Chapter 4

Electrochemically Designed Self-Assembled Monolayers for the Selective Immobilization and Release of Ligands, Proteins, and Cells

Omar Azzaroni* **and Roberto C. Salvarezza****
Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Universidad Nacional de La Plata, CONICET, CC 16 Suc. 4 (1900) La Plata, Argentina
*oazzaroni@yahoo.com and **robsalva@inifta.unlp.edu.ar

4.1 Introduction

The discovery of self-assembled monolayers (SAMs), in the 1980s, created a whole new scientific field and resulted in robust, simple, and highly reproducible technologies that strongly contributed to the molecular design of biointerfaces [1–3]. Since then, many exciting advancements shaped the evolution of modern biomaterials science, which itself originated several new subfields, with physical chemistry and biology playing a major role, and where the boundaries between surface science and life sciences sometimes are hardly distinguishable. One of the key advantages of SAMs is that their properties, that is, thickness, structure, surface energy, and stability, can be easily controlled, and specific functionalities can also be introduced into the building blocks in

Handbook of Biofunctional Surfaces
Edited by Wolfgang Knoll

ISBN 978-981-4316-63-7 (Hardcover), 978-981-4364-18-8 (eBook)
www.panstanford.com

order to expose active groups that could interact with biological materials in a controlled fashion [4, 5]. Hence, to some extent, SAMs can be considered ideal platforms for bioengineering surfaces on a molecular level.

In this context, an emerging research area in biointerface science is the development of substrates that dynamically regulate biological functions in response to applied external stimuli [6]. The ability to trigger the delivery or capture of bioactive molecules upon the emergence of external stimulus is a current challenge in biomaterials science that demands the convergence of tools and concepts from complementary disciplines. One of the most challenging goals in SAMs is the creation of monolayers that could undergo surface reactions only when needed, or, in other words, to promote "surface reactions on demand" that could generate dynamic surfaces in which the physical, chemical, and biological properties of surfaces are tuned at will [6]. Furthermore, provided that SAMs are fully compatible with a wide variety of patterning techniques, this approach would also enable the independent addressability of surface reactions on the substrate.

In this respect, electrochemically commanded surfaces have some advantages over other methods for surface reactions on demand that make them a very interesting approach. First, dynamic control of surface chemistry could be easily achieved by potential-induced redox reactions. Second, electrochemical equipment is readily available in any laboratory and its implementation is very straightforward. Chemically modified surfaces can be easily incorporated into electronic devices, thus generating independently addressable electrodes because the electrochemical reaction can be triggered on a designated electrode [6].

Even though the term "electrochemistry" evokes a wide variety of electrochemical processes, ranging from electrolysis to electrodeposition, in our case this term refers to a scenario in which electron transfer chemistry of species confined at solid-liquid interfaces can be controlled with accuracy and convenience. This is particularly obvious when we think of an electrode surface as a macrosize molecule whose ability to provide or accept electrons may be precisely adjusted by fine-tuning of the electrode potential [7]. Exciting opportunities are revealed when we think in this manner. Electrochemistry provides a complementary perspective from

which to consider the manipulation of the organic groups through the formation of carbon-carbon bonds or bonds between carbon and heteroatoms and ultimately control the chemical features of SAMs. Harnessing the ability to change the chemical nature of terminal groups in SAMs [8, 9] upon modifying the electrochemical potential represents the basis of the dynamic control over the formation of covalent bonds.

Physicists, chemists, and biologists are becoming increasingly interested in electrochemistry as a toolkit with which to tune and modulate the biological activity of biofunctional SAMs. However, the interdisciplinary nature of biointerface science demands the convergence of biology, organic chemistry, and surface science, together with electrochemistry, to take advantage of the strengths of each discipline so as to develop a highly functional SAM displaying electrochemically driven, well-defined biological activity.

Herein a critical review of relevant examples from the literature illustrating the use of electrochemistry as a key enabler to modulate the biological activity of SAMs is presented. From these examples it is hoped that the reader will get an understanding not only of the versatility of electroactive SAMs to promote organic reactions on surfaces but also of the way in which electrochemistry can open new opportunities to design multifunctional biological interfaces.

4.2 Electrochemistry of Thiol Self-Assembled Monolayers on Metal Surfaces

As mentioned in the introduction section electrochemically commanded SAM-based surfaces are a very interesting platform for performing a variety of reactions in a selective way and in confined regions of surfaces that can be patterned by soft lithography [10]. To design these systems one should first consider the nature of the substrate and the SAM to be used for each particular case. SAM-based systems (devices) under electrochemical control for biological applications require the use of conducting, biocompatible substrates able to work in aqueous solutions at physiological pH values.

Metallic substrates are particularly useful for this purpose because they can be polarized in a wide range of potentials within the stability window of water. Among metals we are interested in

those exhibiting extremely good stability in the working conditions, thus avoiding metal dissolution, oxide formation, and other faradaic processes such as hydrogen or oxygen evolution reactions among others. Gold is the best option because it satisfies all these requirements. Gold is chemically inert in ambient conditions, is easy to clean, and exhibits an extended double-layer region only limited by the HER from the cathodic side and gold oxide monolayer formation from the anodic side. Anion adsorption could take place in the double-layer region, although chemisorbed SAMs completely displace these species from the Au surface.

Concerning the SAMs, molecules such as thiol, alkyldisulfides, and alkylsulfides have been widely used to form dense, well-ordered monolayers on gold [11]. Also, carbamates [12] and thiocyanates can form thiol SAMs on gold surfaces [13].

4.2.1 Electrochemical Stability of Self-Assembled Monolayers

The stability of SAMs in aqueous electrolytes is a crucial point for their application in electrochemistry. It is well known that SAMs of alkanethiolates on Au, Ag, and Cu exhibit reductive electrodesorption [14] and oxidative desorption [15], thus defining a potential window where the SAM is stable on the metal surfaces. This potential window depends on several factors such as the hydrocarbon chain length, the nature of the terminal group, the pH and ionic strength of the electrolyte, and the metal substrate. Therefore, before discussing applications we will briefly discuss the electrochemical stability range of SAMs.

The reductive desorption limits the cathodic stability of thiols. This reaction can be written as follows:

$$\text{R-S-Me} + \text{e} \rightarrow \text{RS}^- + \text{Me} \qquad (1)$$

where R-S-Me stands for the adsorbed alkanethiolate. The typical current (j) potential (E) profiles recorded for alkanethiolate-covered Au or Ag substrates in electrochemical cells containing aqueous electrolytes exhibit well-defined current peaks related to reaction (1) (Fig. 4.1). The peak labeled as CI corresponds to the reductive electrodesorption reaction, while peak AI corresponds to the oxidative electroadsorption of molecules produced at CI. The charge density (q) involved in peak CI is that expected for desorption of a

complete monolayer of thiol in standing-up configuration ($q \approx 0.075$ mC cm^{-2}).

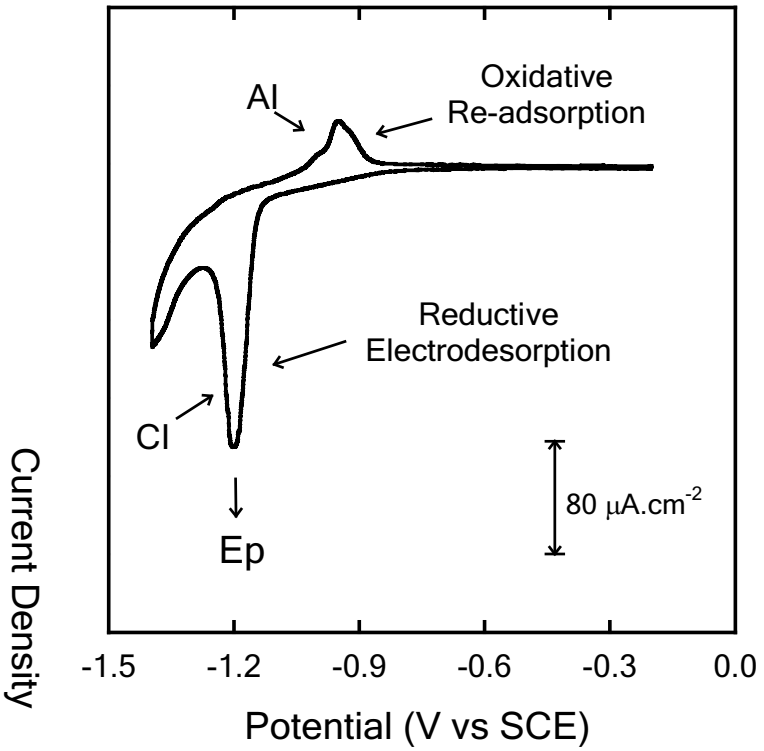

Figure 4.1 Typical current density versus potential profile recorded for a dodecnaethiolate SAM on Au surfaces in 0.1 M NaOH. The potential scan starts in the negative direction. The electrodesorption peak, peak potential (E_p), and oxidative readsorption are indicated. *Abbreviation*: SCE, saturated calomel electrode (reference electrode).

In situ scanning tunneling microscopy (STM) imaging has shown that the SAM structure remains practically unaltered for applied potentials more positive than those corresponding to peak CI, that is, the preceding SAM's desorption [16]. Therefore, the peak potential (E_p) has been widely used to test the stability of SAMs against reductive electrodesorption. However, electrochemical measurements have shown that SAM disorder takes place at potentials near the electrodesorption peaks [17].

In the case of SAMs on Cu, Pd, Ni, and Pt, the E_p measurements are not possible because SAM electrodesorption takes place simultaneously with the hydrogen evolution reaction (HER). In this case, the rotating disc-ring technique has been used for the

E_p determination [18]. However, H atoms can also produce thiol desorption not only for SAMs on these metals but also for SAMs on Au surfaces [19]. The reductive electrodesorption, reaction (1), depends on the pH value. While E_p remains nearly constant in the 3 < pH < 14 range, it moves in the positive direction for pH values lower than 3, that is, alkanethiolates are less stable in strong acid media [20–22]. On the other hand, for a constant pH the E_p value moves in the negative direction as n, the number of C atoms in the alkanethiol chain, is increased. The E_p versus n plots in neutral and alkaline solutions gives straight lines with a slope ≈ 4 kJmol^{-1} per C atom. It means that the SAM stability increases with n, due to the increase in van der Waals interactions between the hydrocarbon chains and hydrophobic forces between the molecules and water [23]. A detailed inspection of the E_p versus n plot for pH less than 3 shows that the straight line has a slope slightly higher than that observed in alkaline and neutral solutions [14]. This fact arises from the lower solubility of alkanethiolate molecules in acidic media that might be an additional factor for stabilizing the SAM against desorption. However, for a given thiolate the E_p value in acidic media is always more positive than that measured in neutral and alkaline solutions, that is, alkanethiolate SAMs are more stable in neutral and alkaline solutions [14].

The terminal group of the thiol plays also an important role in SAM stability. In neutral and alkaline solutions E_p values for COOH-terminated alkanethiols are 110 mV more positive than those for *n*-alkanethiols having the same alkyl chains, due to repulsive interaction between the negatively charged carboxylate groups in a COOH-terminated alkanethiol monolayer [24]. Also the ionic strength of the electrolyte plays a role. In fact, the positive shift of E_p, that is, the smaller stability range, for mercaptopropionic acid with decreasing concentration of KOH solution has been ascribed to the increasing electrostatic repulsive interactions between carboxylate groups.

The metal substrate has also a key role in determining SAM stability. At a constant pH and for a given thiol, the electrochemical stability range increases as Au [24] < Ag [25] ≈ Ni [26] < Pd [27] ≈ Cu [28]. Therefore for electrochemical applications one can select the metal considering the stability range. It has been proposed that the electrodesorption potential for a given alkanethiol on different metals results from a balance between the adsorption energy of

the organic molecule on the metal surface (which varies in the 40–60 kcal mol^{-1} range), the energy to introduce an electron into the alkanethiolate–metal system, and the solvation of the metal surface [29].

Much less attention has been paid to SAM oxidative desorption than that paid to the reductive desorption process. In contrast to the reductive desorption that occurs over a small potential window (current peak CI in Fig. 4.1), removal by oxidative desorption takes place over a wide range of positive potentials related to gold oxide monolayer formation [30]. The oxidative removal of the nonanethiol layer is found to be a slow multiple-step process in which the carbon-sulfur bond can be broken and up to 11 electrons can be involved in the oxidation of a single chemisorbed thiol [15]. In contrast to the reductive process, the oxidatively desorbed molecules have very weak CH stretching bands. In the case of gold, reductive desorption and oxidative desorption define a wide potential range where thiol SAMs are stable and then suitable for SAM-commanded electrochemistry.

4.2.2 Charge Transfer through Self-Assembled Monolayers

The electron transfer properties of SAMs are also a relevant point in order to build electroactive surfaces, particularly in those situations where the redox-active species constitutes the functional end group in the alkyl chain. Charge transfer measurements through alkanethiol and aromatic thiol molecules chemisorbed on Au surfaces have been extensively studied in electrochemical environments under potential control. Charge transfer processes from the metal electrode to redox centers in solution [31, 32], immobilized or covalently bound to the terminal group of the SAMs [33–38], have been reported.

