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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.

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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

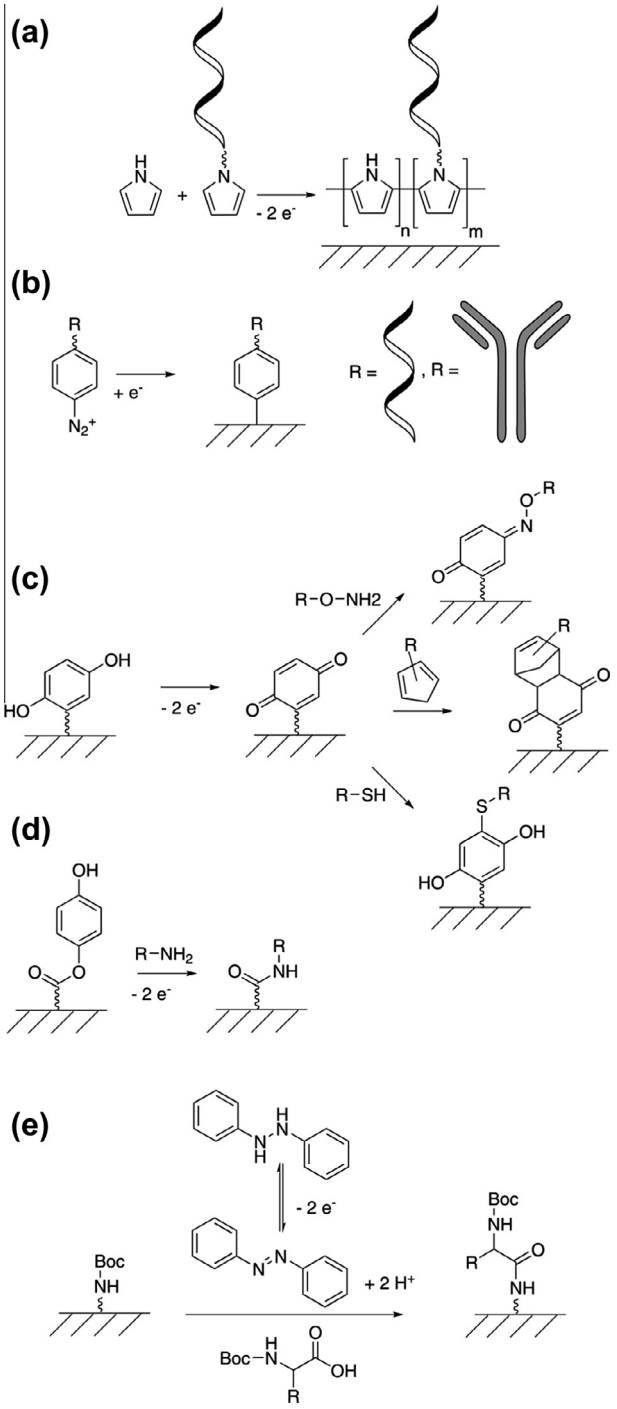

Fig. 1. Immobilization and synthesis of biomolecules can be triggered electrochemically. Reaction schemes for surface modification according to: (a) [11]; (b) [25]; (c) [20–22]; (d) [17]; and, (e) [42].

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],