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Review

Electrochemical patterning as a tool for fabricating biomolecule microarrays

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ABSTRACT

High-density biomolecule arrays are powerful tools for the screening of pharmaceuticals, investigation of biomolecule interactions and patient diagnostics. Surfaces modified with electrochemically addressable films combined with electrochemical surface patterning techniques allow local triggering of DNA and protein immobilization. After a brief overview of classical patterning methods, such as printing, dip-pen nanolithography (DPN) and photolithography, we critically assess electrochemical strategies for local surface modification, such as the use of electrode arrays, electro-DPN and scanning electrochemical microscopy regarding their potential for fabrication and read-out of bioarrays. Capillary-based scanning probe methods are especially promising tools for truly chemoselective microarray and nanoarray generation due to their high patterning resolution and the possibility for directly probing the surface chemistry.

Contents

1. Introduction

Microarrays of biomolecules patterned onto a solid support are powerful tools for high-throughput investigation of biomolecules interactions [1–4]. DNA and protein arrays were implemented for function determination, diagnostics and drug screening. In recent years, much effort was spent to reduce the dimensions of the biomolecule patterns generated in order to increase the density of information on a given surface area. However, it is often neglected that function and activity of a biomolecule may change drastically when confined to a surface as compared with its behavior in solution [5]. Thus, for patterning surfaces with sensitive, complex biomolecules to generate high-density microarrays, well-defined chemistry with no side reactions is required. The binding chemistry should be controlled to ensure not only anisotropic orientation of the biomolecule to maintain access to its binding site, but also to

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avoid side-reactions inducing denaturation during array fabrication [6,7].

The design of suitable surface functionalities and patterning techniques aiming to achieve defined, specific binding of biomolecules to the surface will play an increasingly important role. In many cases, binding events are detected with the help of secondary reporter molecules, which do not provide information about the nature of binding. Hence, unspecific adsorption and loss of biocatalytic activity may result in erroneous interpretation of the data obtained. To overcome this uncertainty, additional analytical tools are needed to probe the surface chemistry by giving direct evidence for critical bonds or interactions formed upon immobilization. With decreasing patterning dimensions, it becomes increasingly challenging to maintain and to demonstrate the chemoselectivity of the immobilization procedure. Moreover, many characterization techniques fail to provide information about the identity of the nature of the surface modification itself.

In the light of these considerations, this review is dedicated to highlight concepts that push forward the limits of array generation with high spatial resolution and to point out novel analytical methods for localized characterization of patterned surfaces. Special attention is given to a critical assessment of the surface chemistry and chemoselectivity of immobilization procedures. The huge potential of electrochemical techniques has been demonstrated recently due to their ability for both surface patterning at the microscale and the nanoscale and high-resolution visualization of the patterned surface chemistry. Whereas classical strategies for fabrication and analysis of bioarrays were previously reviewed [1,2,6,8], this work focuses on electrochemical methods for localized surface patterning and read-out of the structures obtained [9].

2. Switchable reactivity allows biomolecule immobilization

Generally, the surface chemistry has to retain the activity, the function and the accessibility of the biomolecule and simultaneously prevent non-specific adsorption. Whereas any type of array that fails in the first task will result in false negative results, failure in the second leads to false positive results. To prevent unspecific adsorption and loss of activity, the solid-liquid interface has to be well balanced between hydrophilicity and hydrophobicity [5]. We need to take into account that charged surfaces may unspecifically attract or repel biomolecules, especially polyanionic DNA strands [10].

The most commonly used strategy to suppress protein adsorption is coating the surfaces with oligoethyleneglycol (OEG) groups, proteins – typically bovine serum albumin (BSA) – or polysaccharide matrices. Generally, protein attachment to the surface at specific sites of the protein rather than random attachment is more likely to result in retention of protein activity. Various covalent, non-covalent, site-specific and non-specific immobilization strategies for proteins have been reviewed elsewhere [7].

Electrochemical approaches have the inherent possibility for reagentless activation of the surface, after which the molecule to be immobilized from bulk solution may be captured at the solid-liquid interface. Alternatively, biomolecules may be entrapped in a polymer matrix that is deposited by electrochemical conversion of the monomers or by electrochemically generated local changes of the pH value.