After Chidsey et al. reported the heterogeneous electron transfer rate and electron tunneling constant for the ferrocene-terminated alkanethiol monolayers with different alkyl chain lengths [39], a number of groups have reported values for SAMs containing redox couples. A description of the charge transfer between a metallic electrode and a redox-active moiety separated by a SAM involves a chemical kinetics approach to defining a bridge-mediated reaction between the electron donor and the acceptor species [38]. It has been found that the current decreases exponentially with the hydrocarbon chain length with a decay factor β ranging from 0.5 to

1 A^{-1}, suggesting a low tunneling efficiency. The low and high values [40] reflect different transport mechanisms: current flows following the bond overlap along the molecules (through-bond mechanism) [35, 41] and the direct component from one electrode to the acceptor across the molecules acting as a dielectric medium (through-space tunneling). It has been considered that both mechanisms could be also simultaneously present. On the other hand, it has also been proposed that charge transport through the monolayer film is tunneling through σ-bonds [34, 42] and that a small amount of charge is also transported by a process of electron hopping between alkane chains in the monolayer film [42].

Furthermore, similar β values have been reported in aromatic single-molecule junctions [43]. It has been proposed that the charge transfer occurs via the aromatic molecules of the SAMs to the Au surface [44]. It should be noted that aromatic SAMs are often more disordered and less packed than alkanethiolate SAMs.

In general, the dependence of the current with distance is estimated from the SAM thickness or molecular length, assuming ordered chains and defect-free SAMs. However, chain ordering depends strongly on many factors such as hydrocarbon chain length, preparation conditions, and substrate topography. Also defect in SAMs, such as vacancies and domain boundaries, could strongly affect the charge transfer across the SAMs by introducing the preferred path for electron transfer [45]. Therefore, electrochemically designed SAMs require a careful preparation and characterization to minimize the presence of structural and conformational defects that could affect their performance.

The charge transfer from quinone (Q)/quinol groups to Au electrodes through different thiol bridges has been extensively studied because of their importance in many fields of chemistry and biology. As seen in the next sections, this reaction is very important in the design of electrochemically commanded SAMs.

The electrochemical reduction of quinones involves a coupled transfer of protons and electrons. Theoretical treatments of proton-coupled electron transfer indicate that both apparent formal potential and apparent standard heterogeneous rate constant quantities should be highly pH dependent [46]. It has been shown that the SAM-aminobenzoquinone monolayer exhibits a nearly ideal Nernstian behavior. In neutral and weakly acidic electrolytes, this redox species is reduced in a kinetically slow, two-electron, two-

proton (2e + 2H) process. On the other hand, in acid solutions, the observed reaction is consistent with an overall 2e + 3H transfer. Kinetic measurements have shown that the apparent standard rate constants range from 0.1 to 0.01 s^{-1}.

The local pH affects the redox behavior of Q groups. In fact, pyrroloquinoline Q diluted by alkanethiols of different chain lengths showed electrochemical reversibility and electron transfer rate constants greatly affected by the local proton concentration near the electroactive group when protons are coupled with electrochemical reactions. By blocking the proton access to the redox moiety, the reaction becomes irreversible and the electron transfer rate slows accordingly [47].

Dialkyl disulfide–linked naphthoquinone, $(NQ\text{-}C_n\text{-}S)_2$, and anthraquinone, $(AQ\text{-}C_n\text{-}S)_2$, derivatives with different spacer alkyl chains (C_n: n =2, 6, 12) were synthesized, and these Q derivatives were self-assembled on a gold electrode [48]. Voltammograms show a reversible redox reaction between Q and hydroquinone (QH_2) with formal potentials at −0.48 and −0.58 V, for NQ and AQ, respectively, irrespective of the spacer length. In contrast, the oxidation and reduction peak currents were strongly dependent on the spacer alkyl chain length. The pH dependence of the redox couple was in agreement with a $H^+/2e^-$ process. The β values for NQ and AQ SAMs were determined to be 0.12 and 0.73 per CH_2, respectively. The Q terminal group, present in a mixed SAM on gold, reacts with a nucleophile, dissolved in the bulk phase. The reaction rate depends on the chemical nature of the tether, being seven times faster for quinones attached via a delocalized bridge as compared to a saturated alkane chain [49]. Cyclic voltammetry (CV) of the Q/QH_2 redox couple was used to monitor the nucleophilic addition. CV data also suggests that the π-delocalized oligo(phenylene ethynylene) bridge facilitates the addition of two mercaptoethanol molecules as compared to the alkane bridge, where only one molecule is being added.

It has also been observed that specific and directed changes in structure between the bridge and headgroup can significantly change the rate for proton-coupled electron transfer of SAMs containing Q derivatives with varying electronic conjugation [50]. The apparent proton-coupled electron transfer rates of Q SAMs on gold electrodes in which the Q compounds contained different bridging groups (i.e., a single, double, or triple bond) connecting the headgroup to an oligo(phenylene vinylene) (OPV) thiol anchor

were studied in the pH range 1–12. The Q compounds in the SAMs were diluted by octanethiol, and cyclic voltammograms of the Q/QH_2 couple were measured as a function of scan rate. It was found that the two-electron, two-proton couple describing a nine-member square scheme can fit the data with an average single-electron transfer rate constant reflecting the electron tunneling parameter, β, of the different bridging structures. Results have also shown that double-bond bridge gives the fastest electron transfer compared to single- or triple-bond bridges.

Reversible conductance switching in single Q-oligo(phenylene vinylene) (Q-OPV) molecules was demonstrated using electrochemical STM [51]. The switching was achieved by application of electrochemical potential to the substrate supporting the molecule. The ratio of conductances between the high- and low-conductivity states is over 40. The high-conductivity state is ascribed to strong electron delocalization of the fully conjugated QH_2-OPV structure, whereas the low-conductivity state is characterized by disruption of electron delocalization in the Q-OPV structure

4.3 Controlling Biological Activity Using Electroactive Self-Assembled Monolayers

4.3.1 The Diels-Alder Reaction in Two Dimensions

In a seminal work Yousaf and Mrksich described the use of SAMs presenting a Q group to demonstrate that the Diels-Alder reaction of this group with cyclopentadiene (cp) could be exploited as a versatile route to covalently anchor diverse functional groups [52]. One of the attractive features relied on the modulation of the reactivity of the Q group through electrochemical means. This was easily achieved by electrosynthesizing (reduction) of the QH_2, which does not participate in the Diels-Alder reaction. Electrochemical studies revealed that mixed SAM presenting QH_2 and hydroxyl groups exhibited a well-defined electrochemical response, indicating that QH_2 undergoes oxidation at 220 mV to give the Q and reduction at –150 mV (vs. Ag/AgCl—reference electrode) (Fig. 4.2) [52]. Long-term potential cycling also confirmed that the electrochemical process is reversible and highly reproducible.

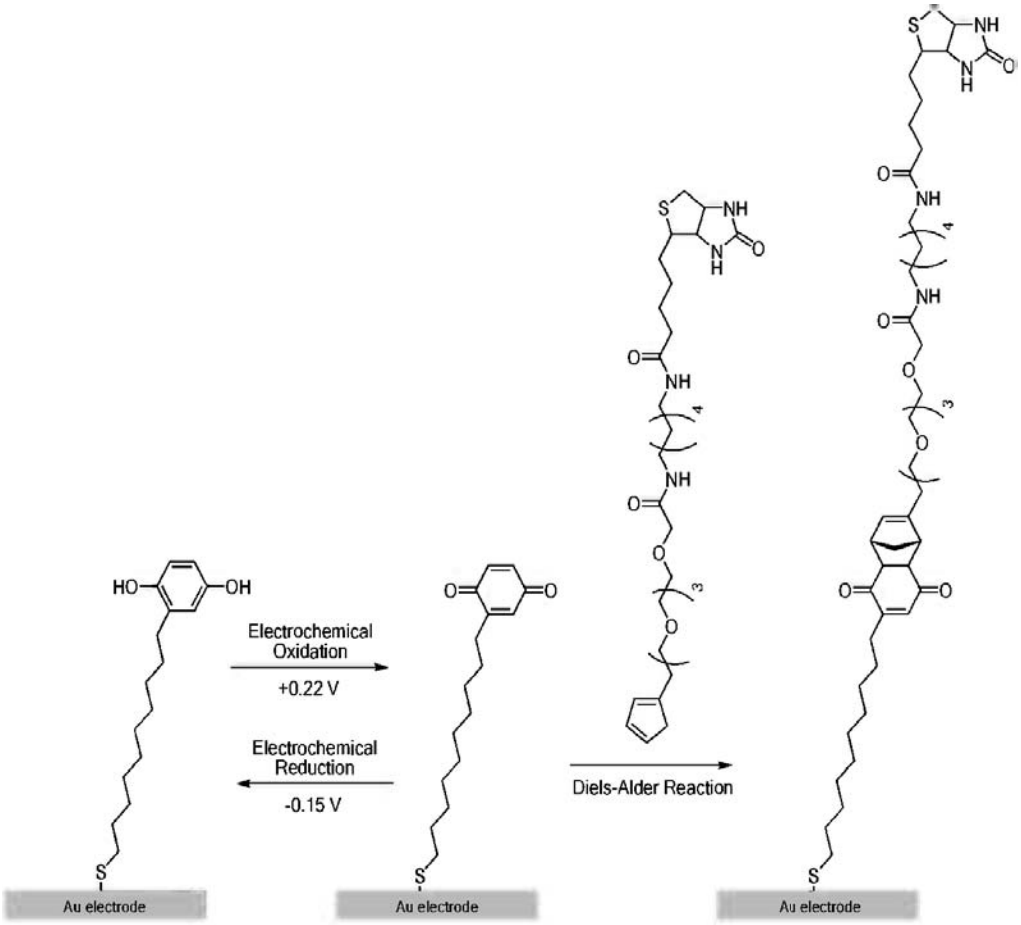

Figure 4.2 Covalent anchoring of biotin onto an electroactive SAM via a Diels-Alder reaction.

Addition of cp to the electrolyte promoted a significant decrease in the peak current for both reduction and oxidation owing to the Diels-Alder reaction of cp with the Q-terminated SAM. Blank experiments involving the addition of other dienes to the electrolyte gave similar losses in current over consecutive cycles, but immersion of a monolayer presenting QH_2 groups in electrolyte containing cp indicated no alterations on the voltammetric response, demonstrating that only the quinine form underwent reaction with cp. The electroformation of a self-assembled platform for the Diels-Alder reaction was extended to the anchoring of biospecific ligands in order to selectively immobilize proteins via recognition-directed assembly (Fig. 4.2). The association of streptavidin to surface-anchored biotin was studied as a model system to demonstrate the validity of the approach. To suppress nonspecific protein

adsorption, SAMs were prepared from alkanethiolates terminated in tetra(ethylene glycol) groups, which resist entirely the adsorption of protein, and an extended linker to the QH_2. Then, the oxidized substrates bearing Q groups were treated with a conjugate of cp and biotin to immobilize the ligand, and subsequently the samples were placed into contact with streptavidin. Surface plasmon resonance (SPR) spectroscopy measurements confirmed that the binding of streptavidin to the immobilized biotin was irreversible. Control experiments using streptavidin presaturated with biotin indicated that the binding of the protein was not feasible, thus demonstrating the biospecific character of the interaction. Furthermore, streptavidin did not immobilize to monolayers that presented either Q or QH_2 groups mixed with glycol groups, which indicated the absence of nonspecific adsorption in the electroactive platform.

The use of electroactive SAMs was proposed as a tool to turn on the migration of mammalian cells [53]. This approach was based on the use of QH_2-terminated alkanethiolates diluted into SAMs derivatized with penta(ethylene glycol) groups. The penta(ethylene glycol) groups, which comprise the major component of the monolayer, are critical to hinder the nonspecific adsorption of proteins to the monolayer and ensure that the interaction of cells with the substrate is mediated by the immobilized ligands alone. As described above, the QH_2 group undergoes oxidation when an anodic potential is applied to the gold substrate to give the corresponding Q, which subsequently undergoes an efficient Diels-Alder reaction with cp to afford a covalent adduct. This electrochemical route enables the facile formation of an electrically switched surface changing from a state inhibiting cell attachment to a state promoting cell attachment. The QH_2 groups were electro-oxidized to Q and then reacted with a conjugate of cp and the peptide Gly-Arg-Gly-Asp-Ser-NH2 (i.e., RGD-cp) to covalently bind the peptide to the gold surface (Fig. 4.3). The RGD peptide mediates cell adhesion by binding to cellular integrin receptors, and it is routinely used as a ligand to promote cell adhesion to different substrates. Experiments performed using Swiss 3T3 fibroblasts demonstrated that cells were able to attach and spread efficiently onto the peptide-derivatized substrates [53]. Blank experiments also showed that cells were released when soluble GRGDS was added to the medium, demonstrating that adhesion to the surface was mediated by biospecific interactions.

