signal-on device.[57,58] In the first example, which utilized a surface-immobilised, ferrocene-labeled oligodeoxynucleotide–poly(ethylene glycol)–oligodeoxynucleotide triblock macromolecule—the signal arose as a consequence of a large conformational change induced by the hybridization of the target with both the top and bottom oligonucleotide of the immobilised triblock probe (Fig. 4b).[57] This simultaneous hybridization forced the terminally linked ferrocene redox tag into proximity with the electrode surface, affording an electrochemical signal. Later, Xiao *et al.*[58] developed another signal-on E-DNA sensor based on a conformational change in a methylene blue (MeB)-modified duplex DNA that occurred after target-induced strand displacement (Fig. 4c). In the absence of target, the two double-stranded regions formed between the capture and signaling probes sequestered the redox moiety from the electrode surface, limiting the observed redox current. On hybridization with the target, an enhanced electrochemical signal was generated, presumably because the flexible, single-stranded element liberated in the signaling probe increased the efficiency with which the MeB can transfer electrons to the electrode surface. The sensitivity of this signal-on E-DNA sensor was largely enhanced over earlier, signal-off E-DNA architectures, with a demonstrated detection limit of 400 fM.[58]

Other directly related sensors based on binding-induced folding of aptamers have been developed.[93–97] Aptamers are DNA or RNA sequences selected *in vitro* from combinatorial libraries by systematic evolution of ligands by exponential enrichment (SELEX). Aptamers can be selected against diverse targets, such as dyes, proteins, peptides, aromatic small molecules, antibiotics and other biomolecules, with high specificity and affinity,[97] and thus they are particularly useful as the basic sensing element for biosensor applications. Consistent with this claim, a series of novel electrochemical aptamer-based (E-AB) sensors, an analogous version to the E-DNA sensor, have been reported for such diverse targets as the blood-clotting enzyme thrombin,[93,94] the small molecule cocaine[95] and adenosine triphosphate (ATP).[96]

Cell-based sensors have also been exploited. An elegant approach to establish molecular communication between cells and material surfaces based on enzyme-responsive SAMs was introduced by Mrksich *et al.*[98,99] The authors demonstrated[98] first that 4-hydroxyphenyl valerate-terminated SAMs on gold electrodes could be switched enzymatically by cutinase from a redox inactive surface to a redox active surface (Fig. 5). Cutinase is a fungal esterase that efficiently removed the acyl groups from the 4-hydroxyphenyl valerate-terminated monolayers to afford the reversible redox active hydroquinone–quinone couple that could be detected by cyclic voltammetry.[98] The same group expanded[99] this approach to transduce cellular activity into a measurable electrochemical signal. When engineered mammalian cells that express cutinase at the cell surface were cultured in contact with the 4-hydroxyphenyl valerate-terminated SAMs on gold electrodes, an electrochemical signal was generated as a result of hydroquinone produced on the monolayer by cell-surface cutinase action (Fig. 5).[99]

Control over the surface properties for bioadhesion has been achieved by exposure of the surface to an oxidizing agent such as HBrO,[100] and changes in pH.[101,102] Nishizawa *et al.*[100] demonstrated a strategy wherein a conductive probe was used to locally generate the oxidizing agent HBrO (from a Br^--containing aqueous solution), which acted on an albumin-coated substrate to render these regions cell-adhesive. pH-Switchable assembly of bis-benzamidine derivatives on pre-formed SAMs of a mercaptoalkanoic acid on gold was used to reversibly control the adsorption of different biomolecules on surfaces.[101,102] Above neutral pH, the positively charged amidinium-terminated surface selectively adsorbed different negatively charged biomolecules such as oligonucleotides, fibrinogen proteins and adenosine phosphates (adenosine monophosphate, AMP; adenosine diphosphate, ADP; adenosine triphosphate, ATP). Under acidic conditions, the bis-benzamidine–biomolecule bilayer was displaced from the

surface. The reassembly–disassembly was reversible and could be repeated several times.

Harnessing the ability to change molecular structures in such a way that they can cover or uncover porous materials, and thus either trap or release their contents, forms the basis of a molecular nanovalve. Such nanovalves are ideal systems for solving the problem of targeted drug delivery because the release can be initiated by an external or cellular stimulus once the target has been reached. Progress in designing and synthesizing molecular nanovalves has been proceeding apace and several examples, using a variety of methods of operation and activation, have been described in the literature.[103–105] For instance, control of mass transport in nanoporous films have been achieved by photodriven motions involving *cis–trans* isomerisation of the azobenzene[106,107] or intermolecular dimerization of coumarins.[108,109] Azobenzene and coumarin derivatives were tethered to the surface walls of nanoporous materials in such a way that their configurable changes upon photoirradiation could control the access of guest molecules to and from the nanopores. Another means by which the orifices of porous materials have been gated is through the use of supramolecular systems. Stoddart and co-workers have developed several supramolecular valves based on [2]pseudorotaxanes or [2]rotaxanes,[110,111] which are placed at pore entrances of mesoporous silica films and particles to function as gatekeepers that can trap and release molecules from the pores when stimulated. Typically, the [2]pseudorotaxanes or [2]rotaxanes are tethered to the silica surface through the use of silane linkers, which are bifunctional coupling agents with one end able to create a monolayer with the silica and the other able to immobilize the supramolecular assembly. The opening of the nanovalves only requires mechanical movement of the rings from the stalks on the [2]pseudorotaxanes or [2]rotaxanes attached to the mesoporous materials. These supramolecular nanovalves were designed in such a way that they could be regulated by either redox chemistry,[112–114] a light source,[115] pH stimulation,[116–118] activation by competitive binding,[117] or enzymatic hydrolysis.[119] Although most of these systems were shown to operate in nonbiological environments (*i.e.* controlled-release of guest molecules from mesoporous silica using organic solvents), recent efforts have been directed towards the development of biocompatible supramolecular nanovalves that can operate under physiological conditions.[118,119] One of the biocompatible nanovalve designs, which could be controlled by pH stimulation, was based on a [2]pseudorotaxane composed of a cucurbit[6]uril ring that encircles a bisammonium stalk (Fig. 6).[118] Spherical mesoporous silica particles were functionalised with the [2]pseudorotaxane in which the bisammonium stalk acted as a gatepost and the cucurbit[6]uril unit served as the gate that controlled access of luminescent guest molecules (rhodamine B) into or out of the nanopores of the silica particles. The mode of action of this valve relied on the pH-dependent binding of cucurbit[6]uril with the bisammonium stalk. At neutral and acidic pH values, the cucurbit[6]uril encircled the bisammonium stalk, thereby blocking the nanopores and trapping the guest molecules inside it. Deprotonation of the bisammonium stalk upon addition of a base resulted in dethreading of the ring and releasing of the contents trapped inside the silica pores. Enzyme-responsive

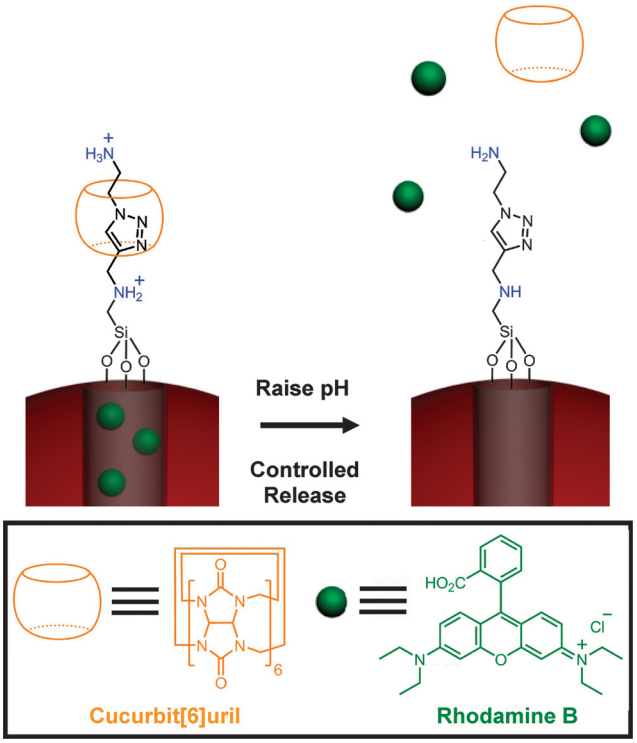

Fig. 6 Structure and graphical representation of a pH-controllable supramolecular nanovalve based on mesoporous silica nanoparticles, using a cucurbit[6]uril–dialkylammonium pseudorotaxane as the gatekeeper. The controlled release of luminescent molecules (opening of the nanovalve) from the supramolecular nanovalve is triggered by switching off the ion–dipole interactions between the cucurbit[6]uril ring and the bisammonium stalk upon pH stimulation.[118]