For DNA immobilization, oligonucleotide-modified monomers may be integrated into the polymer backbone (see Fig. 1a) [11], but proteins have to be physically incorporated [12] or adsorbed [13], or a sequence of bioconjugation steps is necessary to couple proteins to a deposited polymer [14]. There are no restrictions regarding the nature of immobilized molecules; there is especially no need for specific chemical pre-modification. Nevertheless, due to the entrapment in the polymer film, the accessibility for possible binding partners may be altered. However, a monolayer of the biological recognition element adsorbed, covalently or non-covalently bound to the sample surface is more accessible for a potential binding partner. As anchor for biomolecule attachment, redox-active surface-confined groups that reveal reactivity upon applying an electrical stimulus are of particular interest [15]. The hydroquinone/benzoquinone redox couple has found wide application because it can be employed as an electrochemically-removable protecting group {e.g., for biotin [16], carboxyl groups [17],

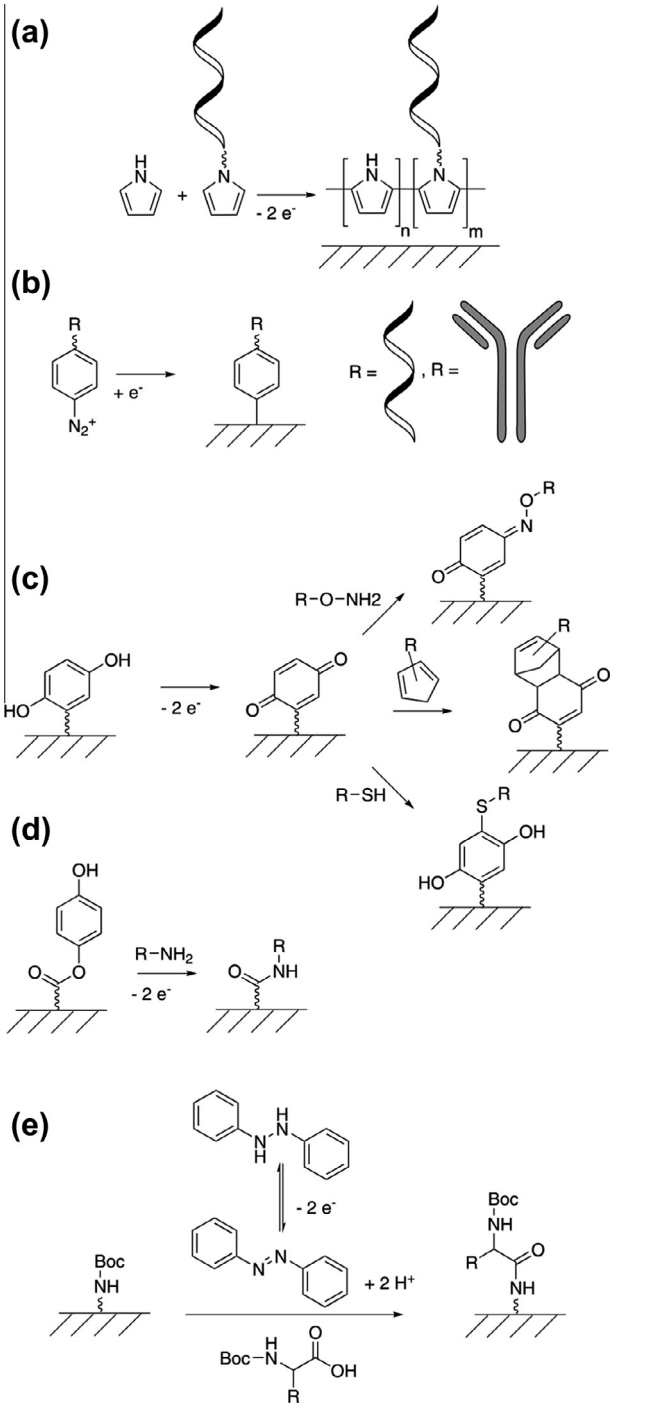

Fig. 1. Immobilization and synthesis of biomolecules can be triggered electrochemically. Reaction schemes for surface modification according to: (a) [11]; (b) [25]; (c) [20–22]; (d) [17]; and, (e) [42].

aldehyde groups [18] and amino groups [19] (see Fig. 1d)}. Moreover, a quinone moiety can serve as the anchoring group itself and capture molecules via electrochemically-induced Michael addition [20], 1,2-addition [21] or Diels-Alder cycloaddition reactions [22] (see Fig. 1c). All these reactions take place at comparatively mild conditions, which are suitable for the immobilization of sensitive biomolecules. The Cu(I)-catalyzed Huisgen cycloaddition or "click reaction" may be controlled by electrochemically triggered unmasking of the alkyne reactant [23] or generation of the catalyst [24].

Another approach, referred to as "electrografting", exploits the electrochemical reduction of aryl-diazonium salts under concomitant formation of a covalent bond to the electrode surface (see Fig. 1b). Antibodies and DNA modified with aryl-diazonium moieties may be grafted to a suitable surface under electrochemical control [25,26].

All these techniques require pre-modification of the biomolecules before their binding to the surface. However, they are suitable for the oriented immobilization of proteins on a surface [27], which prevents the loss of activity upon immobilization and may ensure accessibility to the active site.