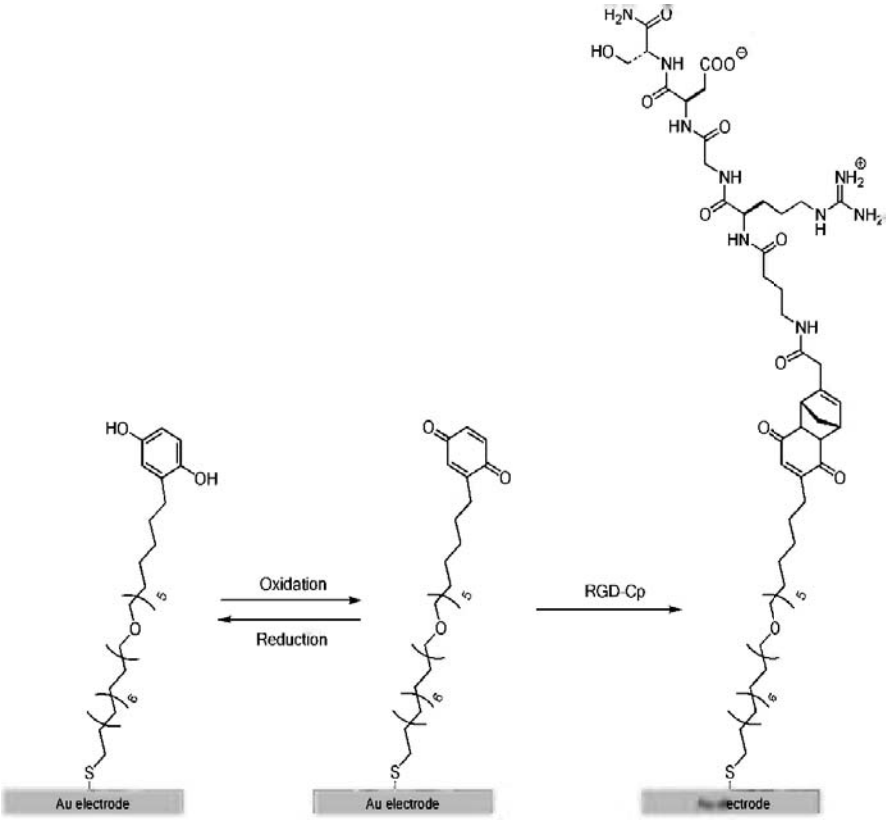

Figure 4.3 Immobilization of RGD peptides to Q-terminated electroactive SAMs through a Diels-Alder reaction.

The ability to confine cells on peptide-derivatized monolayers was combined with a microcontact printing approach in order to tune and manipulate the surface migration of the supported cells. Gold surfaces were modified with microcontact-printed domains of hexadecanethiolate monolayers, whilst the rest of the surface was backfilled with a mixed monolayer presenting QH_2 and penta(ethylene glycol) groups. Immersion of the microcontact-printed surface in a fibronectin solution resulted in the protein adsorption on the hexadecanethiolate domains, which was followed by selective attachment of Swiss 3T3 fibroblasts onto these regions [53]. Thereafter, the nonactive regions of the substrate were electrochemically activated by electro-oxidizing the QH_2 groups in the presence of RGD-cp, thus resulting in the selective peptide immobilization into the inert domains. Afterwards, surface-confined cells began to migrate from the hexadecanethiolate domains onto the regions that were previously inert, leading to an even distribution of cells onto the patterned substrate (Fig. 4.4).

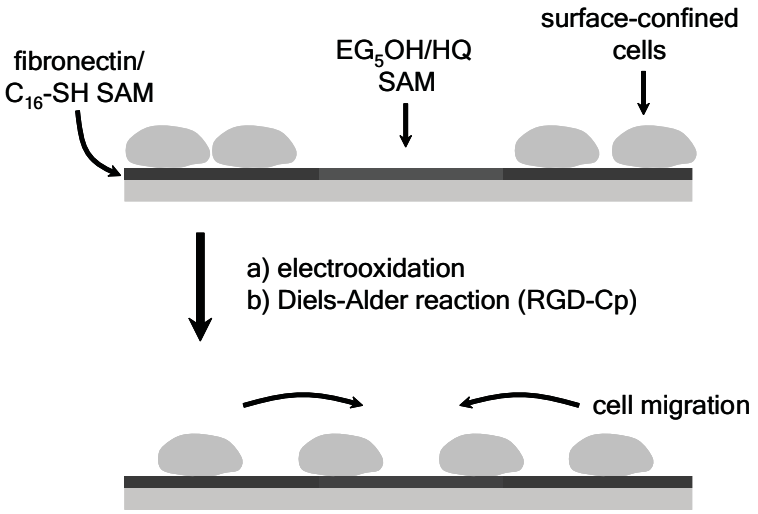

Figure 4.4 Immobilization of RGD peptides to Q-terminated electroactive SAMs through a Diels-Alder reaction.

In a similar vein, Kwak et al. reported a method for the covalent tethering of biological ligands to surfaces using electrochemical activation of QH_2 monoester SAMs [54]. The reaction generates benzoquinone as a good leaving group, followed by nucleophilic acyl substitution with a primary amine to form an amide in high yield (Fig. 4.5). The method allows the very rapid site-selective immobilization of ligands (one-step process) using mild electrochemical conditions for the formation of covalent bonds on the SAM-modified surface [54].

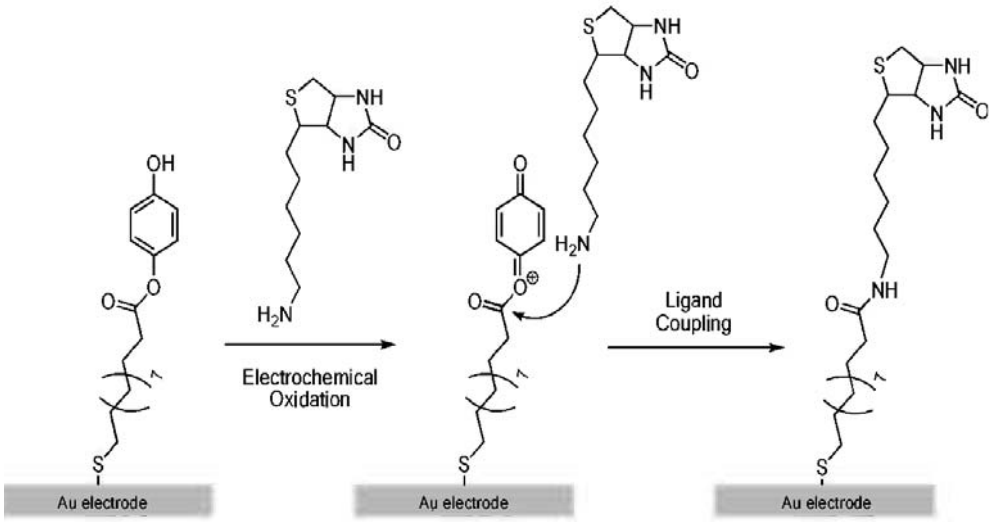

Figure 4.5 Immobilization of RGD peptides to Q-terminated electroactive SAMs through a Diels-Alder reaction.

4.3.2 Electroreductive Release of Ligands from Redox-Active SAMs

One important aspect in the molecular design of biointerfaces is the creation of platforms displaying dynamic properties that can be altered upon the influence of an external stimulus. In this context, Hodneland and Mrksich described the use of SAMs designed to release the ligands when a reductive potential is applied to the gold substrate [55]. This dynamic property arises from the clever use of the Q propionic esters to covalently bind ligands, for example, biotin, to the SAM. Q propionic esters and amides have been used as protecting groups for alcohols and amines, respectively. The Q groups can be reduced under mild conditions to give QH_2, which rapidly lactonizes with liberation of an alcohol or amine [55]. The two methyl groups at the benzylic position together with the proximal methyl group on the rings are important factors to increase the rate of the lactonization reaction and consequently the release of the ligand. This interesting strategy to release ligands and bioconjugated proteins was demonstrated through the electrochemically triggered release of biotin (Fig. 4.6) [55].

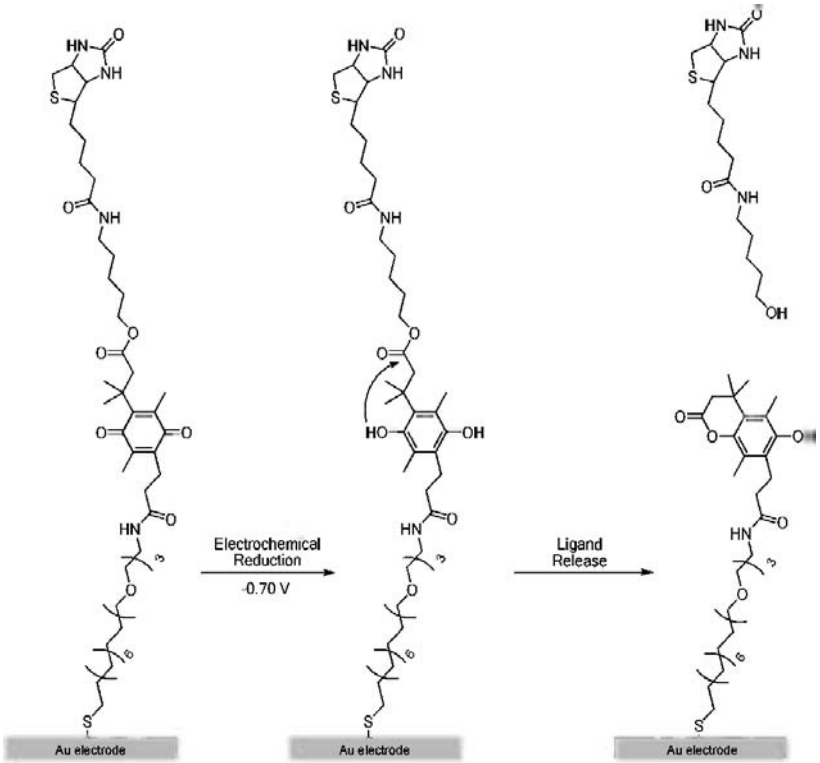

Figure 4.6 Electrochemical release of ligand biotin from an electroactive SAM.

Upon assembling streptavidin onto biotin-derivatized SAMs, SPR experiments confirmed that reduction and subsequent lactonization and release of biotin were triggered by application of a cathodic potential and the amount of streptavidin that bound to the resulting SAM decreased by 95%.

This platform is particularly useful, provided the substrate can selectively release immobilized ligands and regulate ligand-receptor interactions upon predefined external stimuli. Yeo and Mrksich demonstrated that this methodology is also compatible with the manipulation of cells deposited on SAMs bearing RGD peptide ligands tethered to the monolayer through an electroactive Q ester moiety [56]. Upon applying a cathodic potential the Q ester underwent two-electron reduction to give the QH_2, which then rapidly cyclized to give a lactone and the subsequent release of the tripeptide RGD [56]. This electroactive SAM displaying dynamic properties introduces a versatile approach to tune the attachment of cells, provided they can be combined with other nonelectroactive ligand-modified SAMs. One interesting example was the modification of gold surfaces with RGD-terminated SAMs in which two regions differed only in the linkage used to tether the peptides to the monolayer. In one domain, the peptide was tethered through the Q ester (electrochemical release), and in the other domain the peptide was tethered with a linker that was not electrically active. The RGD-modified substrates placed into contact with cell-culture media (3T3 Swiss fibroblasts cells) efficiently attached cells to both regions of the patterned monolayer. Upon applying a cathodic potential (–0.7 V vs. Ag wire pseudo-reference electrode), more than 70% of the cells on the electroactive remained unattached on the substrate and were released from the surface upon changing the media with fresh media (Fig. 4.7).

4.3.3 Electro-Oxidative Release of Ligands from Redox-Active SAMs

Electrochemical oxidation of redox-active SAMs can be also used for release and subsequent immobilization of biospecific ligands. This approach was demonstrated by using SAMs presenting RGD ligands tethered to the monolayer through an *O*-silyl QH_2 moiety [57]. The *O*-silyl QH_2 ether constitutes an electroactive functional group, thus providing a functional platform for selective release of the peptide from the monolayer-modified substrate. In particular,

the electro-oxidation of this organic group promotes the formation of benzoquinone and the corresponding hydrolysis of the silyl ether functionalized with the biospecific ligand (Fig. 4.8) [57].

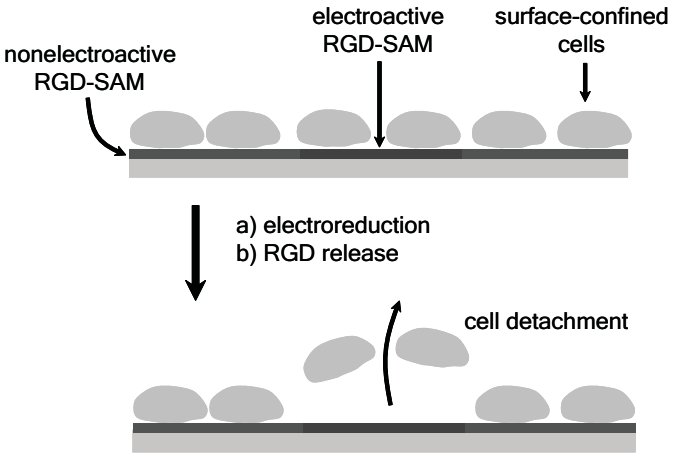

Figure 4.7 Immobilization of RGD peptides to Q-terminated electroactive SAMs through a Diels-Alder reaction.