supramolecular nanovalves that also operate in aqueous media have been demonstrated.[119] In this supramolecular system, the controlled-release of guest molecules from the silica nanopores was based on a [2]rotaxane in which an α-cyclodextrin torus encircled a poly(ethylene glycol) thread and was held in place by an adamantyl ester stopper. The bulky stopper was stable under physiological conditions, but could be cleaved by the catalytic action of porcine liver esterase enzyme. Hydrolysis of the adamantyl ester stopper resulted in dethreading of the torus, and release of the guest molecules (rhodamine B) from the pores of the nanoparticles. Although luminescent guest molecules were employed to demonstrate the effectiveness of the reported supramolecular nanovalves in regulating molecular transport into or out of the containers, these highly controllable systems can potentially be used to control the release of a variety of drug molecules.

4.2. Polymer films

Enzyme-triggered activation of surfaces shows great promise as a method for altering surface biological properties in a controlled manner.[120,121] Ulijn *et al.*[121] reported a strategy which could enzymatically switch polymer surfaces from a state that prevented cell adhesion to another state in which cell adhesion and spreading were promoted (Fig. 7). This strategy was based on the fabrication of poly(ethylene glycol) (PEG) acrylamide films on epoxy-coated glass slides by spin coating

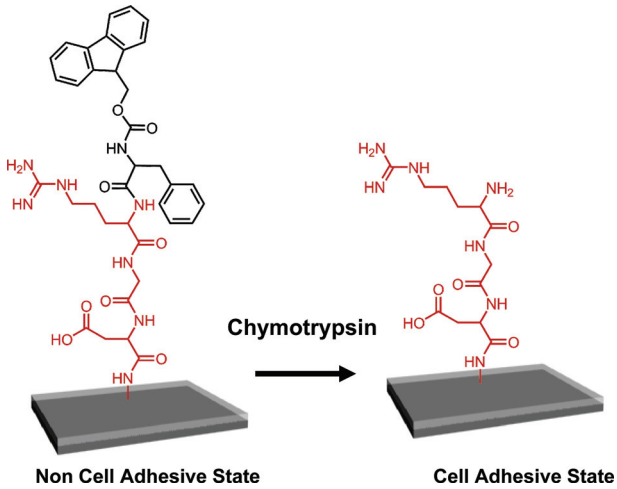

Fig. 7 Chymotrypsin enzyme triggers activation of a surface-tethered RGD peptide, thereby promoting cell adhesion.[121]

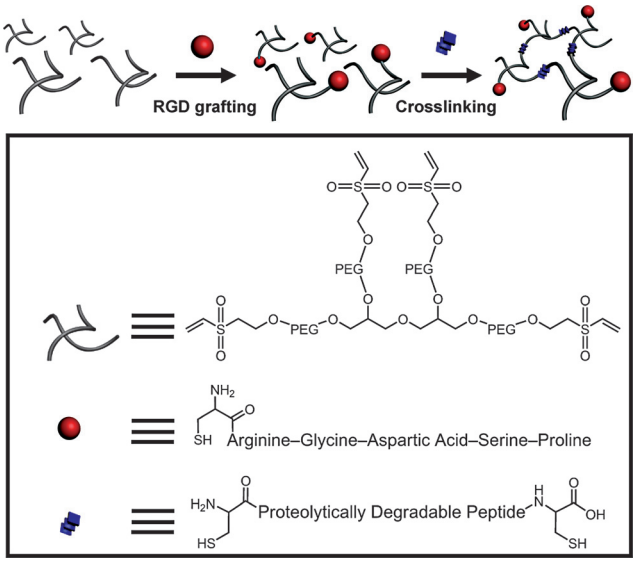

Fig. 8 Structure and graphical representation of the Hubbell *et al.*[128] hydrogel networks. PEG-based gels are first functionalised with the RGD peptide, and subsequently crosslinked by the use of a bifunctional peptide, which is cleaved by cell-secreted matrix metalloproteinases.

and UV curing, followed by the functionalisation of the polymer film with the peptide arginine-glycine-aspartic acid (RGD), which was initially protected with a bulky blocking group, 9-fluorenylmethoxycarbonylphenylalanine. The RGD peptide is present in several cell-contacting ECM proteins such as fibronectin, vitronectin, type I collagen, and it has been widely studied as an immobilised cell adhesion ligand specific for integrin-mediated cell adhesion.[66,122,123] Surface tethered RGD sequences were shown to be inactive to cell adhesion when capped with the blocking group, which was selectively removed upon exposure to the serine protease chymotrypsin, thereby activating the RGD and triggering cell attachment *in situ*.

Macromolecular components of natural ECM are degraded by cell-secreted and cell-activated proteases, including matrix metalloproteinases (*e.g.* Gelatinase-A (MMP-2), fibroblast collagenase (MMP-1), collagenase-3 (MMP-13)) and serine proteases (*e.g.* plasmin, chymotrypsin, elastase and trypsin). This behaviour creates a dynamic two-way communication between the ECM and cell, with the ECM conveying essential signalling cues to the cell and cellular proteases remodeling the ECM and releasing bioactive components such as growth factors from it. Considerable progress has been made in developing bioactive polymer gels that can mimic the protease-mediated invasion of the natural ECM matrix.[51] Synthetic hydrogels have been molecularly engineered to have sensitivity to different cell-associate proteases, such as elastase,[124] plasmin,[125–127] and matrix metalloproteinases.[124,127–131] The hydrogel networks were rendered protease sensitive either by inclusion of proteolytically sensitive peptides, or proteins in the polymer backbone, or by grafting the bioactive molecules into the hydrogel network during its formation. Hubbell *et al.*[128] reacted bifunctional bis-cysteine oligopeptides, which are recognized and cleaved by cell-secreted matrix metalloproteases, with bifunctional PEG–bis-vinyl sulfone polymers grafted with RGD to form a crosslinked network structure (Fig. 8) that was successfully used to mimic the invasive characteristics of native provisional ECM. The hydrogel network not only exhibited sites for proteolytic activity, but also cell adhesive peptides to achieve biospecific cell adhesion. This innovative approach was further explored as a cell-responsive growth factor delivery system in which a potent stimulator of tissue formation, *i.e.* bone morphogenic protein-2 (rhBMP-2), was entrapped within the PEG-based matrix. Release of the growth factor was triggered by cell invasion of the hydrogel, which resulted in efficient and localised bone regeneration.[131] In another example involving tailoring of proteolytic cleavage for bone regeneration, Healy *et al.*[132] prepared responsive poly(*N*-isopropylacrylamide-*co*-acrylic acid) hydrogels incorporating matrix metalloproteinase-13 (MMP-13) degradable crosslinkers and RGD peptides. When implanted in a rat femoral ablation model, the hydrogels were shown to enhance bone regeneration.