In the future, electrochemically-cleavable protecting groups and electrochemically-triggered reactions may be expanded to the *in-situ* synthesis of biopolymers.

3. Surface patterning: site-selectivity *versus* chemoselectivity

The goal in fabricating bioarrays is to assemble as many different biological recognition elements as possible on a given surface area to increase the throughput of the biological assay. At the same time, the amount of sample consumed for each spot reduces with decreasing pattern dimensions.

The spatial information to create a laterally heterogeneous surface, the patterning, may originate from various sources. Classically, different specimens to immobilize are dispensed to discrete areas on a surface with the help of a nozzle or a pin for printing or spotting, respectively [3,4]. These approaches imply a serial patterning process, as opposed to parallel patterning employing photolithographic techniques [8]. Even though there are attempts to increase the patterning speed by using multiple tips [28,29], serial patterning is intrinsically slower than parallel patterning, so it is less suitable for large-scale fabrication of especially high-density arrays. Parallel patterning techniques typically use a photomask, and light is irradiated to restricted areas on the sample, triggering chemical reactions to crosslink [30] or to remove material, such as photo-cleavable protection groups or biomolecule-repelling films from the surface [31]. The necessity for a template limits the flexibility when designing the biomolecule array. Also, protocols from classical photolithography, as used in microelectronics fabrication, cannot be easily adapted to sensitive biomolecules, because the reaction conditions may affect the structure and the activity of biomolecules already immobilized on the array surface. As a consequence, soft lithography, which typically involves elastomeric stamps as template, has been developed [32].

Template-free, but photolithographic patterning was achieved by local ablation of protein-repelling coatings based on oligoethyleneglycol (OEG) [33] or bovine serum albumin (BSA) [34] with an electron beam or UV light. These techniques are limited with respect to the maximum achievable patterning resolution, which roughly amounts to half of the wavelength of the irradiated light. While this method yields local modifications larger than ~100 nm (using UV light), scanning probe techniques boost the patterning resolution for serial patterning methods.

Dip-pen nanolithography [35] consists of an atomic force microscope tip, which is used to dispense a reagent or the molecules to immobilize (see Fig. 2a).

3.1. Electrode arrays

The distinction between serial and parallel patterning also applies to electrochemical techniques. In the case of parallel patterning, photolithography is used to fabricate arrays of small electrodes rather than to trigger local attachment or detachment of biomolecules to the sample surface directly. The electrode array then takes over the role of the photomask. Individual electrodes at different locations on the sample may be contacted to trigger an electrochemical reaction to immobilize biomolecules. The individual on/off switching allows for the fabrication of arrays with multiple biological molecules on multiple electrodes. Various electrochemically triggered immobilization strategies discussed above have been exploited, including electrografting of aryldiazonium salts [25,26], various reactions using surface-confined quinone groups [36,37], quinone-based protecting groups [16,17], polymer entrapment [11–13] and unspecific adsorption [38].

The read-out of binding events at individual electrodes without relying – as classical bioarrays do – on fluorescence microscopy can be achieved using electrode arrays. As most proteins and DNA are not redox-active by themselves, labeling with redox-active reporter molecules is often required. On commercialized electrode arrays consisting of up to 12,544 individually addressable

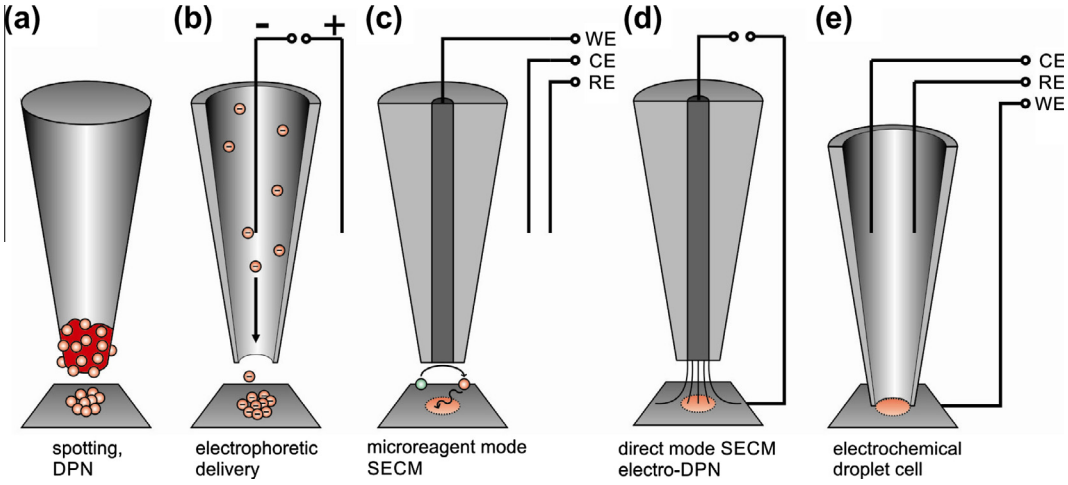

Fig. 2. Scanning probe techniques in various configurations are employed to induce local surface modifications. Patterning schemes as for: (a) spotting/DPN; (b) electrophoretic delivery; (c) microreagent mode SECM; (d) direct mode SECM/electro-DPN; and, (e) the electrochemical droplet cell.