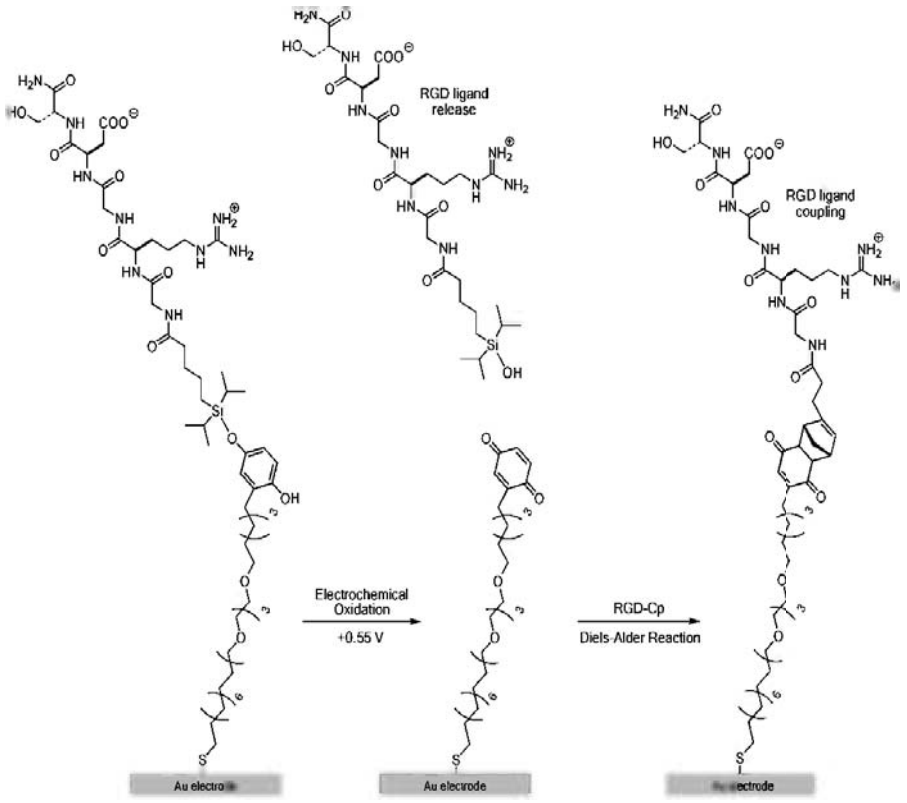

Figure 4.8 Electro-oxidative release of RGD ligand and subsequent immobilization onto the Q-terminated SAM via a Diels-Alder reaction.

On the other hand, as discussed earlier, the electrochemical benzoquinone group may undergo a selective Diels-Alder reaction to covalently anchor diene-tagged ligands, for example, RGD peptide. The accurate control over the formation of reactive chemical species on the electrode surface provides the basis for the manipulation of the electrode dynamic activity. For example, the electrochemical control over the benzoquinone-QH_2 equilibrium may prevent or trigger the immobilization diene-labeled ligands.

Its application to the manipulation of cell adhesion was demonstrated by patterning a gold surface with hexadecanethiol and electroactive RGD-terminated SAMs [57]. Peptide-modified modified domains were diluted (0.02%) into tri(ethylene glycol)-terminated alkanethiolate monolayers. The patterned substrate was incubated in fibronectin, leading to the formation of protein domains on the regions modified with hexadecanethiol (nonspecific adsorption). Incubation of patterned samples into cell culture media containing Swiss 3T3 fibroblast cells followed by microscopy-based analysis revealed that cells were evenly adhered to the substrate regardless of the nature of the underlying film (adsorbed fibronectin or RGD-terminated SAMs). However, the application of an anodic potential promoted the detachment and release of most of the cells confined on the RGD-terminated domains due to the release of the RGD from the *O*-silyl ether-tethered SAM, whilst the cells immobilized on the fibronectin domains remained unaffected (Fig. 4.9).

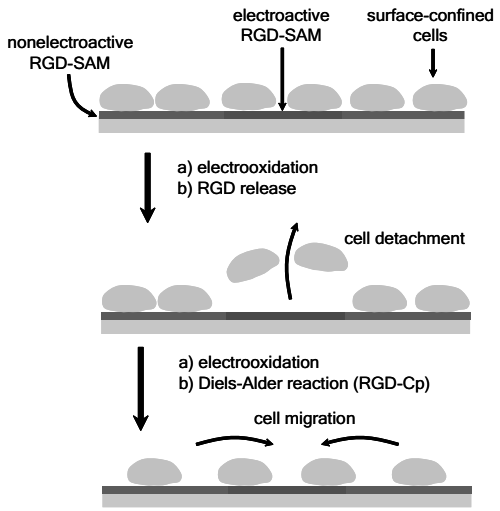

Figure 4.9 Electro-oxidative release of RGD ligand and subsequent immobilization onto the Q-terminated SAM via a Diels-Alder reaction.

4.3.4 Dynamic Control over Cell Adhesion Using Ligands Tethered to Redox-Active SAMs

Electrochemical strategies can be further extended to substrates displaying diverse independent dynamic functions for controlling cell adhesion, which are based on the formation of SAMs displaying two electroactive groups that release the tethered ligands in response to either reductive or oxidative potentials. For example, electroactive Q esters and *O*-silyl QH_2 groups release ligands upon electroreduction (Fig. 4.10) and electro-oxidation (Fig. 4.11), respectively [58].

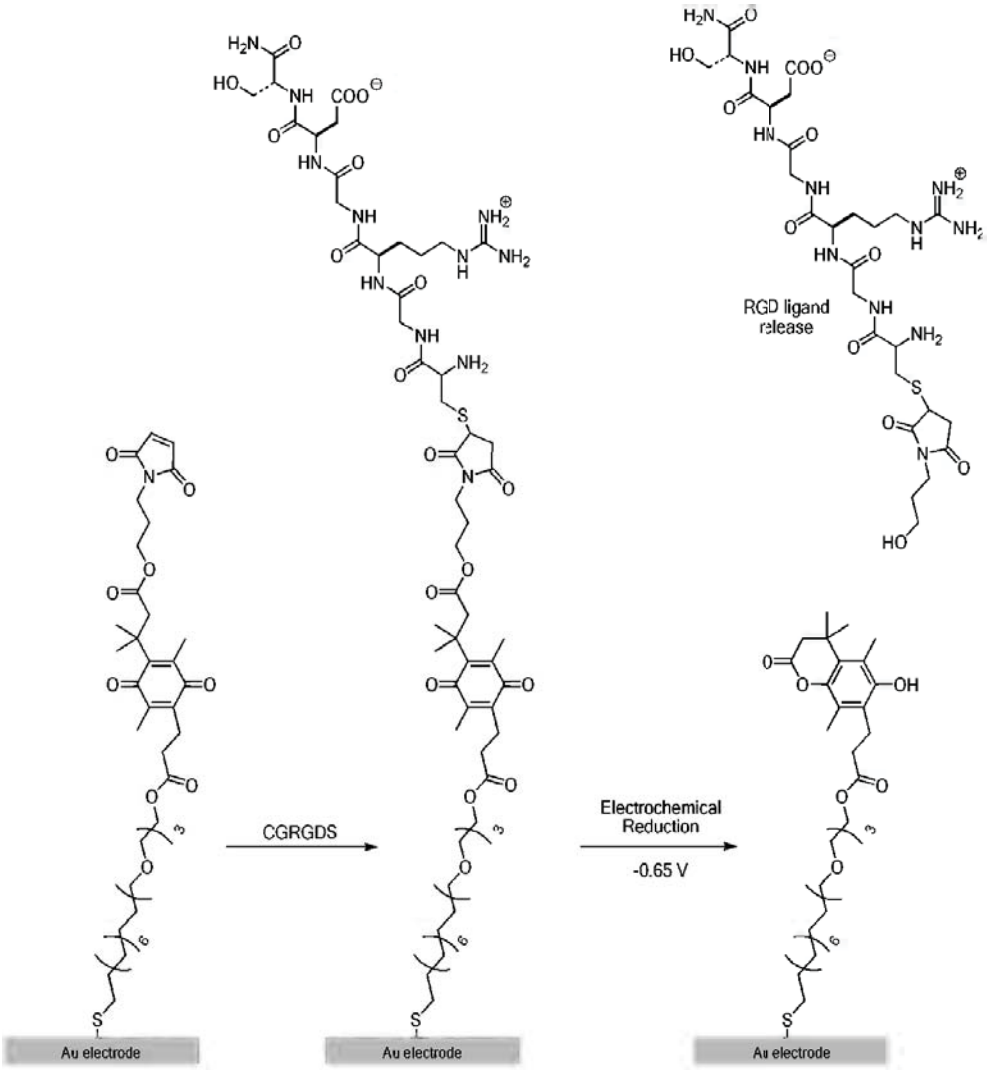

Figure 4.10 Coupling and electroreductive release of RGD peptides from electroactive maleimide-terminated SAMs.

Furthermore, the use of thiols bearing maleimide groups tethered to the electroactive Q esters confers even more versatility to the molecular film, provided this chemical function enables the conjugation of a wide variety of biological building blocks, for example, cysteine-terminated RGD peptide (Figs. 4.10 and 4.11).

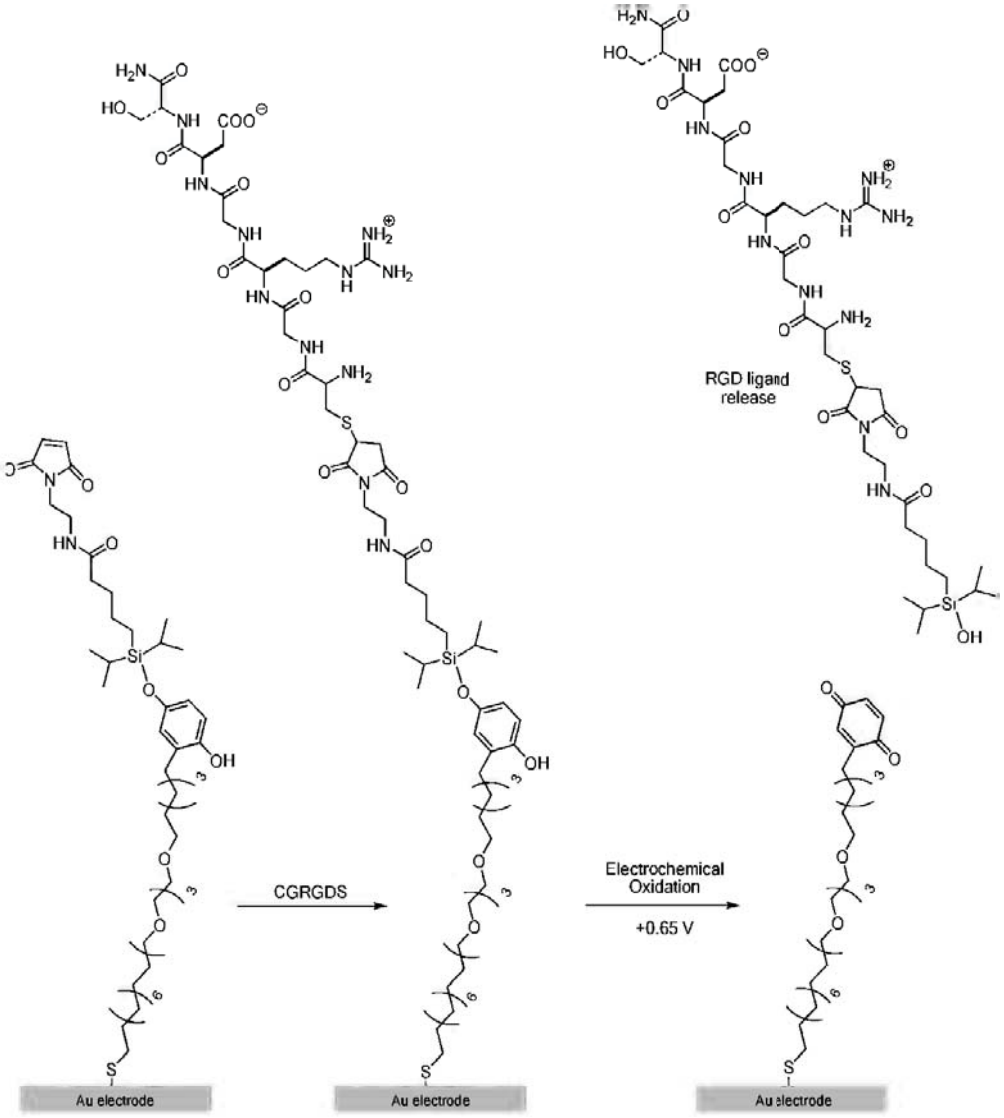

Figure 4.11 Coupling and electro-oxidative release of RGD peptides from electroactive maleimide-terminated SAMs.

The versatility of this approach to tune the electrochemical release of cells was demonstrated by incubating patterned RGD-modified substrates involving different surface chemistries into cell culture media containing Swiss 3T3 fibroblast cells. Substrate

anodic polarization at 0.65 V promoted the selective release of the cells from regions modified with electroactive *O*-silyl QH_2, whilst cells confined on electroactive Q groups were only detached upon applying a cathodic potential (–0.65 V) (Fig. 4.12) [58].