Ionic strength-induced responses in polymers have been used to regulate protein interactions with surfaces. Elastin-like polypeptides (ELPs) are stimuli-responsive biopolymers that undergo an LCST phase transition in aqueous solution.[85,133] These polypeptides comprise oligomeric repeats of the pentapeptide Val-Pro-Gly-X-Gly (*i.e.* a motif found in the structural protein elastin), where the "guest residue" X is any amino acid with the exception of proline. The choice of X determines the phase transition temperature.[85,133] Below their LCST, ELPs are highly solvated, and therefore soluble in aqueous solution, but when the temperature is raised above their LCST, they undergo a sharp phase transition leading to desolvation and aggregation of the polypeptide. Changes in the ambient temperature, ionic strength, or pH can trigger this completely reversible transition. At a molecular level, the LCST transition of an ELP is accompanied by a conformational change in the polymer chain from a disordered, random hydrophilic coil to a more ordered, collapsed hydrophobic globule. This phase transition behaviour has been used to dynamically control the immobilisation of proteins on solid surfaces.[134–136] In response to changes in ionic strength, ELPs,

which were tethered onto either glass[135] or gold[136] surfaces by the ''grafting-to'' approach, have been shown to undergo a switchable and reversible, hydrophilic–hydrophobic phase transition.[135,136] The LCST transition resulted in the reversible capture of an ELP fusion protein, thioredoxin (Trx), onto the ELP surfaces directly from cell lysate.[135] The steric accessibility of Trx was confirmed by its binding to an antibody specific to Trx—anti-thioredoxin monoclonal antibody.[135] The LCST-triggered adsorption and desorption of the Trx–ELP fusion protein has also been demonstrated on ELP nanopatterned surfaces.[136] Also by the manipulation of ionic strength, polyelectrolyte brush nanoparticles immobilised on glass substrates have been used to function as nanocontainers for the controlled uptake and release of proteins while preserving their structural integrity.[137] The polyelectrolyte brush nanoparticles consisted of poly(styrene) core particles of 110 nm diameter onto which long chains of poly(styrene-sulfonate) were grafted. The individual immobilised nanoparticles could be loaded with up to 30 000 green fluorescent proteins in a buffer solution of low ionic strength. The bound proteins were fully released upon increasing the ionic strength by adding 250 mM NaCl to the buffer solution. Their high storage density, good retention, and controlled uptake and release of the proteins make these polyelectrolyte brush nanoparticles promising candidates for applications in drug delivery systems.

Efforts have also been directed toward the development of pH-responsive switchable surfaces. Higashi *et al.*[138] have pursued a strategy that uses acidic (poly(L-glutamic acid), PLGA) and basic (poly(L-lysine), PLL) block-polypeptides immobilised on gold surfaces to undergo a pH induced phase transition between peptide surfaces with different charge distributions. Two types of diblock-polypeptides, PLGA-*block*-PLL and PLL-*block*-PLGA, were prepared on gold substrates *via* the ''grafting-from'' method. The surface charge distribution on both polypeptide brushes has been found to be switchable by changing pH and to be strongly dependent on the conformation and ionised state of the outer peptide-block.

5. Temperature-controlled switchable surfaces

5.1. SAMs

Temperature stimulation is another convenient method widely used for controlling the biological properties of surfaces. The smart thermo-responsive surfaces that have been explicitly designed for bio-applications are mostly based on polymers.[42–44,139] However, mixed SAMs of OEG-terminated alkylthiolates and methyl (CH_3)-terminated alkanethiols have been demonstrated to exhibit different tendencies for protein adsorption and bacteria attachment at room temperature and at 37 °C.[140] This reversible switching between non-adhesive (room temperature) and adhesive (37 °C) states was not observed either on pure OEG-terminated SAMs or CH_3-terminated SAMs. It is well established[70] that immobilised OEG can prevent or greatly reduce protein adsorption and, consequently, render surfaces resistant to cell adhesion. However, the exact mechanism by which OEG resists protein adhesion is still unclear. What is known is that the protein adsorption behaviour is strongly influenced by such molecular parameters as packing density, chain length and chain conformation.[141,142] In line with these observations, thermally induced conformational changes in the OEG chains in the OEG–CH_3 mixed monolayers were suggested to be responsible for the changes in bioadhesion on the mixed monolayers at room temperature and at 37 °C.

5.2. Polymer films

A significant body of work during the past five years has established a class of thermally-responsive polymer surfaces that regulate molecular recognition events and control cell attachment and detachment without cell damage. The most widely studied system is poly(*N*-isopropylacrylamide) (PNIPAM), a thermo-responsive polymer that has a low critical solution temperature (LCST) of 32 °C in aqueous solution. Below its LCST, PNIPAM polymer is in an extended, solvent-swelled conformation, but when heated up above the LCST, the polymer undergoes a phase transition to

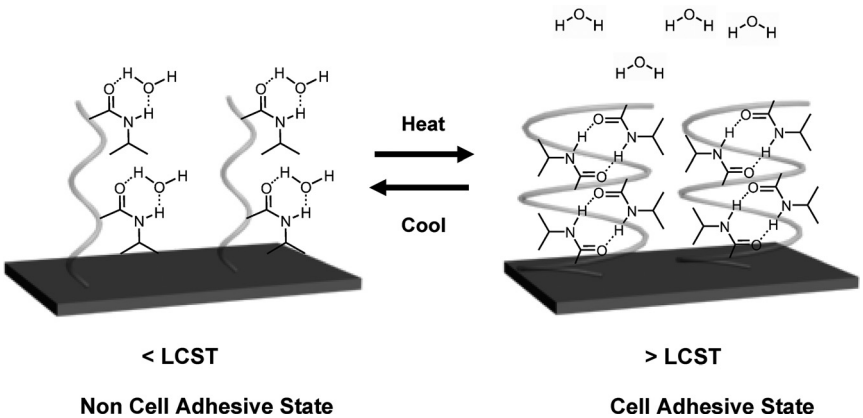

Fig. 9 Diagram illustrating the temperature-induced switching of a PNIPAM-modified surface. PNIPAM chains are shown forming intermolecular hydrogen bonds with water molecules at temperatures below the LCST (left) and forming intramolecular hydrogen bonds between C═O and N–H groups at temperatures above the LCST (right). The PNIPAM surfaces exhibit different cell adhesion below and above the LCST.[45]

yield a collapsed morphology that excludes solvent. This behaviour is based on a widespread hydrogen bond network between the amide groups and water molecules at lower temperatures, whereas at higher temperatures the stabilising H-bonds break up and the hydrophobic interactions become predominant (Fig. 9).[30,143]

The fact that PNIPAM undergoes a sharp property change in response to a moderate thermal stimulus near physiological temperatures has generated great interest in the biomaterials community. The reversible volume phase transition of surface-grafted PNIPAM has been utilised to develop thermo-responsive culture dishes for cells.[42–44] Okano *et al.*[45] demonstrated that e-beam-grafted PNIPAM on tissue culture polystyrene dishes allowed cells to adhere, spread and proliferate above the LCST of the polymer. A decrease in culture temperature below the LCST resulted in detachment of cells. Further investigation into the mechanism of cell attachment revealed that strong interactions between hydrophobic PNIPAM and fibronectin, an ECM protein, mediated cell attachment above the LCST.[42] Decreasing the temperature below the LCST makes the surface hydrophilic and resulted in desorption of the ECM along with the cultured cells. In an extension of these studies, Okano *et al.*[144,145] grafted PNIPAM in a pattern on tissue culture polystyrene (TCPS) dish surfaces to control the shape of the cell sheets[145] and to co-culture cells.[144]

Temperature-triggered cell desorption from PNIPAM surfaces provides a gentler alternative to traditional cell removal methods such as mechanical dissociation and enzymatic digestion.[146,147] The removal of cells cultured on tissue culture polystyrene by mechanical dissociation and enzymatic digestion have been shown to be damaging to both cells and ECM.[146] On the other hand, by culturing cells on PNIPAM-grafted surfaces, it was possible to recover intact cell monolayers and most of the underlying ECM by using a modest temperature drop as the sole stimulant for detachment.[42,146–148] The released cells maintained their substrate adhesivity, growth and secretion activities.[44,146,147] This non-invasive cell recovery method has promising applications in tissue engineering.[144,145,147]

In order to induce biospecific interactions between the culture dish and the cell, PNIPAM polymers have been further modified with biologically active synthetic peptides containing sequences that interact with cell components.[43,129,149,150] When the RGD moiety was immobilised to temperature-responsive poly(*N*-isopropylacrylamide-*co*-2-carboxyisopropylacrylamide) (P(NIPAM-*co*-CIPAAm)) copolymer-grafted surfaces, these surfaces promoted cell adhesion and spreading under serum-free conditions at 37 °C (above the LCST of the copolymer).[43] At this temperature, the copolymer collapsed and formed a compact structure, allowing the integrin receptors on the cell membrane to recognize the conjugated RGD sequences to promote cell adhesion. By lowering culture temperature below the LCST, the copolymer chains were swollen, shielding immobilised RGD peptides from integrin access, limiting cell–surface attachment tension, and mechanically disrupting cell–surface contacts. This dissociation of specific interactions between cell integrins and RGD-conjugated surfaces upon hydration was demonstrated to be similar to results using soluble RGD in culture as a competitive substitution for immobilised ligands. These studies showed that specific interactions between cell integrins and immobilised RGD moieties can be non-invasively thermally regulated for cell attachment/detachment under serum-free conditions.