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electrodes, each 44 μm in diameter, DNA-hybridization events [39] and antibody recognition [38] were successfully detected.

Ultimately, instead of classical immobilization of pre-synthesized biopolymers on a surface, the electrode arrays were utilized for the *in-situ* synthesis of both DNA [40,41] and peptide arrays [42] (see Fig. 1e). Electrochemical generation of protons replaced the addition of acids to deprotect the pH-sensitive protective group at the growing chain, as in standard solid-phase synthesis procedures. This makes array fabrication faster than serial spotting of reagents [43] and more flexible and less costly than array synthesis based on photochemical cleavage of protecting groups using photomasks [44–46].

3.2. Electrophoretic delivery

Because most biomolecules are charged, they may be locally delivered from micropipettes and nanopipettes to the surface by applying a voltage between an electrode inside the pipette and an external one (see Fig. 2b). This configuration is also used in scanning ion-conductance microscopy (SICM) [47] to control precisely the distance between the fragile nanopipette tip and the sample surface by measuring the ionic current through the pipette orifice. The advantage of this technique is that, e.g., immobilized DNA [48] or proteins [49] are in solution at all times which prevents possible denaturation. The requirement is a surface chemistry that is reactive enough to capture instantaneously the molecules delivered in order to avoid dilution or broadening of the biomolecule patterns.

Instead of using SICM-based distance control, the nanopipette was also coupled to atomic force microscopes (AFMs) [50]. This technique, often called nanofountain, also allowed parallel patterning using multiple tips [51], but in air.

3.3. Surface patterning in scanning electrochemical microscopy (SECM)

Most scanning-probe microscopy techniques rely on a purely physical signal to characterize a sample surface with spatial resolution (i.e., usually the topography is mapped but the chemical properties or identity of the specimen remain unknown). However, SECM yields localized chemical information (see Fig. 2c, d). SECM employs a microelectrode or a nanoelectrode, which can be moved with high precision along the three directions in space. Typically, an electrochemical reaction is carried out at the microelectrode tip and the tip is brought close to the sample surface. This results in perturbation of the typical electrochemical behavior of the microelectrode observed in bulk solution. For instance, kinetics of electrochemical reactions or production of electrochemically-active species at the sample can be detected, quantified and visualized [52].

The advantage of SECM for patterning of surfaces is that interfacial reactions with biomolecules can be locally triggered without prior loading of the tip, as it is necessary in dip-pen nanolithography or nanofountain technology. Surface patterning using SECM has two modes of operation, whose advantages and disadvantages we discuss below:

- microreagent mode (see Fig. 2c) and,
- direct mode (see Fig. 2d).

3.3.1. Local production of reagents

Active reagents can be locally generated at a microelectrode or a nanoelectrode by electrochemical conversion of precursors available in bulk solution (see Fig. 2c). The chemistry at the microelectrode tip is precisely controlled by the applied potential. A follow-up chemical reaction with specific functional groups at the sample surface may promote localized attachment, detachment or deactivation of biomolecules. This principle is referred to as microreagent mode or indirect mode of SECM.

In the microreagent mode, changes in surface functionality may be induced by local changes in the pH value invoked by electrochemical splitting of water at the microelectrode tip. For example, the protective SiO_2 layer on silicon was etched by anodic generation of H^+ in an electrolyte solution containing F^- ions [53]. The resulting corrosion pits were backfilled by the reduction of aryldiazonium salts. The modified areas obtained could be further (bio)-functionalized in subsequent steps.

Localized cleavage of ester functionalities from self-assembled monolayer (SAM)-modified gold electrodes induced by proton production at the SECM tip was demonstrated. An alkylalcohol residue was removed from a sample surface modified with an ester-terminated SAM, while carboxyl functionalities remained at the surface and were later activated and used to attach proteins covalently (see Fig. 3b). To prevent unspecific adsorption of proteins to non-activated areas, a chemical lift-off process was suggested [54]. After specific local immobilization of the protein to the carboxyl functions, the remaining ester groups were cleaved globally and, by this, removed together with the proteins that were unspecifically adsorbed to areas surrounding the activated spots.