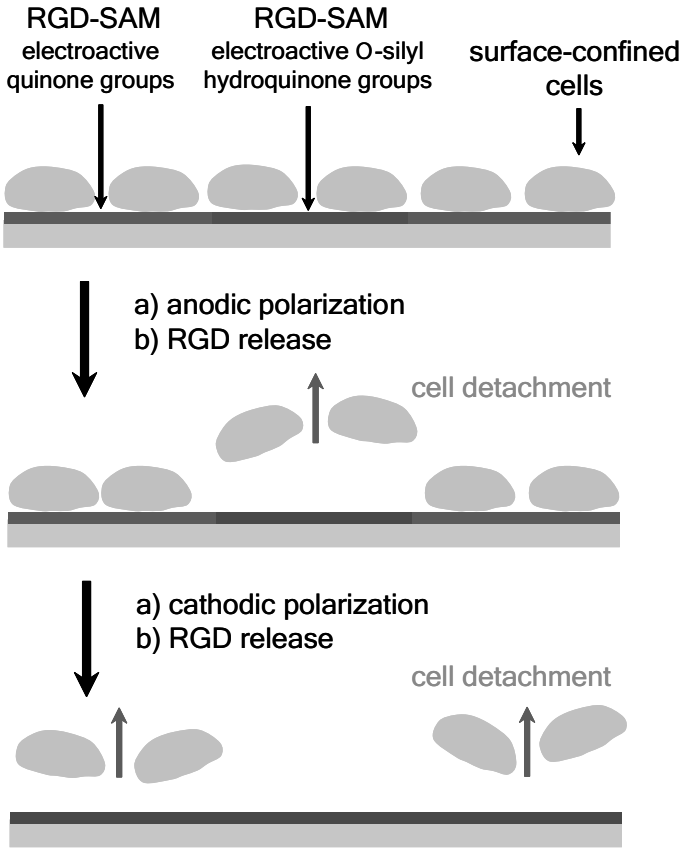

Figure 4.12 Oxidative (first step) and reductive (second step) electrochemical release of RGD ligands, leading to site-selective detachment of surface-confined cells.

4.3.5 Tethering of Ligands to Electroactive SAMs through the Formation of Oxime Linkages

Chen and Yousaf demonstrated a straightforward approach to immobilize ligands onto an electroactive Q monolayer with precise control of ligand density by using the coupling between the ketone-bearing Q monolayer and soluble amino-oxy-terminated ligands as a general route for the preparation of substrates presenting a variety of biological ligands [59]. The use of oxyamines introduces

several attractive advantages, provided they readily react with Q in high yield at physiological pH and room temperature, they form stable oxime linkages, and the derivatization of biological functional units with amino-oxy groups can be easily achieved through simple experimental protocols [59]. In addition, the surface-confined oxime is also redox active but at a different potential and therefore allows for real-time monitoring of the immobilization reaction. Detailed electrochemical studies showed that QH_2-terminated SAMs displayed reversible oxidation and reduction features that were affected by addition of amino-oxy acetic acid. The marked loss of peak currents corresponding to the Q groups and the increase in peak currents corresponding to the redox process of the oxime product confirmed the successful surface immobilization.

The oxime conjugate is chemically stable in aqueous environments; however, its electrochemical reduction at pH 7 results in the regeneration of the QH_2 and subsequent release of the ligand from the monolayer (Fig. 4.13). It is worth indicating that the electroduction of the oxime not only releases the ligand but also regenerates the catalytic QH_2 form for subsequent ligand immobilization [60].

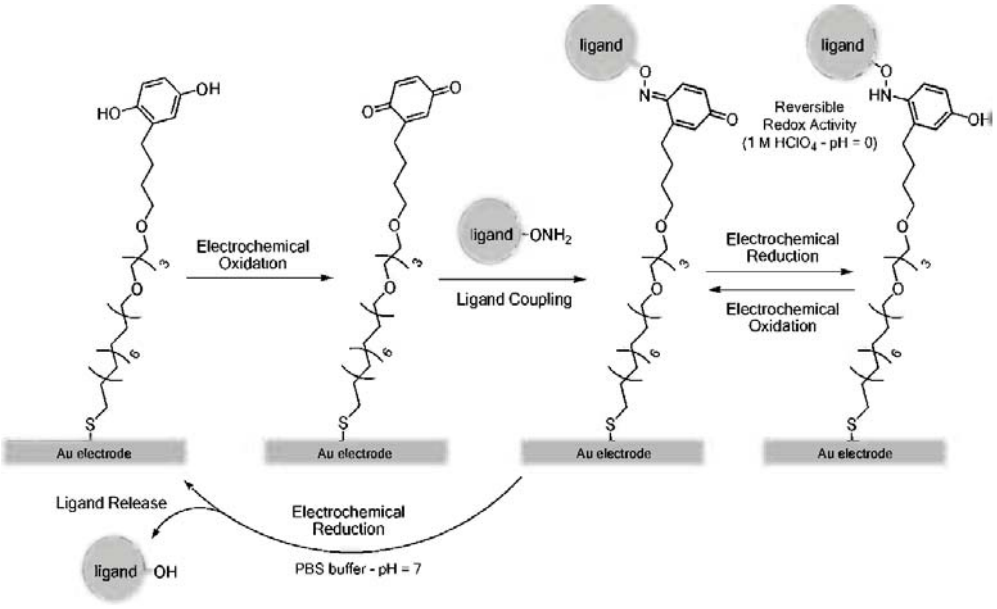

Figure 4.13 Schematic depiction describing the formation of the redox-active oxime conjugate and the electrochemical release of the conjugated ligand.

The formation of oxime linkages to confine bioactive elements on surfaces was extended to biological ligand immobilization through the association of an anti-FLAG antibody to a surface bearing a FLAG peptide. A mixed monolayer containing 1% QH_2 groups and 99% tetra(ethylene glycol) groups was electrochemically oxidized to produce the corresponding Q that was subsequently reacted with an amino-oxy-functionalized FLAG peptide. SPR spectroscopy confirmed the selective binding of anti-FLAG onto the SAM presenting the conjugated FLAG peptides [59]. Control experiments were performed on conjugated peptides displaying a nonbinding sequence, and no protein association to the surface was observed, thus confirming that the anti-FLAG binding to the immobilized FLAG peptide was biospecific.

Wescott and Yousaf combined the use of electroactive SAMs and microfluidic devices as a straightforward and flexible route to pattern a variety of ligands and cells [61]. Spatial control over the surface functionalization was achieved through the implementation of poly(dimethylsiloxane) (PDMS) microchips that confined the flow of electrolyte within the channels in contact with the substrate. The regions on the surface under the channels were electrochemically activated by the oxidation of the QH_2 groups to quinine [61]. Or in other words, the surface was selectively activated only in regions where the electrolyte contacts the surface. After the removal of the elastomeric chip, the substrate was placed into contact with an oxyamine-terminated ligand solution. This process enables the chemo- and site-selective reaction of the Q, resulting in the patterned immobilization of the ligands. The low oxidative potential does not damage the inert properties of the surface and oxidizes only the QH_2 to the reactive Q within the channels for subsequent rapid conjugation of oxyamine derivatives. The dynamic nature of the QH_2-Q redox couple allows for the construction of platforms that can be easily manipulated to generate different surface chemistries within a modular approach [61].

4.3.6 Photochemistry as a Tool to Control the Spatial Distribution of Electroactive Groups

The use of SAMs bearing nitroveratryloxycarbonyl (NVOC)-protected QH_2 introduces the possibility of exploiting light as a

stimulus to trigger the desired functionality. Upon UV irradiation (NVOC)-protected QH_2-modified surfaces are able to expose electroactive QH_2 groups [62]. The photogenerated QH_2 moiety is then electrochemically oxidized to the corresponding Q, which subsequently reacts with the cp-tagged ligand (Fig. 4.14) [62].

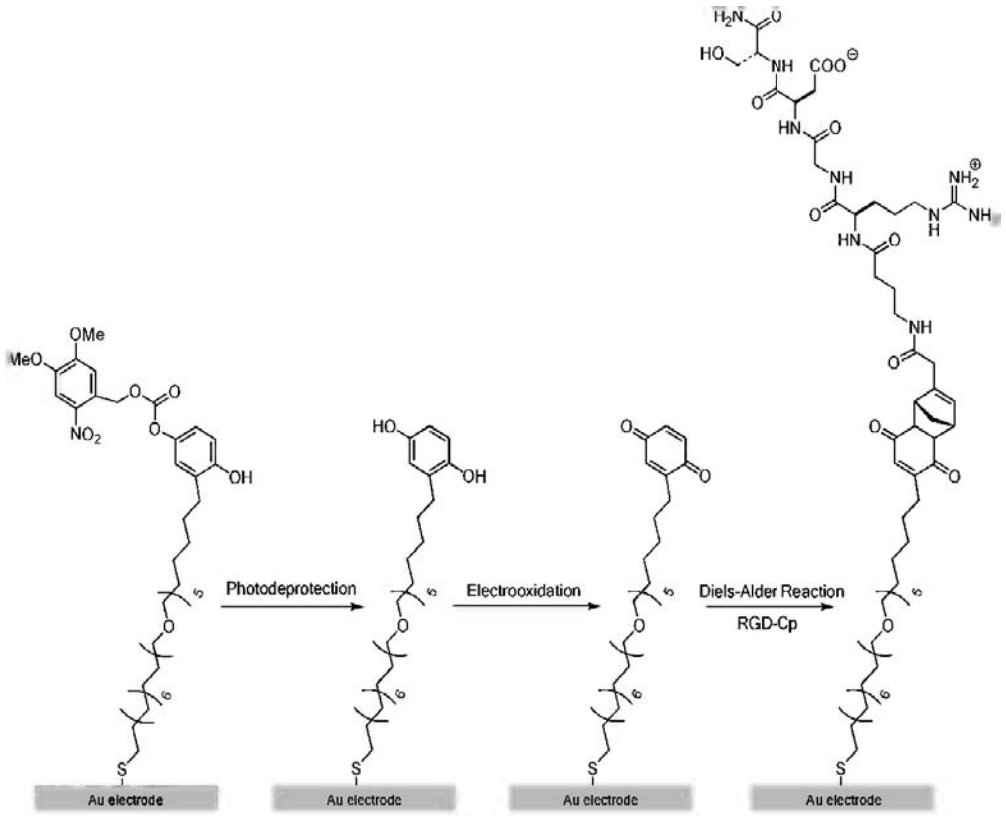

Figure 4.14 Coupling of RGD peptides onto photoactive QH_2-terminated SAMs.

The photogeneration of reactive domains was achieved by simple illumination through a mask (Fig. 4.15) or by using an optical microscope with a programmable translational stage to write patterns of deprotection [62]. One interesting aspect of this approach relies on the use of chemistries like photodeprotection, electro-oxidation, and surface Diels-Alder reactions that proceed with high yield, and can be readily accomplished using simple experimental protocols. The combination of diverse chemical strategies allows for the design of biointerfaces with excellent control over spatial distribution of ligands.

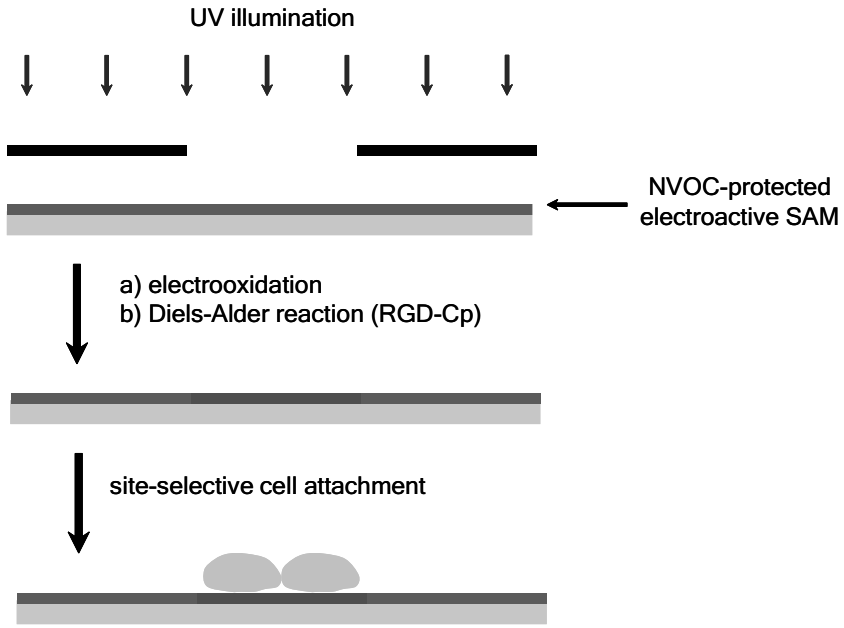

Figure 4.15 Photopatterning and subsequent electrochemical coupling of RGD ligands leading to site-selective immobilization of cells.

In a similar way, Yousaf et al. used NVOC-protected QH_2-modified SAMs to photo-pattern a variety of oxyamines onto Q-terminated domains (Fig. 4.16) [60].