PNIPAM polymers have also been exploited as bacterial,[151] protein,[59–61,151,152] peptide[62] and steroid[62] adhesion mediators. As with cell adhesion, changes in surface properties governed by polymer transitions from hydrophilic to hydrophobic states mediated bioadhesion. Such regulatory control over adhesion of different bioactive analyte classes can be

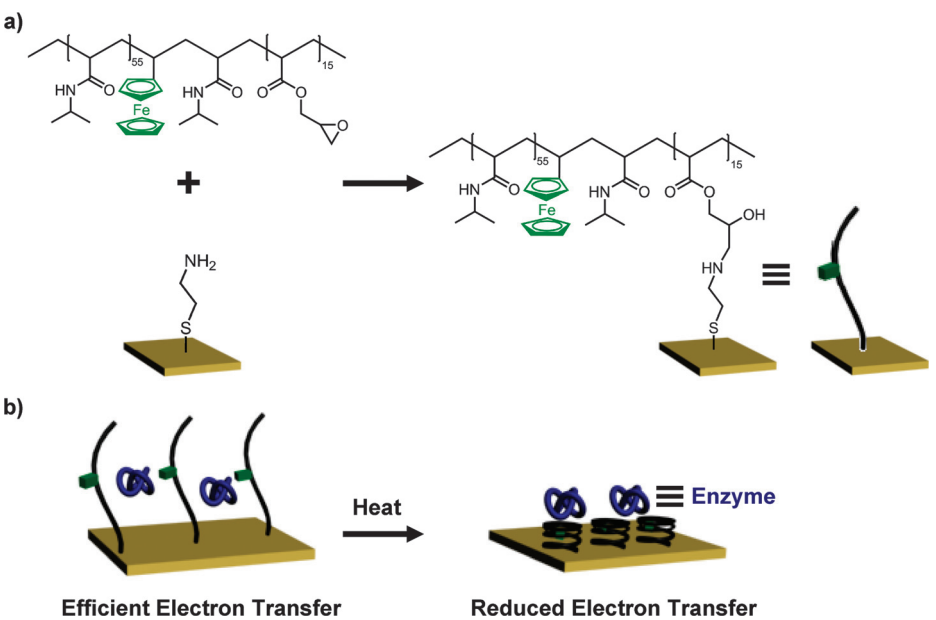

Fig. 10 (a) Covalent coupling of PNIPAM–ferrocene polymer to an amino-terminated monolayer on a gold surface. (b) Schematic model of the electron transfer behaviour between the sGDH enzyme and the PNIPAM–ferrocene polymer brush surface upon temperature changes.[155]

applied to develop novel chromatographic matrixes.[60–62] Other researchers have exploited temperature-responsive properties of PNIPAM to create a "smart" biofouling system,[153] and a microfluidic device with the capacity to adsorb small and large proteins from solution and subsequently release these proteins on demand.[59] The microfluidic device, which could be switched by programmed on-chip heating, was fabricated using a 4 nm thick PNIPAM layer with a network of gold heater wires on a silicon chip. A surface tethered azo-radical initiator, AIBN, was used to create the 4 nm polymer brushes. Each gold wire on the chip could be heated or cooled separately on demand, and thus it was employed to capture or release proteins, such as myoglobin, BSA, haemoglobin and cytochrome C, at specific sites on command in less than 1 s.[59] This study demonstrates that temperature stimulus can also offer the prospect for spatio-temporal control of the surface properties by activating restricted micrometre-scale regions on a surface.

Thermo-responsive polymers containing redox groups have also been employed as dynamic enzyme supports[154] and electron mediators.[155] The poly(*N*-isopropylacrylamide-*co*-vinylferrocene) thermo-shrinking redox gel was used to control the loading and unloading of both glucose oxidase (GOx) and lactate oxidase (LOx) enzymes from electrode surfaces. The copolymer-coated indium tin oxide (ITO) electrode was loaded with either GOx or LOx enzyme at a temperature below 10 °C, followed by subsequent elevation of the temperature to 35 °C. This increase in temperature caused the gel film to shrink and entrap the enzyme. In order to unload the enzyme from the redox gel-coated electrode, the temperature was decreased to <10 °C and a fresh or different enzyme could be reloaded.[154] A PNIPAM–ferrocene polymer bearing oxirane side groups has also been covalently immobilised by the "grafting-to" approach to an amino-terminated self-assembled monolayer on a gold surface (Fig. 10a), such that the polymer could act as a mediator for electron transfer between the cofactor pyrroloquinoline quinone (PQQ) of soluble glucose dehydrogenase (sGDH) and the gold electrode.[155] In the swollen PNIPAM state, the PNIPAM–ferrocene polymer formed brush-like structures, offering optimal conditions for mediated electron transfer between the enzyme and electrode surface (Fig. 10b). At higher temperatures, the brush-like structure disappeared and the polymer showed a collapsed state as the hydrophobic interactions dominate. As a result, the enzyme molecules could not penetrate the polymer network and the interactions and electron transfer decreased.[155] The examples reported herein demonstrate the versatility and broad potential of polymers that undergo phase transitions in physiologically relevant conditions of temperature.

6. Electrically-controlled switchable biological surfaces

6.1. SAMs

SAMs with a number of different electroactive groups have been successfully employed to switch on functionalities *in situ*, offering an unprecedented ability to manipulate the interactions of peptides,[33–38] DNA,[156–158] proteins,[39] and cells[33–37] with surfaces. By employing the electrochemical reaction in which aromatic nitro (NO_2) groups can be chemically modified by a redox process to amino (NH_2) groups, site-selective and reaction-controlled immobilisation of DNA[156,158] and proteins[39] on surfaces have been achieved. For instance, Mendes *et al.*[39] demonstrated that the NO_2-terminated groups in the SAMs of 4-nitrothiophenol on gold surfaces could be reduced electrochemically and selectively to NH_2 groups by applying a negative voltage between the addressed electrode and its counter electrode in the presence of an electrolyte. By employing a homo-bifunctional activated ester linker, proteins were immobilised with high affinity and selectivity onto the NH_2 regions, after activation. Electrochemical active coatings based on thiol chemistry have also been used to programme the electrochemical release of immobilised proteins from selected micropatterned gold electrodes.[159] Since the thiol bond formed between the thiol-containing monolayer and the gold surface is electrochemically reversible, thiols can be electrochemically reduced on the gold surface to form thiolates that are easily displaced from the gold surface. By immobilising first the proteins on the thiol containing SAMs, it was possible to desorb the SAM–biomolecule system from the gold surface upon application of a negative potential (−1.5 V). A powerful tool for the programmed loading or/and release of one or more proteins from a surface could be developed by combining the flexibility, at the level of controlling protein adsorption and desorption, of these strategies[39,159] with high-density arrays of electrically addressable nanoscale electrodes.