Since the electrochemical reaction takes place at the microelectrode, an advantage of patterning in the microreagent mode of SECM is that the sample surface does not necessarily have to be electrically conductive. For example, glass substrates were locally functionalized through click chemistry [24] or polystyrene slides were oxidized by the generation of reactive radicals from Ag^+ or nitrate in solution [55]. The resulting functional groups on the polystyrene surface were suitable for the unspecific attachment of proteins and cells. A cathodic pathway was demonstrated by performing a localized Fenton's reaction, which gives rise to hydroxyl radicals to corrode various alkylsilane layers [56]. Through unspecific adsorption or after further bioconjugation steps, the sample surface was patterned with an enzyme.

The most widespread patterning scheme is to use the microelectrode for the electrochemical conversion of bromide into bromine/hypobromous acid. A homogenous layer of enzyme immobilized on the surface was locally inactivated through local oxidation by bromine [57]. When the sample surface was covered with protein-repelling coatings prior to patterning, the locally produced bromine degraded the film and allowed spatially restricted immobilization of cells at these positions. Bio-resistant layers based on BSA [58] or OEG [59] have been used to suppress unspecific adsorption. While probing the nature of the local surface modification induced in the microreagent mode (see section below) was challenging, it was possible to perform control experiments on macroscopic electrodes globally treated with electrogenerated bromine. Extensive characterization using IR spectroscopy elucidated the mechanism of degradation and the nature of the final product [60]. Recently, the local generation of bromine was also used, in combination with a scanning multiple tip consisting of eight individually addressable electrodes, which significantly increased the intrinsically low speed of serial patterning techniques [29].

However, the microreagent mode often employs rather aggressive conditions to induce the local surface modification. Either the precursors in bulk solution or the electrogenerated species themselves, which diffuse away from the microelectrode, may harm the sensitive biomolecules. For arrays modified with multiple and different (bio)molecules, where repeated activation and immobilization steps are necessary, the conditions for local activation may affect the activity of the molecules that were already immobilized on the array surface. To limit such side reactions, diffusion of the active species may be controlled with a quenching

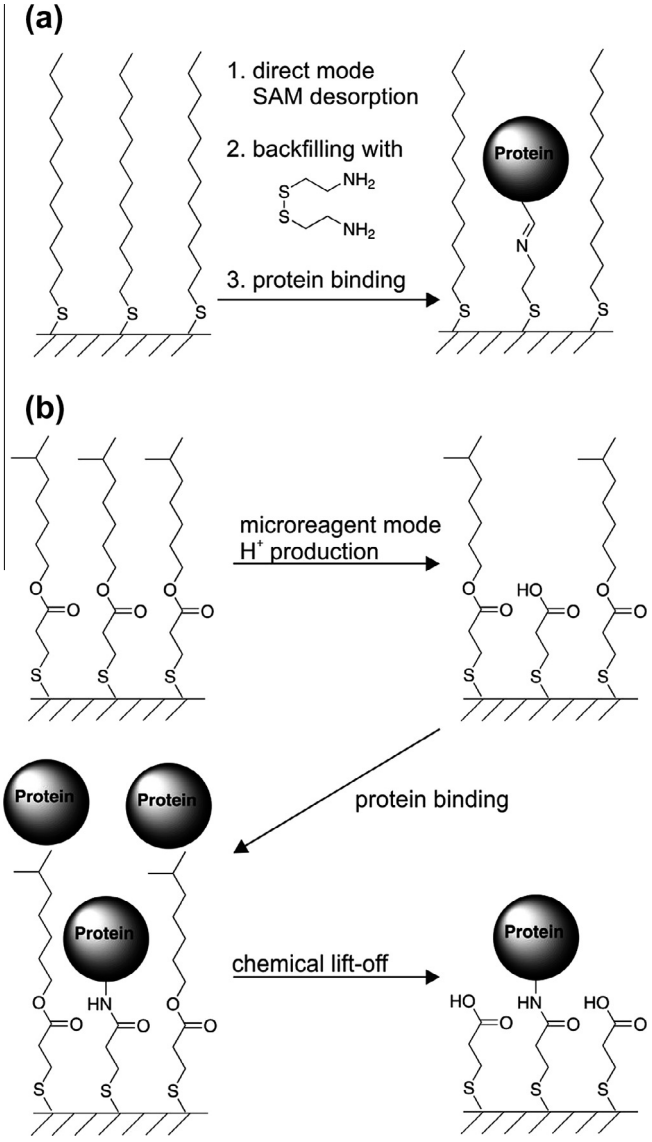

Fig. 3. Scanning electrochemical microscopy (SECM) allows for localized biomolecule attachment in the direct and microreagent modes of operation. Patterning schemes according to: (a) [62]; and, (b) [54].

agent. For example, in an application involving electrochemically-generated acids, adjusting the buffer strength or the pH value of the electrolyte enables fine tuning of the extent of diffusional transport of the protons to the restricted area targeted for modification [42,61].