The potential of this approach was demonstrated by attaching and releasing cells from tailored gradient surfaces containing photo- and electroactive functional groups [60]. Hexadecanethiol-patterned SAMs were microcontact-printed on gold surfaces and backfilled with a mixed monolayer bearing both the NVOC-protected QH_2 and tetraethylene glycol groups. UV photopatterning through a mask revealed well-defined domains containing QH_2 groups that were electro-oxidized to Q and subsequently reacted with soluble RGD-oxyamine conjugates to form bioactive domains onto the gold surface. Addition of Swiss 3T3 fibroblasts to the patterned substrate resulted in the immobilization of cells only on the hexadecanethiolate domains (due to nonspecific hydrophobic interactions) and the photoactivated regions (due to the conjugation of the RGD peptides). The application of a reductive potential promoted the release of the cells from the domains presenting the RGD peptides, whereas cells confined on the hexadecanethiolate domains remained unperturbed.

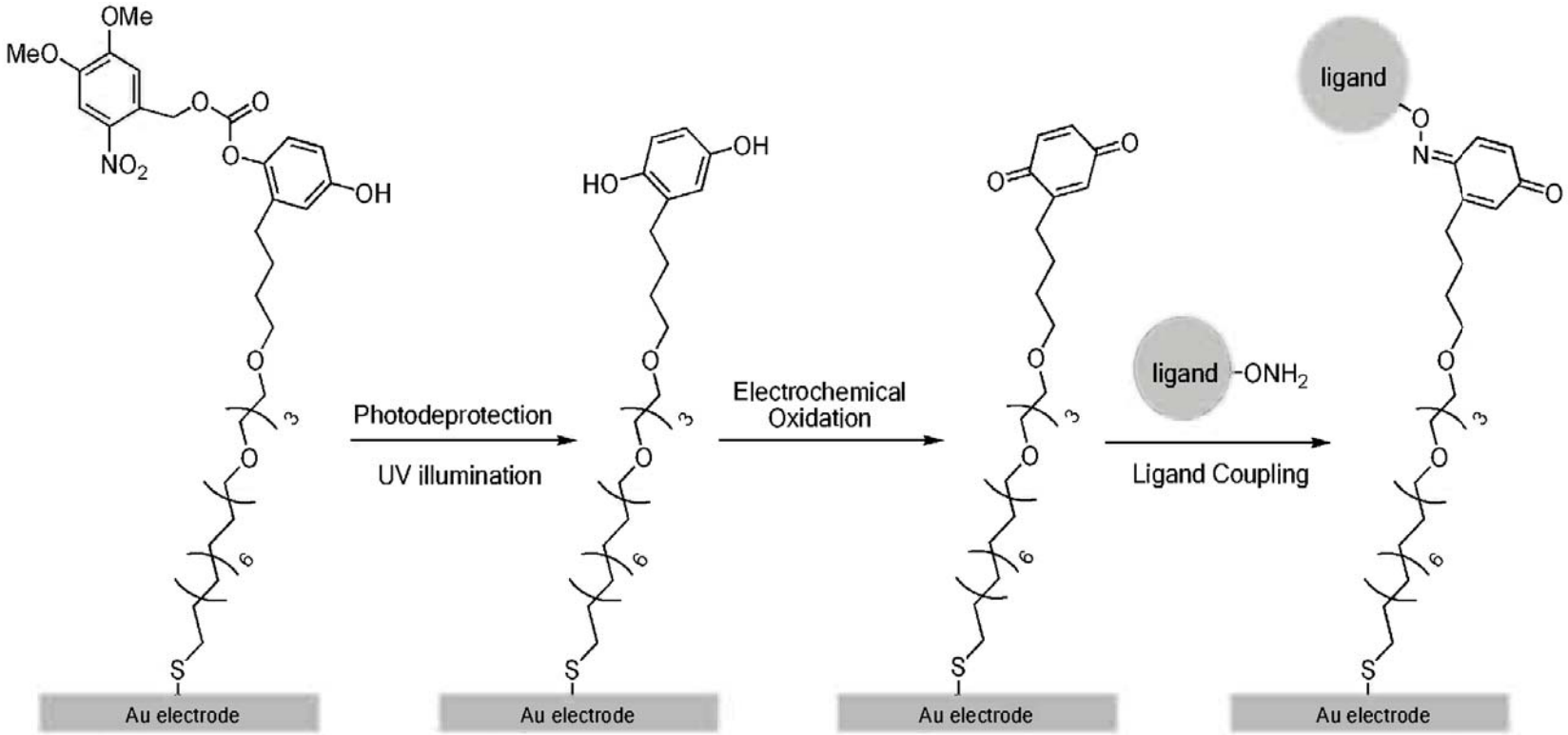

Figure 4.16 Photoactivation and subsequent formation of oxime conjugates taking place after electro-oxidation of QH_2-terminated SAMs in the presence of oxyamine-derivatized ligands.

4.3.7 Electrochemical Deprotection of "Caged" Ligands Immobilized on Self-Assembled Monolayers

Kwak et al. reported a very elegant method for activating ligands on surfaces based on an electrochemically active biotin derivative that generates biotinylated surfaces after mild electro-oxidation of the SAM [63]. The electrochemical deprotection of the "caged" biotin takes place after electro-oxidizing the QH_2 moiety that is converted to a benzoquinonium cation and subsequently released after nucleophilic acyl substitution in the presence of water (Fig. 4.17) [63]. Detailed SPR studies corroborated that this electrochemical strategy permits the modulation of the biological activity of modified gold surfaces in the presence of streptavidin (biotin receptor).

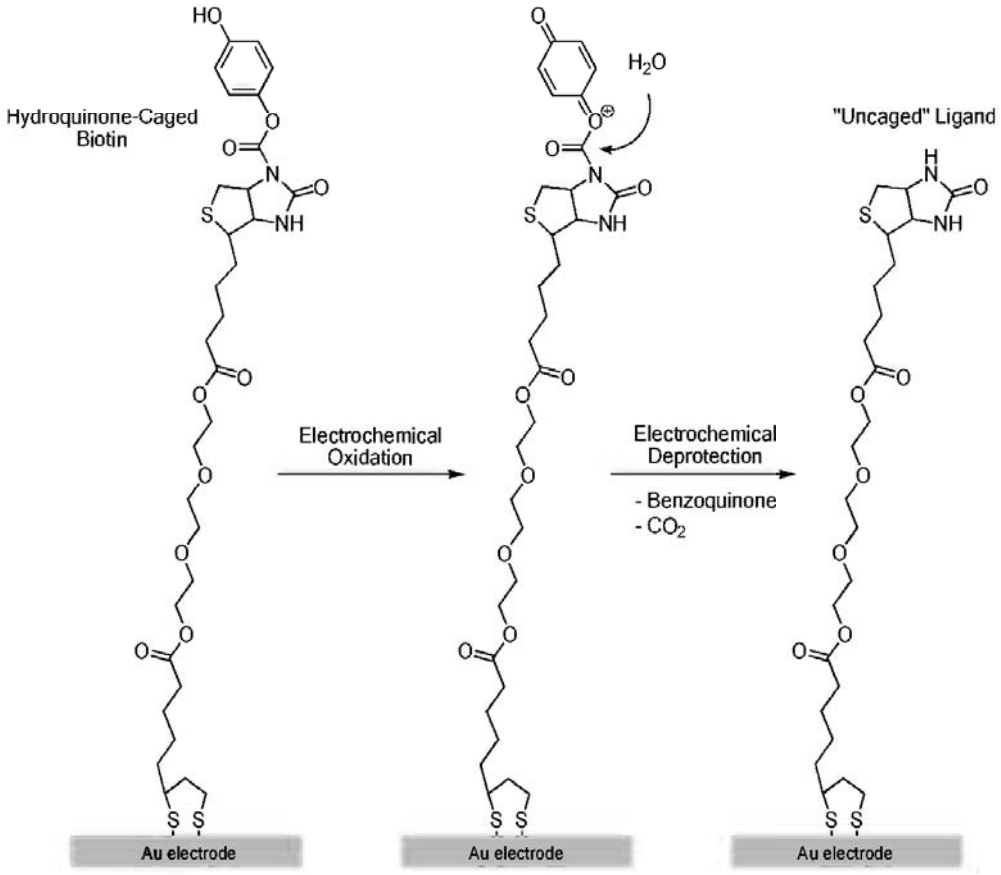

Figure 4.17 Electroinduced exposure of biotin ligands upon controlled electrochemical oxidation of SAMs containing QH_2-caged biotin.

4.3.8 Electrochemical Cleavage of Azo Linkages for Site-Selective Immobilization of Biofunctional Units

Chung et al. proposed an interesting electrochemical strategy focused on the use of azobenzene-derivatives as redox-active platforms for the site-selective immobilization of ligands and biomolecules, which undergoes a four-electron reduction to yield the corresponding anilines at a low reduction potential. The application of reductive potentials to azobenzene-bearing SAMs produces hydrazobenzene species that, upon further electroreduction, leads to complete cleavage of the hydrazo bond at low pH. The result of the whole electroreduction process is the formation of amine-terminated SAMs from an azobenzene-modified SAM (Fig. 4.18) [64].

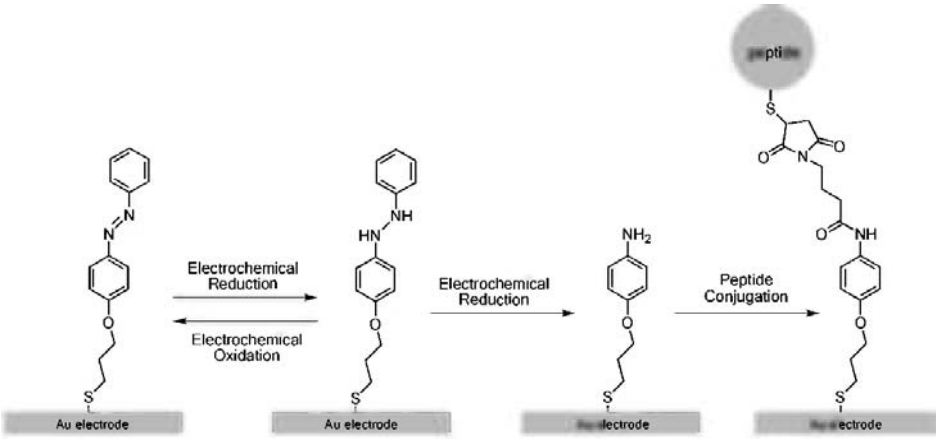

Figure 4.18 Electroformation of amine-terminated SAMs followed by peptide conjugation.

To demonstrate the potential of this approach to manipulate surface bioaffinity, these authors cultured primary hippocampal neurons on triethylene glycol (TEG)-tethered 3-(4-(phenyldiazonyl) phenoxy)propane-1-thiol SAMs, which prevents the nonspecific adhesion of cells. Then, the Cys-Gly-Gly (CGG)-linked Ile-Lys-Val-Ala-Val (IKVAV) peptide (a hippocampal neuron adhesive peptide that originates from laminin) was conjugated to electrochemically activated domains on the TEG-terminated surface using a heterobifunctional N-hydroxysuccinimide (NHS)-maleimide linker [64]. Microscopy imaging of hippocampal neuronal cells two days after seeding on the activated TEG-tethered azobenzene surface showed that neurons with extensive neurites were only grown on

the IKVAV-conjugated, electrochemically activated domains of the SAM-modified surface [64].

4.4 Controlling the Release of Bioactive Elements by Electrochemical Desorption of Self-Assembled Monolayers

4.4.1 Reductive Electrodesorption of Self-Assembled Monolayers as a Strategy to Release Cells and Proteins from Surfaces

While organic electrosynthesis on SAMs provides an elegant approach that allows sophisticated manipulation of the chemical groups at the biointerface, the synthesis of electroactive alkanethiols is not very straightforward. Along these lines, electrodesorption of alkanethiolate monolayers, through the application of cathodic potentials that ultimately leads to the reduction of the gold-thiolate bond, offers a simple avenue for controlling biointerfacial properties of gold electrodes.

Whitesides et al. exploited the electrodesorption of alkanethiolate SAMs to release patterned mammalian cells from the constraints of these patterns [65]. Microcontact-printed domains presenting octadecanethiolate and oligo(ethyleneglycol)-terminated SAMs were used to confine cells into well-defined regions of the modified surface. These authors demonstrated that electrochemical desorption of oligo(ethyleneglycol)-terminated SAMs promotes the release of patterned bovine capillary endothelial (BCE) cells from their confinements. Oligo(ethyleneglycol)-terminated SAMs resist the adsorption of proteins, and since mammalian cells attach to and spread on surfaces only if suitable extracellular matrix (ECM) proteins are present, these SAMs also resist the attachment and spreading of cells. Cells were confined on these micropatterns in normal growth media for 24 hours. After application of a cathodic voltage pulse (< -1.2 V), cells began to spread perceptibly from the microdomains (Fig. 4.19). The electrodesorption of oligo(ethyleneglycol)-terminated SAMs in the presence of ECM proteins such as fibronectin (secreted by cells) rapidly adsorbed onto regions that had been previously rendered inert by these SAMs, and consequently, cells migrated

across the entire surface upon electrodesorption [65]. This strategy has been also extended to the release of different antibody- and RGD-bound cells [66, 67].