Dynamic control of cell adhesion onto substrates during cell cultivation is important for various biological and medical applications, including tissue engineering, cell-based drug screening, and fundamental cellular studies. In order to realise such dynamic control, Mrksich *et al.*[33–35,160] have investigated SAMs of alkanethiolates on gold, which presented appropriately designed electroactive groups, that could be selectively modified in response to applied potentials. Electroactive-functionalised surfaces based on the hydroquinone–quinone redox couple have been shown to give real-time control over the molecular interactions that mediate peptide attachment, and consequently the adhesion of cells. The electroactive monolayers were able to directly switch peptide ligand activities on and off, and subsequently to influence the behaviour of attached cells *in situ* and in real time.[36] This dynamic property was based on the use of the electroactive *O*-silyl hydroquinone moiety to tether the RGD peptide ligand to the monolayer. Upon electrochemical oxidation of the *O*-silyl hydroquinone to the corresponding benzoquinone moiety, the silyl ether was hydrolyzed and the RGD peptides were selectively released from the surface (Fig. 11). Subsequently, the resulting benzoquinone-terminated SAMs were coupled with a second ligand, diene-tagged RGD peptide, *via* a Diels–Alder reaction. In order to demonstrate that these dynamic substrates could be used to modulate specific cell receptor–ECM ligand interactions, a monolayer was patterned using microcontact printing into circles of hexadecanethiolate, with the intervening regions presenting the RGD peptide ligands tethered to the surface by way of *O*-silyl hydroquinone

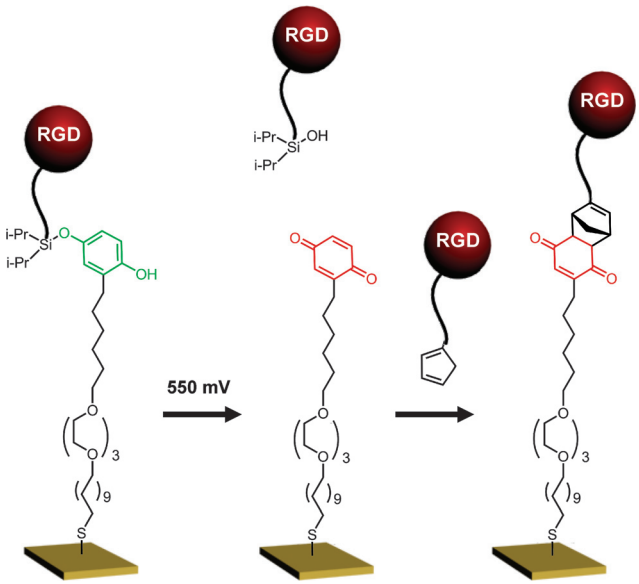

Fig. 11 Selective release of the RGD peptide from a monolayer presenting the *O*-silyl hydroquinone by electrochemical oxidation and subsequent immobilisation of a second RGD peptide by a Diels–Alder reaction.[36]

groups. The surface was treated with ECM protein fibronectin that selectively adsorbed to the hexadecanethiolate circular regions. Cell addition to the substrate resulted in the attachment and growth of cells evenly distributed across the regions presenting fibronectin and RGD peptide. On application of an electrical potential to the gold (550 mV for 5 min), the hydroquinone groups were converted to benzoquinone groups, which selectively released the cells only from the

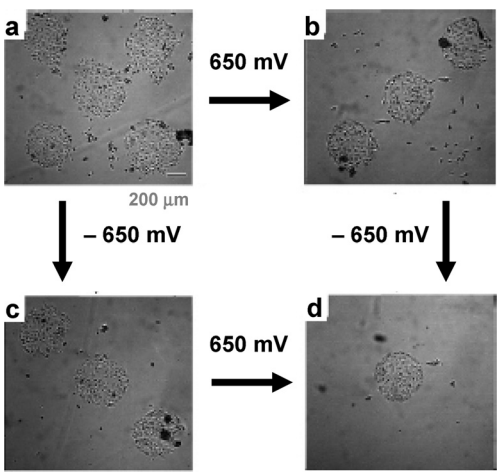

Fig. 12 Electrochemical control of cell adhesion on RGD-patterned gold surfaces. The patterned regions differ in the electrochemical properties of the redox active linkers (*i.e.* *O*-silyl hydroquinone or quinone ester) that tether the RGD to the gold surfaces. Application of an electrical potential of 650 mV releases the cells from regions presenting the electroactive *O*-silyl hydroquinone (a → b and c → d), whereas −650 mV induces the release of the cells from regions presenting the electroactive quinone ester linker (a → c and b → d). (Reprinted with permission from ref. 37. Copyright (2006) American Chemical Society).

RGD regions. Subsequent treatment of the monolayer with diene-tagged RGD peptide resulted in ligand immobilisation and initiated cell migration from fibronectin-coated circular regions onto remaining regions of the substrate.

A related strategy was later used to design substrates that displayed two independent dynamic functions for controlling cell adhesion (Fig. 12).[37] By using patterned surfaces with two different electroactive tethers that release the RGD ligands in response to either reductive or oxidative potentials, it was possible to trigger the selective release of cells from the surface. *O*-Silyl hydroquinone and quinone ester moieties were employed as the electroactive linkers that tethered the peptides to the monolayers. In contrast with *O*-silyl hydroquinone that was oxidised to release the RGD ligands, a reductive potential was applied on the gold surface to release the RGD ligands from the quinone ester patterned regions. The electrochemical reduction converted the quinone ester to the corresponding hydroquinone on the surface. These studies demonstrated that the electrical potentials applied to the surfaces containing cells appear to be non-invasive and compatible with the conditions of cell culture,[34,35,161] and that the electroactive monolayers can indeed be designed to influence the adhesion of different cells *in situ* and in real time.

Following the initial studies by Mrksich *et al.*,[33–35] several other research groups have been exploiting the hydroquinone–quinone redox system for site-selective generation of bioactive surfaces.[157,162–165] Oxidised hydroquinone (quinone) reacts with a range of functional groups, such as thiol,[157] cyclopentadiene[33] and aminooxy groups,[38] which can be relatively easily incorporated into biomolecules. Heath *et al.*[163] demonstrated the use of electrochemical activation of hydroquinone for site-selective conjugation of cyclopentadiene- or thiol-terminated biotin molecules onto silicon micro- and nanoelectrodes.[163] In another example, Yousaf *et al.*[38,164,165] have demonstrated the ability to utilise electroactive quinone monolayers to control and determine the density of immobilised biomolecules on a surface. This strategy was based on the coupling of aminooxy-terminated biomolecules with the electroactive quinone-terminated SAMs. The surface chemistry product oxime was also redox active, but at a different potential, allowing for real-time monitoring of the immobilisation reaction, and therefore determination of the density of immobilised biomolecules by cyclic voltammetry. This type of experimental control can be very useful for the preparation of molecularly well-defined biological surfaces for studies of cell behaviour.[165]

SAMs comprised of redox-active rotaxanes[110,111] have also been used to establish electrical communication between the redox centres of enzymes and the electrode surfaces.[166] Redox enzymes usually lack direct electron transfer communication with electrode surfaces, owing to the spatial separation of their redox centres from the conductive surface. In order to overcome this lack of communication, enzymes should be properly aligned in respect to the electrodes and incorporate redox relay units acting as mediators. Willner *et al.*[166] employed the electrochemically stimulated shuttling of a tetracationic macrocycle on a molecular wire as the charge-transport track for wiring of the enzyme glucose oxidase (GOx). The electron acceptor cyclobis(paraquat-*p*-phenylene) macrocycle was threaded on a molecular wire that included a