3.3.2. Direct electrochemical reaction at the sample surface

Instead of producing or delivering a reagent from a scanned tip in proximity to the sample surface, SECM also allows to perform a localized electrochemical reaction at the sample surface directly, using a rather peculiar configuration of electrodes, namely the direct mode of SECM. The sample surface to be locally modified is used as the working electrode whereas the SECM tip serves as the counter electrode (see Fig. 2d). If a short potential pulse is applied to the sample, the current necessary to drive the localized electrochemical reaction is restricted to the area directly underneath the microelectrode tip. While the potential at the sample is controlled, the potential applied to the counter electrode is driven to whatever value is necessary to provide the necessary current to charge the large sample electrode and to carry out the Faradaic reaction at it. Hence, in most cases, the electrochemical reaction taking place at the microelectrode tip is electrolysis of the solvent.

For patterning with biomolecules the direct mode of SECM was first applied to remove a SAM locally and backfill the resulting holes with a differently functionalized alkanethiol to which glucose oxidase was coupled [62] (see Fig. 3a). Also exploiting SAM formation, gold was locally deposited and redox enzymes were bound to the gold spots [63]. A SAM terminated with nitro groups was locally reduced electrochemically to give rise to spots of amino/hydroxylamine groups. Using classical coupling reagents, enzymes could be immobilized exclusively to the modified areas [64]. These examples all required further ligation steps after patterning of the sample surface.

Alternatively, the direct mode allows local deposition of biomolecules in one step by incorporation into electrodeposited polymers. As tested for macroscopic electrodes and electrode arrays, incorporation of oligonucleotide-modified pyrrol monomers into a polypyrrol backbone may be used to generate DNA arrays [65]. Similarly, robust enzymes, such as glucose oxidase, can be co-deposited physically inside a chitosan matrix by generating a pH gradient in the gap between the microelectrode and the sample surface through proton reduction at the sample and water oxidation at the counter electrode [66]. The same enzyme was also deposited by the more specific avidin-biotin interaction with biotinylated electropolymerized polypyrrol [67].

In general, the direct mode allows patterning without reagents in solution. A surface uniformly modified with a redox-active species may be locally activated to capture biomolecules from solution. Nevertheless, its limitations are mainly due to the processes taking place at the counter electrode. Aggressive species generated during electrolysis of the solvent at the SECM tip may (as in the microreagent mode) reach the sample surface and damage sensitive surface groups used for immobilization of biomolecules.

We have found that the attempt to activate surface-confined quinone groups locally for subsequent biofunctionalization leads to massive corrosion of the electrode material [68] (see Fig. 4). Moreover, patterning in the direct mode is restricted exclusively to conducting surfaces. However, to the present day, this particular configuration is probably the one with the highest patterning resolution of all electrochemical techniques. By applying a voltage between an AFM or scanning tunneling microscopy (STM) tip and the sample, nanostructured surfaces suitable for biofunctionalization were generated through localized metal reduction [69] or changes in organic surface functionalities [70,71]. In these cases, high voltages have to be applied and the problem of an ill-defined surface chemistry is even more pronounced because of the absence of supporting electrolyte and a reference electrode.

3.4. Electrochemical droplet cells

In a droplet cell, the electrochemically active area on the sample is defined by the dimensions of a droplet of electrolyte located on the sample surface [72]. Some authors refer to the scanning droplet cell (SDC) as scanning electrochemical cell microscopy (SECCM) or electrospotting, emphasizing its possibilities for imaging and patterning applications, respectively. Usually, a glass or plastic capillary filled with the electrolyte and housing the counter and reference electrodes is brought in close proximity to the sample surface (see Fig. 2e).

Because the sample serves as the working electrode, electrochemical activation is precisely controlled by the potential applied to it. The droplet size and, thus, the patterning resolution are defined by the dimensions of the orifice of the capillary. Just as in direct mode patterning, application of the droplet cell is restricted to conducting surfaces but also exhibits the possibility for local reagentless electroactivation on previously uniformly modified