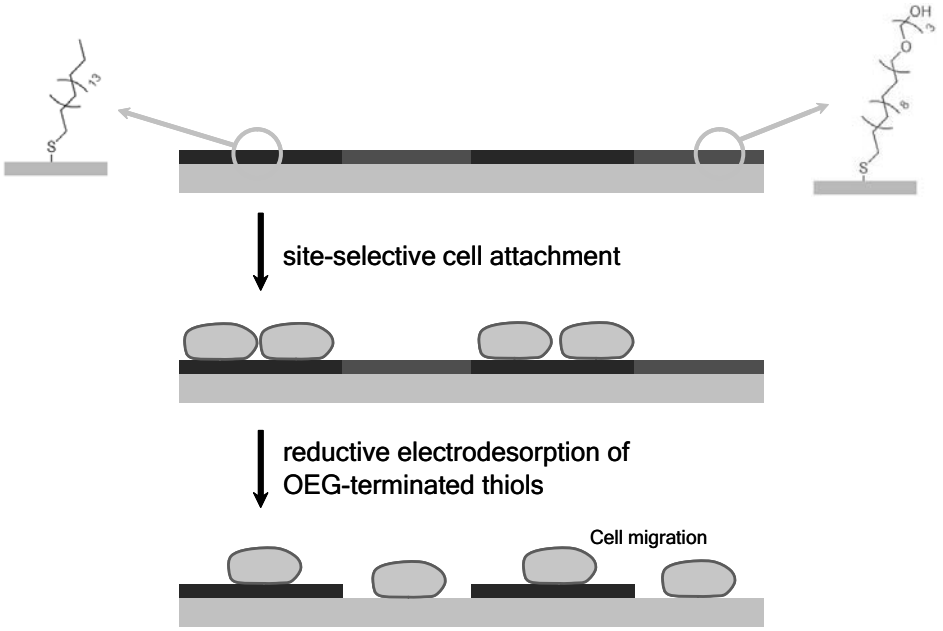

Figure 4.19 A simplified cartoon describing the migration of cells on surfaces upon reductive electrodesorption of oligo(ethyleneglycol)-terminated SAMs.

In a similar vein, Jian et al. reported a method for patterning multiple types of adherent cells on the same substrate by electrochemical desorption of SAMs in localized areas defined by a microfluidic system [68]. This method also employs an oligo(ethyleneglycol)-terminated thiol chemisorbed on a gold surface, which resists adsorption of proteins and adhesion of cells. A micropatterned PDMS stamp with predefined microfluidic channels is used to carry out selective electrochemical desorption of the thiol from the gold substrate. This strategy enables the site-selective "activation" of the inert surface, promoting the adsorption of proteins, for example, fibronectin, and the subsequent adhesion of cells. Because electrodesorption only takes place in areas exposed to microfluidic channels, patterned cells are selectively confined to activated regions, which are defined by these channels upon removal of the stamp that carries the fluidic system [68].

Simonian et al. explored the use SAM electroreduction as a methodology to release surface-bound proteins [69]. Model

proteins like bovine serum albumin (BSA) and immunoglobulin G (IgG) were conjugated via carbodiimide (EDC) chemistry to a mercaptoundecanoic acid (MUA) SAM. Deposition of alkanethiols and proteins was monitored by ellipsometry and SPR techniques and was further confirmed by cyclic voltammetry using $[Fe(CN)_6]^{3-}$ as a redox probe. The surface-bound proteins were completely removed by applying a reductive potential of −1.2 V (vs. Pt electrode) in phosphate buffered saline (PBS) buffer. Importantly, these authors demonstrated that the protocol involving immobilization and release of the proteins could be repeated multiple times, thus evidencing the robustness and reproducibility of the approach [69].

4.5 Conclusions

Biosurface organic chemistry is an emerging research field at the boundaries of surface science, organic chemistry, and biophysics, in which SAMs are essential building blocks to design with molecular detail a wide variety surfaces [70, 71]. However, this scenario becomes more fascinating when electrified interfaces come into play. Electrochemistry introduces a complementary perspective from which to consider the manipulation biochemical features of tailor-made interfaces. Electrochemically induced interfacial reactions are becoming an increasingly important subject for studies in biofunctional surfaces [72–84], provided the electrode potential represents an alternative variable to manipulate in a predictable manner the chemical characteristics of terminal organic groups in SAMs as well as the surface stability of the whole self-assembled film. In this chapter we reviewed some recent findings in the field of biosurface organic chemistry by focusing on electrochemically induced interfacial chemical reactions on SAMs. These examples illustrate the combination of electrochemistry and SAMs as a versatile platform for the manipulation of biological phenomena on surfaces that may include electrochemically stimulated protein release or potential controlled cell migration, just to name some examples. As electrochemistry is becoming more available to a wide range of research groups in biology, organic chemistry, and surface science, we fully expect SAMs on electrode supports to become a versatile toolbox to design and manipulate biofunctional interfaces.

Acknowledgments

The authors gratefully acknowledge financial support from Universidad Nacional de la Plata (UNLP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) (PIP 2009-0362), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) (projects: PRH 2007 No. 74 – PIDRI No. 74 – PICT-PRH 163/08, PICT Bicentenario 2010-2554), the Guggenheim Foundation, Alexander von Humboldt Stiftung, Max-Planck-Gesellschaft (Max Planck Partner Group for Functional Supramolecular Bioconjugates – INIFTA/MPIP), and Centro Interdisciplinario de Nanociencia y Nanotecnología (CINN – ANPCyT – Argentina). O.A. and R.C.S. are CONICET fellows.

References

1. Mrksich, M., Whitesides, G.M. (1996). Using self-assembled monolayers to understand the interactions of man-made surfaces with proteins and cells, *Annu. Rev. Biophys. Biomol. Struc.*, **25**, pp. 55–78.
2. Chen, C.S., Mrksich, M., Huang, S., Whitesides, G.M., Ingber, D.E. (1998). Micropatterned surfaces for control of cell shape, position, and function, *Biotechnol. Prog.*, **14**, pp. 356–363.
3. Mrksich, M. (2009). Using self-assembled monolayers to model the extracellular matrix, *Acta Biomater*., **5**, pp. 832–841.
4. Jonkheijm, P., Weinrich, D., Schröder, H., Niemeyer, C.M., Waldmann, H. (2008). Chemical strategies for generating protein biochips, *Angew. Chem., Int. Ed.*, **47**, pp. 9618–9647.
5. Chi, Y.S., Lee, J.K., Lee, K.-B., Kim, D.J., Choi, I.S. (2005). Biosurface organic chemistry: Interfacial chemical reactions for applications to nanobiotechnology and biomedical sciences, *Bull. Korean Chem. Soc.*, **26**, pp. 361–370.
6. Choi, I.S., Chi, Y.S. (2006). Surface reactions on demand: Electrochemical control of SAM-based reactions, *Angew. Chem., Int. Ed.*, **45**, pp. 4894–4897.
7. Henning, L. and Hammerich, O. (2001). *Organic Electrochemistry*, 4th ed. (Marcel Dekker, USA).
8. Sullivan, T.P., Huck W.T.S. (2003). Reactions on monolayers: Organic synthesis in two dimensions, *Eur. J. Org. Chem.*, pp. 17–29.
9. Chechik, V., Crooks, R.M., Stirling, C.J.M. (2000). Reactions and reactivity in self-assembled monolayers, *Adv. Mater.*, **12**, pp. 1161–1171.

10. Qin, D., Xia, Y.N., Whitesides, G.M. (2010). Soft lithography for micro- and nanoscale patterning, *Nat. Protocols*, **5**, pp. 491–502.
11. Love, J.C., Estroff, L.A., Kriebel, J.K., Nuzzo, R.G., Whitesides, G.M. (2005). Self-assembled monolayers of thiolates on metals as a form of nanotechnology, *Chem. Rev.*, **105**, pp. 1103–1169.
12. Zhao, Y., Perez-Segarra, W., Shi, Q., Wei, A. (2005). Dithiocarbamate assembly on gold, *J. Am. Chem. Soc.*, **127**, pp. 7328–7329.
13 Ciszek, J.W., Tour, J.M. (2005). Mechanistic implications of the assembly of organic thiocyanates on precious metals, *Chem. Mater.*, **17**, pp. 5684–5690.
14. Schilardi, P.L., Dip, P., Dos Santos Claro, P.C., Benítez, G.A., Fonticelli, M.H., Azzaroni, O., Salvarezza, R.C. (2006), Electrochemical deposition on self-assembled monolayers: New insights into micro- and nanofabrication, *Chem. Eur. J.*, **12**, pp. 38–49.
15. Yang, D.-F., Al-Maznai, H., Morin, M. (1997). Vibrational study of the fast reductive and the slow oxidative desorptions of a nonanethiol self-assembled monolayer from a Au(111) single crystal electrode, *J. Phys. Chem. B*, **101**, pp. 1158–1166.
16. Vericat, C., Andreasen, G., Vela, M.E., Martin, H., Salvarezza, R.C. (2001). Following transformation in self-assembled alkanethiol monolayers on Au(111) by in situ scanning tunneling microscopy, *J. Chem. Phys.*, **115**, p. 6672.
17. Boubour E, Lennox RB (2000). Potential-induced defects in *n*-alkanethiol self-assembled monolayers monitored by impedance spectroscopy, *J. Phys. Chem. B*, **104**, pp. 9004–9010.
18. Azzaroni, O. Vela, M.E., Fonticelli, M., Benıtez, G., Carro, P., Blum, B., Salvarezza, R.C. (2003). Electrodesorption potentials of self-assembled alkanethiolate monolayers on copper electrodes. An Experimental and theoretical study, *J. Phys. Chem. B*, **107**, pp. 13446–13454.
19. Kautz, N.A., Kandel, S.A. (2008). Alkanethiol/Au(111) Self-assembled monolayers contain gold adatoms: Scanning tunneling microscopy before and after reaction with atomic hydrogen, *J. Am. Chem. Soc.*, **130**, pp. 6908–6909.
20. Widrig, C.A., Chung, C., Porter, M.D. (1991). The electrochemical desorption of *n*-alkanethiol monolayers from polycrystalline Au and Ag electrodes, *J. Electroanal. Chem.*, **310**, pp. 335–359.
21. Walczak, M.M., Chung, C., Stole, S.M., Widrig, C.A., Porter, M.D. (1991). Structure and interfacial properties of spontaneously adsorbed n-alkanethiolate monolayers on evaporated silver surfaces, *J. Am. Chem. Soc.*, 113, pp. 2370–2378.

22. Munakata, H., Oyamatsu, D., Kuwabata, S. (2004). Effects of ω-functional groups on pH-dependent reductive desorption of alkanethiol self-assembled monolayers, *Langmuir*, **20**, pp. 10123–10128.

23. Hatchett, D.W., Uibel, R.H., Stevenson, K.J., Harris, J.M., White, H.S. (1998). Electrochemical measurement of the free energy of adsorption of n-alkanethiolates at Ag (111), *J. Am. Chem. Soc.*, **120**, pp. 1062–1069.

24. Imabayashi, S., Iida, M., Hobara, D., Feng, Z.Q., Niki, K., Kakiuchi, T. (1997). Reductive desorption of carboxylic-acid-terminated alkanethiol monolayers from Au(111) surfaces, *J. Electroanal. Chem.*, **428**, pp. 33–38.

25. Vela, M.E., Martin, H., Vericat, C., Andreasen, G., Hernández-Creus, A., Salvarezza, R.S. (2000). Electrodesorption kinetics and molecular interactions in well-ordered thiol adlayers, *J. Phys. Chem B*, **104**, pp. 11878–11882.

26. Bengio, S., Fonticelli, M., Benítez, G., Hernández-Creus, A., Carro, P., Ascolani, H., Zampieri, G., Blum, B., Salvarezza, R.C. (2005). Electrochemical self-assembly of alkanethiolate molecules on Ni(111) and polycrystalline Ni surfaces, *J. Phys. Chem. B*, **109**, pp. 23450–23460.

27. Corthey, G., Rubert, A.A., Benitez, G.A., Fonticelli, M.H., Salvarezza, R.C. (2009). Electrochemical and X-ray spectroscopy characterization of alkanethiols adsorbed on palladium surfaces, *J. Phys. Chem. C*, **113**, pp. 6735–6742

28. Azzaroni, O. Vela, M.E., Fonticelli, M., Benıtez, G., Carro, P., Blum, B., Salvarezza, R.C. (2003). electrodesorption potentials of self-assembled monolayers on copper electrodes. An experimental and theoretical study, *J. Phys. Chem. B*, **107**, pp. 13446–13454.

29. Fonticelli, M., Azzaroni, O., Benitez, G., Martins, M.E., Carro, P., Salvarezza, R.C. (2004). Molecular self-assembly on ultrathin metallic surfaces: Alkanethiolate monolayers on Ag(1×1)-Au(111), *J. Phys. Chem. B*, **108**, pp. 1898–1905.

30. Musgrove, A., Kell, A., Bizzotto, D. (2008). Fluorescence imaging of the oxidative desorption of a BODIPY-alkyl-thiol monolayer coated Au bead, *Langmuir*, **24**, pp. 7881–7888.

31. Miller, C., Cuendet, P., Gratzel, M. (1991). Adsorbed omega-hydroxy thiol monolayers on gold electrodes: Evidence for electron tunneling to redox species, *J. Phys. Chem.*, **95**, pp. 877–886.