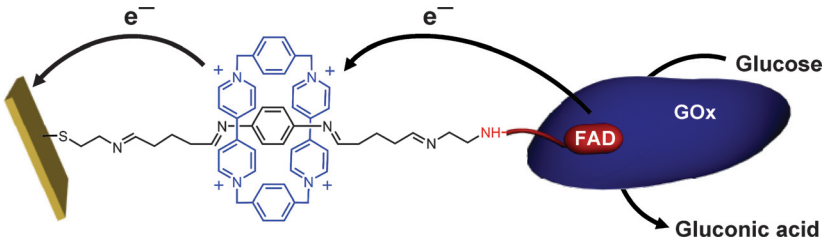

Fig. 13 GOx-reconstituted FAD-stoppered redox-active rotaxane on a gold electrode. The reconstituted GOx is brought into electrical contact with the electrode by the redox-active rotaxane which operates as an electron-transfer mediator to allow the effective bioelectrocatalytic oxidation of glucose.[166]

diiminobenzene π-donor site, and the supramolecular complex was then stoppered with the bulky flavin adenine dinucleotide (FAD) cofactor (Fig. 13). The reconstitution of apo-GOx onto the FAD units resulted in the electrically contacted bioelectrocatalytic system, where the electrochemically induced shuttling of the macrocycle mediated the electron transfer from the enzyme's active site to the electrode surface. This type of electrical communication is a key process in the tailoring of enzyme electrodes for bioelectronic applications.[167]

Low density SAMs on gold have attracted interest for controlling protein adsorption and release under electrical modulation.[40,41] These surfaces display increased interchain distances, enabling the reversible conformational transition of surface-confined molecules. In order to achieve such low density SAMs, different strategies have been exploited.[22,40] Liu *et al.*[40] generated SAMs of a pre-formed inclusion complex—a cyclodextrin (CD)-wrapped alkanethiolate—on gold, followed by the release of the CD space-filling group from the anchored pseudorotaxane. Removal of the non-covalently bound large CD was a means by which a low density, regular monolayer could be formed. Loosely packed carboxylic-terminated and amino-terminated SAMs were shown to induce dynamic changes in the surface properties, such as wettability and charge, in response to an electrical potential.[40] These changes resulted from the electrostatic effect between the ionised terminal groups and the charged gold substrate upon applying an electrical potential. The acid-terminated surfaces were negatively charged and hydrophilic under a negative applied potential (straight chains with carboxylate anions exposed at the surface), whereas a positive potential rendered a neutral and hydrophobic surface (bent chains with greasy alkyl chains exposed at the surface). On the other hand, amino-terminated monolayers induced a neutral and hydrophobic surface under a negative potential and a positively charged and hydrophilic surface under a positive potential. These low density SAMs have been successfully integrated in microfluid chips to reversibly control the assembly of two proteins with different isoelectric points (Fig. 14).[41] By employing carboxylic-functionalised microfluidic chips, the positively charged avidin protein has been adsorbed in the microchips under a negative potential and released under the opposite potential. In contrast, a positive potential on the amino-functionalised microchips induced the adsorption of the negatively charged streptavidin protein, which can be released by applying a negative potential. These SAM-modified smart microchips could potentially be useful for

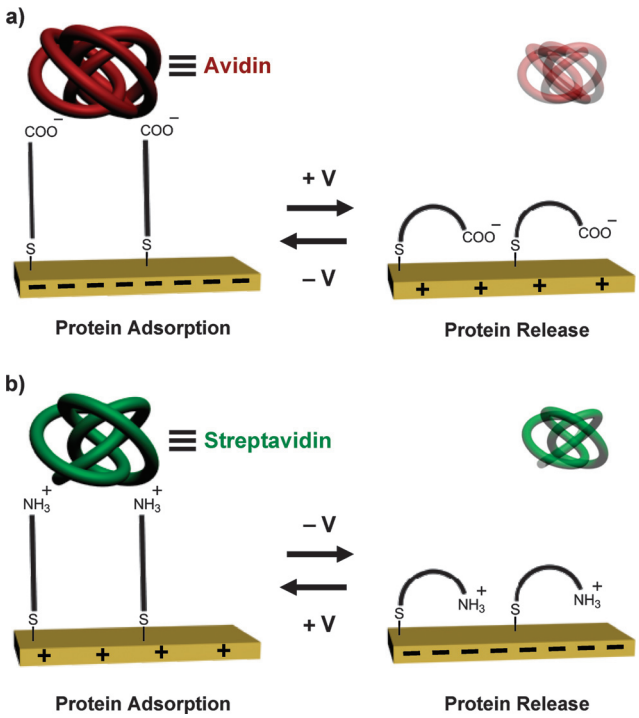

Fig. 14 Electrically controlled adsorption and release of avidin and streptavidin proteins by low density ionisable alkanethiolate SAMs on gold surfaces. (a) Acid-terminated and (b) amino-terminated monolayers show reversible and different conformational reorientation behaviour under negative and positive potential. These switchable surfaces can reversibly and selectively adsorb and release differently charged proteins under electrical control.[41]

controlled "on-chip" capture of target proteins directly from a complex protein mixture.

A similar electrically induced switching process has been applied to modulate the structural conformation of end-tethered DNA molecules on metal substrates. DNA-based SAMs have been shown[88–92,168,169] to be capable of producing reversible, well-defined nanometre-scale motions. DNA molecules exhibit negative electric charges due to the phosphates in the sugar–phosphate backbone and, thus, DNA molecules immobilised on a conductive surface (*e.g.* gold) can be driven away from, or pulled toward the surface, depending on the electrode potential.[88–90,92] At a negative electrical potential, the DNA molecules were shown to stand straight up on the surface, whereas at positive potentials the molecules lay

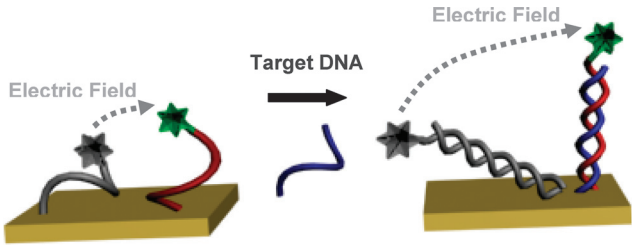

Fig. 15 Schematic representation of the electric-field-induced switching amplitude of a single-stranded oligonucleotide immobilised on a gold surface (left) and upon hybridization of the target sequence to the surface confined-single-stranded oligonucleotide (right). The flexible single-stranded oligonucleotide is shown to be only partially aligned by the electric field, whereas the double-stranded oligonucleotide is oriented more efficiently because of its intrinsic rigidity.[56]

flat.[88–92] The appropriate surface coverage, in order to prevent steric interactions between neighbouring strands, together with the strength of the electric field were key elements to realise electrically switchable surface-tethered DNA.[88–92] Based on the active manipulation of surface-confined DNA molecules, an elegant method to detect label-free oligonucleotide targets has recently been developed by Rant *et al.*[56] Single-stranded oligonucleotides labeled at one end with Cy3 fluorophores and modified at the other end with thiol linkers to chemically graft the molecules to gold surfaces constituted the sensor's basic element. The detection method was based on the orientation and extension of the DNA molecules from the surface, which was inferred from the fluorescence intensity emitted from the fluorophores attached to the DNA's upper end (Fig. 15). When the bias was switched to being positive, the molecules adopted a tilted conformation on the metal surface that partially quenches the fluorescence of the Cy3 dye. Application of a negative potential induced the molecules to extend away from the surface, and thus higher fluorescence intensity was observed. Moreover, hybridization of the target sequence to the surface confined-single-stranded DNA resulted in an amplified fluorescence signal, owing to the lower flexibility of the formed double-stranded DNA molecules. In this regard, single-stranded and double-stranded DNA molecules feature very distinct mechanical properties: single-stranded oligonucleotides act as almost perfectly flexible chains, whereas double-stranded helices behave like rigid rods. This DNA switching technique offers a convenient and sensitive means for identifying specific DNA targets, with a current detection limit of $\approx 3 \times 10^8$ bound targets per cm^2 sensor area.[56] Furthermore, it provided quantitative and sequence-specific data from which binding kinetics, affinity constants and duplex melting transitions could be evaluated.