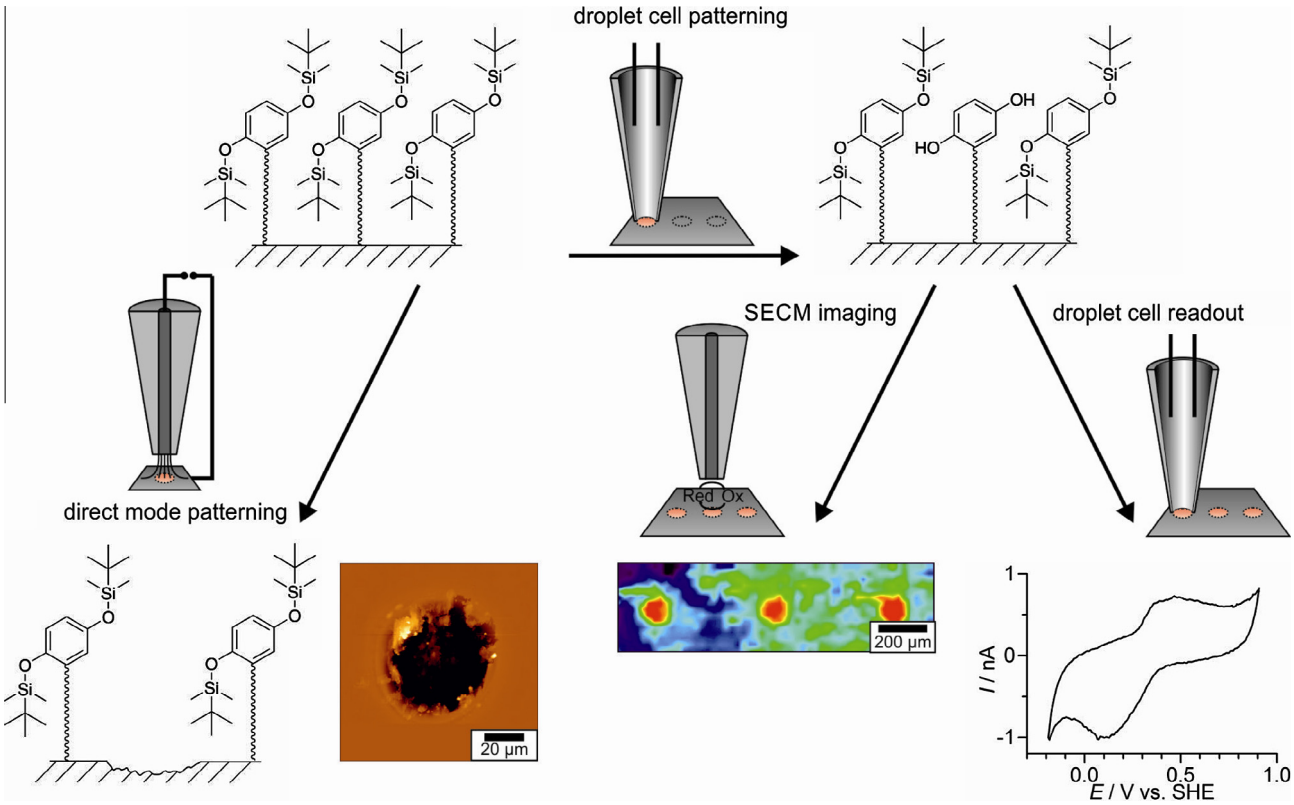

Fig. 4. The scanning droplet cell is used for truly chemoselective patterning. Direct mode SECM patterning results in substrate corrosion (AFM image, left branch). The droplet cell yields successful localized electrochemical cleavage of the TBDMS protecting group as confirmed by SECM imaging and locally recorded cyclic voltammograms showing the typical redox signals for the surface-confined quinone groups (right branch) [68].

surfaces without the need for photolithographic techniques. However, in contrast to the direct mode configuration, electrochemical reactions taking place at the counter electrode do not have to be considered, since the counter electrode is located far away from the patterning site. Consequently, reactive species potentially generated during the counter electrode reaction do not reach the working electrode during the time-scale of the experiment. Hence, the droplet-cell configuration can be applied to the patterning of sensitive surface functionalities suitable for biomolecule attachment.

Until now, SDC patterning for biofunctionalization has been used for local deposition of conducting polymer films [73,74] including oligonucleotide-modified polypyrrol [75]. Nevertheless, the potential of the SDC approach goes far beyond that. We have used the scanning droplet cell for the local electroactivation of surface-confined quinone groups through the cleavage of an electrochemically removable protecting group to demonstrate its applicability for patterning of sensitive surface functionalities [68]. The droplet-cell approach proved to be the only possible patterning tool, while other techniques, namely the direct mode of SECM, failed to provide the anticipated local surface modification but instead led to corrosion of the sample surface (see Fig. 4).