32. Becka, A., Miller, C.J. (1992). Electrochemistry at omega-hydroxy thiol coated electrodes. 3. Voltage independence of the electron tunneling

barrier and measurements of redox kinetics at large overpotentials, *J. Phys. Chem.*, **96**, pp. 2657–2668.

33. Chidsey, C.E.D (1991). Free energy and temperature dependence of electron transfer at the metal-electrolyte interface, *Science*, **251**, pp. 919–922.
34. Finklea, H.O., Hanshew, D.D. (1991). Electron transfer kinetics in organized thiol monolayers with attached pentaammine (pyridine) ruthenium redox centers, *J. Am. Chem. Soc.*, **114**, pp. 3173–3181
35. Smalley, J.F., Finklea, H.O., Chidsey, C.E.D., Linford, M.R., Creager, S.E., Ferraris, J.P., Chalfant, K., Zawodzinsk, T., Feldberg, S.W., Newton, M.D. (2003). Heterogeneous electron-transfer kinetics for ruthenium and ferrocene redox moieties through alkanethiol monolayers on gold, *J. Am. Chem. Soc.*, **125**, pp. 2004–2013.
36. Finklea, H.O. (2001). Theory of coupled electron-proton transfer with potential-dependent transfer coefficients for redox couples attahced to electrodes, *J. Phys. Chem. B*, **105**, pp. 8685–8693.
37. Newton, M.D., Smalley, J.F. (2007). Interfacial bridge-mediated electron transfer: Mechanistic analysis based on electrochemical kinetics and theoretical modeling, *Phys. Chem. Chem. Phys.*, **9**, pp. 555–572.
38. Liu, B., Bard, A.J., Mirkin, M.V., Creager, S.E. (2004). Electron transfer at self-assembled monolayers measured by scanning electrochemical microscopy, *J. Am. Chem. Soc.*, **126**, 1485–1492.
39. Chidsey, C.E.D., Bertozzi, C.R., Putvinski, T.M., Mujsce, A.M. (1990). Coadsorption of ferrocene-terminated and unsubstituted alkanethiols on gold: electroactive self-assembled monolayers, *J. Am. Chem. Soc.*, **112**, pp. 4301–4306.
40. Salomon, A., Cahen, D., Lindsay, S., Tomfohr, J., Engelkes, V.B., Frisbie, C.D. (2003). Comparison of electronic transport measurements on organic molecules, *Adv. Mater.*, **15**, pp. 1881–1890
41. Sumner, J.J., Weber, K.S., Hockett, L.A., Creager, S.E. (2000). Long-range heterogeneous electron transfer between ferrocene and gold mediated by n-alkane and N-alkyl carboxamide bridges, *J. Phys. Chem. B*, **104**, pp. 7449–7454.
42. Slowinski, K., Chamberlain, R.V., Miller, C.J., Majda, M. (1997). Through-bond and chain-to-chain coupling. Two pathways in electron tunneling through liquid alkanethiol monolayers on mercury electrodes, *J. Am. Chem. Soc.*, **119**, pp. 11910–11919.
43. Quek, S.Y., Choi, H.J., Louie, S.G., Neaton, J.B. (2009). Length dependence of conductance in aromatic single-molecule junctions, *Nano Lett.*, **9**, pp. 3949–3953.

44. Scharf, J., Strehblow, H.H., Zeysing, B., Terfort A. (2001). Electrochemical and surface analytical studies of self-assembled monolayers of three aromatic thiols on gold electrodes, *J. Solid State Electrochem.*, **5**, 396–401

45. Vericat, C., Vela, M.E., Benítez, G.A., Carro, P., Salvarezza, R.C. (2010). Self-assembled monolayers of thiols and dithiols on gold: New challenges for a well-known system, *Chem. Soc. Rev.*, **39**, pp. 1805–1834.

46. Zhang, W., Rosendahl, S.M., Burgess, I.J. (2010). Coupled electron/transfer studies of benzoquinone-modified monolayers, *J. Phys. Chem. C*, **114,** pp. 2738–2745.

47. Hong, H., Park, W., Kim, S., Kim, H.J. (2010). Surface diluent effect on the heterogeneous electron-transfer kinetics of PQQ self-assembled monolayers, *Bull. Korean Chem. Soc.*, **31**, pp. 1711–1714.

48. Nagata, M., Kondo, M., Suemori, Y., Ochiai, T., Dewa, T., Ohtsuka, T., Nango, M. (2008). Electron transfer of quinine self-assembled monolayers on a gold electrode, *Colloids Surf. B*, **64**, pp. 16–21.

49. Trammell, S.A., Moore, M., Lowy, D., Lebedev, N. (2008). Surface reactivity of the quinone/hydroquinone redox center tethered to gold: Comparison of delocalized and saturated bridges, *J. Am. Chem. Soc.*, **130**, pp. 5579–5585.

50. Trammell, S.A., Lebedev, V. (2009). Proton-coupled electron transfer in self-assembled monolayers containing quinone compounds with different bridging groups of varying electronic conjugation, *J. Electroanal. Chem.*, **632**, pp. 127–132.

51. Tsoi, S., Griva, I., Trammell, S.A., Blum, A.S., Schnur, J.M., Lebedev, N. (2008). Electrochemically controlled conductance switching in a single molecule: quinone-modified oligo(phenylene vinylene), *ACS Nano*, **2**, pp. 1289–1295.

52. Yousaf, M.N., Mrksich, M. (1999). Diels–Alder reaction for the selective immobilization of protein to electroactive self-assembled monolayers, *J. Am. Chem. Soc.*, **121**, pp. 4286–4287.

53. Yousaf, M.N., Houseman, B.T., Mrksich, M. (2001). Turning on cell migration with electroactive substrates, *Angew. Chem., Int. Ed.*, **40**, pp. 1093–1096.

54. Kim, K., Jang, M., Yang, H., Kim, E., Kim, Y.T., Kwak, J. (2004). Electrochemically induced and controlled one-step covalent coupling reaction on self-assembled monolayers, *Langmuir*, **20**, pp. 3821–3823.

55. Hodneland, C.D., Mrksich, M. (2000). Biomolecular surfaces that release ligands under electrochemical control, *J. Am. Chem. Soc.*, **122**, pp. 4235–4236.

56. Yeo, W.-S., Hodneland, C.D., Mrksich, M. (2001). Electroactive monolayer substrates that selectively release adherent cells, *ChemBioChem*, **7/8**, pp. 590–593.

57. Yeo, W.-S., Yousaf, M.N., Mrksich, M. (2003). Dynamic interfaces between cells and surfaces: Electroactive substrates that sequentially release and attach cells, *J. Am. Chem. Soc.*, **125**, pp. 14994–14995.

58. Yeo, W.-S., Mrksich, M. (2006). Electroactive self-assembled monolayers that permit orthogonal control over the adhesion of cells to patterned substrates, *Langmuir*, **22**, pp. 10816–10820.

59. Chan, E.W.L., Yousaf, M.N. (2006). Immobilization of ligands with precise control of density to electroactive surfaces, *J. Am. Chem. Soc.*, **128**, pp. 15542–15546.

60. Chan, E.W.L., Park, S., Yousaf, M.N. (2008). An electroactive catalytic dynamic substrate that immobilizes and releases patterned ligands, proteins, and cells, *Angew. Chem., Int. Ed.*, **47**, pp. 6267–6271.

61. Westcott, N.P., Yousaf, M.N. (2008). Synergistic microfluidic and electrochemical strategy to activate and pattern surfaces selectively with ligands and cells, *Langmuir*, **24**, pp. 2261–2265.

62. Dillmore, W.S., Yousaf, M.N., Mrksich, M. (2004). A photochemical method for patterning the immobilization of ligands and cells to self-assembled monolayers, *Langmuir*, **20**, pp. 7223–7231.

63. Kim, K., Yang, H., Jon, S., Kim, E., Kwak, J. (2004). Protein patterning based on electrochemical activation of bioinactive surfaces with hydroquinone-caged biotin, *J. Am. Chem. Soc.*, **126**, pp. 15368–15369.

64. Jung, H.J., Min, H, Yu, H, Lee, T.G., Chung, T.G. (2010). Electrochemical cleavage of azo linkage for site-selective immobilization and cell patterning, *Chem. Commun.*, **46**, pp. 3863–3865.

65. Jiang, X., Ferrigno, R., Mrksich, M., Whitesides, G.M. (2003). Electrochemical desorption of self-assembled monolayers noninvasively releases patterned cells from geometrical confinements, *J. Am. Chem. Soc.*, **125**, pp. 2366–2367.

66. Zhu, H., Yan, J., Revzin, A. (2008). Catch and release cell sorting: Electrochemical desorption of T-cells from antibody-modified microelectrodes, *Colloids Surf. B*, **64**, pp. 260–268.

67. Inaba, R., Khademhosseini, A., Suzuki, H., Fukuda, J. (2009). Electrochemical desorption of self-assembled monolayers for engineering cellular tissues, *Biomaterials* **30**, pp. 3573–3579.

68. Li, Y., Yuan, B., Ji, H., Han, D., Chen, S., Tian, F., Jiang, X. (2007). A method for patterning multiple types of cells by using electrochemical desorption of self-assembled monolayers within microfluidic channels, *Angew. Chem., Int. Ed.*, **46**, pp. 1094–1096.

69. Balasubramanian, S., Revzin, A., Simonian, A. (2006). Electrochemical desorption of proteins from gold electrode surface, *Electroanalysis*, **18**, pp. 1885–1892.

70. Mendes, P.M. (2008). Stimuli-responsive surfaces for bio-applications, *Chem. Soc. Rev.*, **37**, pp. 2512–2529.

71. Robertus, J., Browne, W.R., Feringa, B.L. (2010). Dynamic control over cell adhesive properties using molecular-based surface engineering strategies, *Chem. Soc. Rev*., **39**, pp. 354–378.

72. Westcott, N.P., Yousaf, M.N. (2009). Chemoselective ligand patterning of electroactive surfaces using microfluidics, *Electrophoresis*, **30**, pp. 3381–3385.

73. Pulsipher, A., Westcott, N.P., Luo, W., Yousaf, M.N. (2009). Rapid in-situ generation of two patterned chemoselective surface chemistries from a single hydroxy terminated surface using controlled, *J. Am. Chem. Soc.*, **131**, pp. 7626–7632.

74. Pulsipher, A., Westcott, N.P., Luo, W., Yousaf, M.N. (2009). Rapid microfluidic generation of patterned aldehydes from hydroxy-terminated self-assembled monolayers for ligand and cell immobilization on optically transparent indium tin oxide surfaces, *Adv. Mater.*, **21**, pp. 3082–3086.

75. Westcott, N.P., Lamb, B.M., Yousaf, M.N. (2009). Electrochemical and chemical microfluidic gold etching to generate patterned and gradient substrates for cell adhesion and cell migration, *Anal. Chem.*, **81**, pp. 3297–3303.

76. Luo, W., Westcott, N.P., Pulsipher, A., Yousaf, M.N. (2008). Renewable and optically transparent electroactive indium tin oxide surfaces for chemoselective ligand immobilization and biospecific cell adhesion, *Langmuir*, **24**, pp. 13096–13101.

77. Luo, W., Yousaf, M.N. (2009). Tailored electroactive nanorods for biospecific cell adhesion and differentiation, *Chem. Comm*., pp. 1237–1239.

78. Lamb, B.M., Westcott, N.P., Yousaf, M.N. (2008). Live-cell fluorescence microscopy of directed cell migration on partially etched electroactive SAM gold surfaces, *ChemBioChem*, **9**, pp. 2220–2224.

79. Chan, E.W.L., Yousaf, M.N. (2007). Site-selective immobilization of ligands with control of density on electroactive microelectrode arrays, *ChemPhysChem* **8**, pp. 1469–1472.

80. Chan, E.W.L., Yousaf, M.N. (2008). A photo-electroactive surface strategy for immobilizing ligands in patterns and gradients for studies of cell polarization, *Mol. BioSyst.*, **4**, pp. 746–753.

81. Yousaf, M.N., Houseman, B.T., Mrksich, M. (2001). Using electroactive substrates to pattern the attachment of two different cell populations, *Proc. Natl. Acad. Sci. U S A*, **98**, pp. 5992–5996.

82. Dondapati, S.K., Montornes, J.M., Sanchez, P.L., Sanchez, J.L.A., O´Sullivan, C., Katakis, I. (2006). Site-directed immobilization of proteins through electrochemical deprotection on electroactive self-assembled monolayers, *Electroanalysis*, **18**, pp. 1879–1884.

83. Hoover, D.K., Lee, E.-j., Chan, E.W.L., Yousaf, M.N. (2007). Electroactive nanoarrays for biospecific ligand mediated studies of cell adhesion, *ChemBioChem*, **8**, pp. 1920–1923.

84. Mendes, P.M., Christman, K.L., Parthasarathy, P., Schopf, E., Ouyang, J., Yang, Y., Preece, J.A., Maynard, H.D., Chen, Y., Stoddart, J.F. (2007). Electrochemically controllable conjugation of proteins on surfaces, *Bioconjugate Chem.*, **18**, pp. 1919–1923.