6.2. Polymer films

An electrically conducting polymer is generally comprised of a conjugated polymer chain with π electrons delocalised along the backbone, yielding a semiconducting polymer that can be reversibly tuned through doping, an oxidation/reduction process where charge carriers are introduced to the polymeric backbone either chemically or electrochemically. Largely due to the reversibility of doping, conducting polymers are of considerable interest for a variety of biomedical applications.[170] A particular application of conductive polymers is drug delivery, where control of the current allows one to control the amount of ionic drug that can be released from a polymer film on an electrode. On the basis of this process, a variety of anions including biotin,[171] neurotransmitter glutamate,[172,173] and adenosine 5′-triphosphate (ATP)[174,175] have been electrostatically entrapped into conducting polymer films and released by electrical potential stimulus in a controlled way. Furthermore, conductive polymer films have also been developed[176,177] that could act as vehicles for delivery of positively charged drugs. As an example, a conducting, composite polymer, poly(*N*-methylpyrrolylium) poly(styrenesulfonate) (PMP^+PSS^-) has been employed for controlled delivery of the neurotransmitter dopamine.[176] The PMP^+PSS^- films were prepared on glassy carbon disks by the anodic polymerization of *N*-methylpyrrole from an aqueous sodium poly(styrenesulfonate) solution. In its reduced state, the PMP^+PSS^- film was able to bind dopamine cations, which could be released by oxidizing the polymer film.

Polypyrrole is a particular interesting candidate for biomedical applications, by virtue of its chemical and thermal stability, and low cytotoxicity. Apart from being exploited in drug-delivery systems,[171,172,174] polypyrrole polymers may be especially useful as advanced substrates for cell cultures since they provide a non-invasive way to regulate cell form and function (*e.g.* DNA synthesis). By reversibly changing their oxidation state and, consequently, their properties and surface binding characteristics, polypyrrole polymer films on ITO-coated glass substrates have been shown[178] to act as dynamic cell culture substrates. *In vitro* studies demonstrated that extracellular matrix molecules, such as fibronectin, adsorb efficiently onto the oxidised (polycation) polypyrrole thin films, and support cell attachment under serum-free conditions. When aortic endothelial cells were cultured on fibronectin-coated polypyrrole (oxidised) either in chemically defined, serum-free medium or serum-containing medium, the cells spread normally and synthesised DNA. On the other hand, electrochemical reduction of the oxidised polypyrrole film to its neutral state by applying an electrical potential resulted in inhibition of both cell spreading and DNA synthesis, but without adversely affecting cell viability. Application of a similar electrical potential to cells cultured on ITO surfaces had no effect on cell shape or function. The mechanisms by which oxidation and reduction of the polymer substrate influence cell behaviour are not fully understood.

Other potential biomedical application of the polypyrroles include highly localised stimulation of neurite outgrowth and guidance for neural tissue regeneration.[179] The current clinical approach to repair a peripheral nerve over a gap involves the utilisation of autologous nerve grafts.[180,181] However, this procedure has several drawbacks, including loss of function at the donor nerve graft site and mismatch of damaged nerve and graft dimensions. As an alternative to nerve autografts, natural and synthetic tubular guidance channels, which can bridge the gap between severed nerve ends, have been investigated. The electrically conductive polymer—oxidised polypyrrole—appears[179] to be a promising material for this purpose. Langer *et al.*[179] electrochemically synthesised

polypyrrole films on ITO-conductive borosilicate glass and evaluated their use as a substrate to enhance nerve cell interactions in culture. In the absence of electrical stimulation, a nerve-like cell line derived from a rat pheochromocytoma, PC-12 cell, was found to adhere and extend neuritis on it. Furthermore, application of an external electrical stimulus through the polymer film resulted in enhanced neurite outgrowth. The median neurite length for PC-12 cells grown on polypyrrole film subjected to an electrical stimulus was nearly doubled compared with cells grown on polypyrrole without the application of a constant potential. Research *in vivo* revealed that polypyrrole polymers promote little negative tissue or inflammatory response.

7. Photo-controlled switchable surfaces

7.1. SAMs

Molecular approaches that are similar to those already discussed can be used to engineer dynamic substrates that respond to light. Instead of developing molecular groups that undergo, for instance, specific redox reactions, molecules are designed to undergo photochemical reactions that lead to a change in the surface biological properties. Willner *et al.*[182] have created a SAM on gold with a photostimulated redox flavoenzyme to reversibly activate and deactivate the electrobiocatalysed oxidation of glucose. The on–off photostimulated system was achieved by the reconstitution of the apoflavoenzyme (*i.e.* apo-glucose oxidase) with the semi-synthetic nitrospiropyran–FAD cofactor (Fig. 16). The photochemical switch direction of the surface-reconstituted photoisomerisable enzyme was controlled by the electrical charge of the electron-transfer mediator. With ferrocenecarboxylic acid and ferrocenedicarboxylic acid acting as neutral and positively charged electron mediators, respectively, the bioelectrocatalytic functions of the nitrospiropyran–FAD–reconstituted glucose oxidase enzyme electrode were blocked. Upon isomerisation of the monolayers from the spiropyran-state into the merocyanine-state (generated by UV irradiation at 360–380 nm), the protonated nitromerocyanine–FAD–glucose oxidase exhibited bioelectrocatalytic activities for the oxidation of glucose. On the other hand, it contrasted with the positively charged electron mediator 1-[1-(dimethylamino)-ethyl]ferrocene in which the enzyme electrode in the spiropyran-state exhibited bioelectrocatalytic activities for the oxidation of glucose and the merocyanine-state inhibited its bioelectrocatalytic functions. Similar control over the bioelectrocatalytic activity of the glucose oxidase on gold electrodes was later demonstrated[183] by employing a reconstituted glucose oxidase on a FAD–nitrospiropyran mixed monolayer in the presence of the negatively charged electron mediator 1-[1-(dimethylamino)ethyl]ferrocene.

Two additional examples of photochemical control over monolayer substrates include the development of a surface that releases nitric oxide[184] over nanometre length scales for photochemotherapeutic applications, and a surface that can be photoactivated for spatio-temporal control of cell adhesion during cell cultivation.[185,186] The latter strategy was based[185,186] on the UV irradiation of a photocleavable 2-nitrobenzyl ester-terminated monolayer coated with a non-cell adhesive protein or polymer layer. Upon exposure to UV irradiation through a photomask using a mercury lamp equipped on a standard fluorescence microscope, the 2-nitrobenzyl groups were selectively removed and consequently the protein and polymer dissociated from the surface. The incubation of the micropatterned surface with the cell-adhesive protein fibronectin led to its selective adsorption onto the irradiated areas and localised cell adhesion. These investigations demonstrated effectively that new cell-adhesive regions could be formed and controlled at single-cell level during cell cultivation.[185,186]

7.2. Polymer films

Polymer films comprising photo-responsive molecules such as spiropyran[187,188] also represent attractive candidates for controlling protein and cell adhesion on surfaces. Spiropyran isomerises by illumination with UV light from the more hydrophobic spiro conformation to the polar, hydrophilic zwitterionic merocyanine conformation, while reverse isomerisation can be triggered by irradiation with visible light. This change from hydrophobic to hydrophilic state upon isomerisation has been applied to demonstrate UV light-induced detachment of fibrinogen, platelets and mesenchymal stem (KUSA-A1) cells from poly(spiropyran-*co*-methyl methacrylate)-coated glass plates.[187] The other approach by Edahiro *et al.*[188] involved the use of a polymer material composed of poly(*N*-isopropylacrylamide) having spiropyran chromophores as side chains to develop a reversible photo-responsive culture surface. UV irradiation with a wavelength of 365 nm was shown to induce an

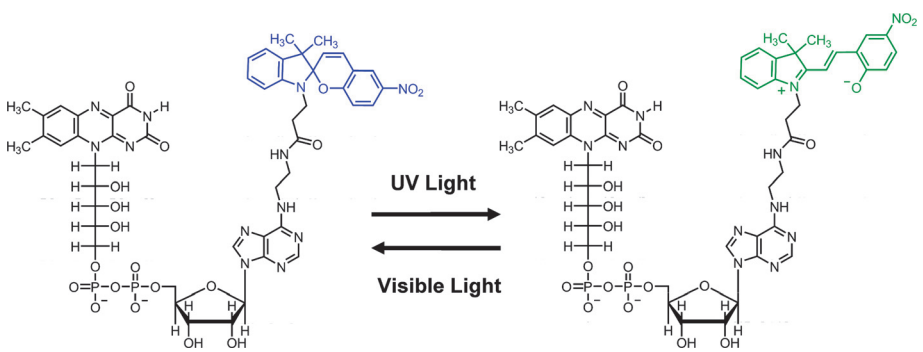

Fig. 16 Molecular structure of semisynthetic nitrospiropyran–FAD cofactor, which can undergo reversible photoisomerisation to the protonated nitromerocyanine–FAD cofactor upon UV irradiation.[182]

enhancement to adhesion of living cells, which could be reversed by visible light irradiation with a wavelength of 400–440 nm followed by thermal annealing at 37 °C.[188] The effect of UV irradiation on cells is dependent on the energy and wavelength of the radiation. Also different cell types and lines have somewhat different sensitivity towards UV radiation. In these cell cultivation studies based on spiropyran-derived polymer films,[187,188] the cells were apparently not significantly affected by the UV irradiation and exhibited sufficient viability after exposure.