Also, the SDC has the potential for further decrease of the dimensions of the structures generated. So far, capillaries with dimensions significantly below 1 µm were employed [76]. Using novel top-down nanofabrication, glass capillaries and nanoelectrodes with dimensions of 10 nm can be fabricated with little technical effort [77–79]. Ultimately, the patterning resolution may almost reach that of techniques based on AFM and STM, but with the additional advantage of the possibility for truly chemoselective surface modification. Instead of using a droplet to confine the electrochemically-activated area, nanocapillaries may also be used to pattern the sample while it is completely immersed in solution. With the counter electrode located inside the capillary, the current upon electroactivation is, as in the direct mode of SECM configuration, restricted to the area underneath the nanopipette. However, reactions at the counter electrode do not interfere with the electrochemical modification at the sample surface. In contrast to SDC, this configuration is envisioned to allow surface patterning with sensitive biomolecules because the molecules already immobilized on the array are surrounded by electrolyte at all times to prevent denaturation during drying.

4. Label-free characterization of surface chemistry

The application of a bioarray usually comprises the immobilization of at least two elements, namely the biological recognition element and the target. For the detection of the binding of the target molecule, fluorescence microscopy is the method predominantly used due to its high sensitivity. The biological target itself or a secondary specific reporter molecule in the case of sandwich assays is labelled with a fluorophore that is detected upon localized binding to the biological recognition element. Label-free detection is desirable because labelling requires tedious purification steps and may affect the molecular recognition event by steric hindrance. Even though label-free detection strategies are difficult to realize for protein arrays, DNA-hybridization events between non-labelled strands can be detected electrochemically. Using SECM, DNA arrays were read out so that spots of single-stranded capture DNA could be distinguished from the double-stranded DNA after complementary hybridization due to an increase of electrostatic repulsion of the negatively-charged redox mediator [80].

When evaluating a strategy for biopatterning, it is also important to assess the nature of binding of the initial biorecognition element. To make the surface-bound biorecognition element

optimally accessible to the target molecules, unspecific interactions between the biomolecule and the surface have to be kept at a minimum and must be restricted to the attachment site without involving other parts, especially the target-binding sites of the molecule. Structural knowledge of the interfacial region, especially the termination of the surface with specific functional groups, is necessary to predict and potentially to influence the nature of biomolecule binding. Because of the difficulty in visualizing the surface chemistry at the microscale or the nanoscale, direct chemical information about local surface modifications induced during the patterning process is often missing. Many studies use the local immobilization of a model biomolecule in combination with negative control experiments as indirect evidence for the local surface modification.

By contrast, additional spectroscopic data containing chemical information are considered direct evidence for the surface modification. This requires surface-sensitive spectroscopic methods with the possibility for lateral resolution of the signals obtained. For example, X-ray photoelectron spectroscopy (XPS) studies confirmed the local removal of protein-repelling coatings [33] and the local oxidation of polystyrene [55]. Similarly, the absence of PEG moieties after patterning was also proved directly by secondary ion mass spectrometry (SIMS) [31]. Also, infrared reflection absorption spectroscopy (IRRAS) has helped to characterize local polystyrene oxidation [55] and corrosion of OEG moieties [60]. The lateral resolution of these three methods is relatively low so that local modifications at the lower μm scale and below cannot be resolved.

SDC or SECCM can be used to investigate the local surface chemistry with high resolution. Even though strictly no structural information is obtained, surface-bound groups may be unequivocally identified by their electrochemical properties. Using SDC, the electrochemistry of locally deposited conducting polymers was directly probed by recording localized cyclic voltammograms [81] or the electrocatalytic activity of single metal nanoparticles was measured [82]. After patterning of hydroquinone-modified surfaces using the SDC, the redox activity of the quinone moieties was exploited to give direct evidence for the anticipated surface modification [68] (see Fig. 4). This reflects the potential of droplet cells as a tool for localized modification of surfaces, because patterning and subsequent characterization of the generated patterns are performed in one device. However, use of the SDC for patterning and biofunctionalization is restricted to electrochemically active surfaces. In future, application of nanosized capillaries will increase the patterning resolution while maintaining high chemoselectivity of the localized surface treatment.

5. Conclusion

Control over the localized immobilization of biomolecules to retain biomolecule activity is necessary to generate functional microarrays for high-throughput bioanalytical applications. SECM techniques are powerful tools both for biological surface patterning and subsequent read-out of the generated patterns at the microscale and the nanoscale. Localized surface activation can be induced using scanning electrochemical droplet cells by triggering surface-confined functional groups to capture biomolecules. This constitutes a truly chemoselective, reagentless route to electrochemical-surface modification.

Further decrease of electrochemical probe dimensions will boost the patterning resolution of bioarray fabrication while still maintaining control over the surface chemistry. This development will be empowered by the recently proposed top-down fabrication of nanopipettes and needle-type nanoelectrodes. Nanocapillaries hosting a counter electrode may be employed for direct electrochemical nanopatterning even with the sample immersed in electrolyte solution to avoid denaturation of previously surface-bound biomolecules by desolvation. Moreover, by combining electrochemically-addressable protective groups and modern SECM techniques, arrays of biomolecules could be fabricated *in situ*.

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