A further means to control biomolecule activity of surfaces by photochemical stimulus relied on the *trans–cis* isomerisation of the azobenzene molecule. The azo chromophore isomerises by illumination with UV light (λ = 300–400 nm) from the stable *trans* form to the *cis* state, while reverse isomerisation can be triggered by irradiation with visible light (λ = 425–500 nm). Isomerisation of azobenzene is accompanied by an appreciable shape change as the *trans* isomer adopts a more linear conformation than the *cis* isomer. Hayashi *et al.*[32] have shown that, when azobenzene moieties were incorporated into a peptide immobilised on a carboxymethylated dextran-coated gold surface, the structural changes in the azobenzene could lead to the photoregulation of peptide binding to its RNA aptamer. By also taking advantage of the change in azobenzene molecular dimensions, which are decreased by approximately 3.4 Å upon isomerisation from the *trans* to *cis* conformation, Kessler *et al.*[46] reported the control of cell adhesion properties on RGD-functionalised surfaces.[46] The azobenzene derivative, 4-[(4-aminophenyl)azo]benzocarbonyl, was incorporated into the RGD peptide and tethered to a poly(methyl methacrylate) surface by UV irradiation. The photoswitchable RGD peptide-coated surfaces exhibited enhanced cell adhesion in the *trans*-azobenzene configuration. On the other hand, the surfaces that had previously been UV irradiated at 366 nm showed a reduced cell plating efficiency as a result of shortening the distances of the RGD peptides to the surface by the *trans–cis* isomerisation of the azobenzene derivative. The azobenzene molecule has also been incorporated into an enzyme inhibitor to provide a means of modulating the binding of α-chymotrypsin to a surface (Fig. 17).[189] Photoregulation was achieved by using a phenylalanine-based trifluoromethyl ketone inhibitor containing a photoisomerisable azobenzene group, an oligoethylene glycol as a spacer linker, and a terminal alkyne for attachment to a surface-bound azide using click chemistry. The azide-modified surface was formed by covalently linking azidooligoethylene glycol amines to the carboxylic acid groups of a dextran polymer matrix coated gold surface. The surface-bound azobenzene inhibitor in the *trans* state exhibited a reduced binding of the α-chymotrypsin enzyme. However, irradiation of the functionalised surface with UV light (>360 nm) induced isomerisation from the *trans* to the *cis* azobenzene configuration, which was accompanied by a significant increase in enzyme binding to the surface.

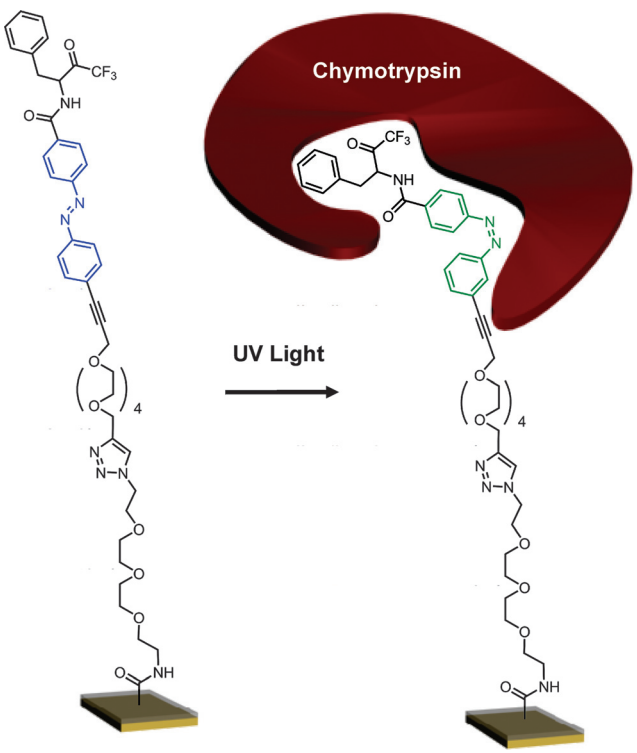

Fig. 17 Photoswitchable surface wherein the photo-induced structural changes in the azobenzene moiety gives rise to changes in the binding of chymotrypsin to the surface.[189]

8. Concluding remarks and perspectives

Whereas important progress on static biological surfaces has been made in the past decade, much research is now focusing on the development of smart biological substrates. A significant number of switchable biological surfaces based on self-assembled monolayers and polymer films have been described in recent years, which modulate interactions with biomolecules, including peptides, DNA and proteins, and change the response of cells and tissues that come into contact with the surface. To date, a variety of stimuli, including chemical/biochemical, thermal, electric and optical stimuli have been used to this goal. Most studies aim to develop a reversible response, in which the properties are switched when stimuli are delivered and then regenerated when the stimuli are removed or an alternate trigger is applied. However, both classes of behaviour—nonreversible and reversible modification—can be usefully exploited. For instance, nonreversible systems can be desirable in drug delivery systems and surgical implants to promote wound healing and regeneration.

The switchable biological surfaces reviewed in this article differ in the complexity of their design and fabrication and the sophistication with which interactions with biomolecules can be dynamically controlled. Nonetheless, whether based on highly complex or simpler systems, switchable surfaces were shown to be of great interest for diverse biological and medical applications. Different methodologies were shown to reversibly modulate protein adsorption, which is important in a variety of applications, including biofouling, chromatography and bioanalytic devices. Synthetic biomimetic materials have been developed to serve as provisional matrices for tissue regeneration *in vivo*. For instance, by incorporating three types of

bioactive signals in biodegradable hydrogels, several properties of the natural ECM were mimicked, such as biospecific cell adhesion, degradation by proteolytic processes involved in cell migration and tissue remodeling, and presentation of growth factors.[128] These hydrogels were successfully employed to promote bone healing *in vivo*. Thermally and electrochemically active substrates have been shown to grow and harvest multicellular tissues. Important advances have been achieved in the development of highly sensitive biosensors and novel drug delivery systems.

Albeit the substantial progress and scientific advances in the field, exciting future developments are ahead of us. An important aim of further work in developing switchable substrates is to increase the biological relevance of the model systems. The dynamic state of the natural ECM is regulated by a highly complex temporal and spatial coordination of many different cell–matrix and cell–cell interactions, and thus more complex biosurfaces are expected to be developed. These surfaces should more closely capture the properties of the natural ECM. An application easily envisaged is for addressing basic problems in biology. These developments will also be extremely important for the future of tissue engineering, repair and regeneration. Other interesting areas for future research are to investigate alternative stimuli and to extend the switchable surface properties to achieve more types of response. It is anticipated that a wider range of applications will be unveiled as the field of switchable surfaces matures, and the full potential of surfaces with dynamic properties is realised. The potential of this exciting research field is enormous, but it will certainly require concerted efforts from scientists in a variety of disciplines. Only with collaborative efforts of chemists, physicists, material scientists, engineers, medical researchers and biologists will we be able to fully explore the potential of switchable surfaces for biological and medical applications.

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