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Protein adsorption and surface patterning

Tobias Ekblad, Bo Liedberg *

Division of Molecular Physics, Department of Physics, Chemistry and Biology, Linköping University, SE-581 83 Linköping, Sweden

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ABSTRACT

Surface patterning has become an important discipline of biologically oriented surface science over the past decades. Many methods have been developed that allow the formation of patterns on the micro- and nanoscale. This Opinion discusses the role of protein adsorption in patterning technologies, highlighting how it can be used as an integrated part of the patterning process, how it can be controlled by patterns with appropriate properties, and how it may lead to disruption of formed patterns if not properly accounted for. Recent examples from literature are used to emphasize some of the most interesting developments in the field, such as novel surface chemistries only allowing specific protein adsorption, directed self-sorting adsorption of proteins on patterned surfaces, and control of protein adsorption through nanopatterning.

1. Introduction

The intention of this Opinion is to give a broad perspective to the field of protein adsorption and surface patterning. Numerous approaches to patterning for applications in biology have been demonstrated over the past decades, often with a strong focus on describing the technical aspects of the patterning method itself. To provide a context for the discussion, this contribution will summarize some of the most important trends in this highly interdisciplinary field, with a current as well as a historical perspective, focusing on the challenges associated with protein adsorption. This particular subject has sometimes been overlooked, or studied using simplified biological model systems (e.g. a solution of a single protein in buffered saline), when the primary goal has been to describe novel patterning methods capable of creating patterns with very small dimensions and/or high packing densities of biological species on a supporting surface. However, if (and this is really an *if* in this rather proof-of-concept-based field) a patterned surface is eventually used in a real biological application, rather than in a simplified model system, protein adsorption will potentially become a major concern, capable of obliterating the results of all prior patterning efforts, no matter how meticulously performed. If instead taken into account as the natural process that it is, and met with an appropriate surface design, protein adsorption may be used as an advantage, and even as an integrated part of the patterning process.

The ability to prepare patterns and structures with small dimensions has been crucial for the development of many modern technologies, in particular microelectronics and photonics. In light of these advances, it is not surprising that inventive researchers have attempted to exploit some of these technologies also for applications in the biological field. Some of the pioneering studies of bio-patterning had ambitious goals, aiming to develop bio-electronic microcircuits [1]. Another early application was the basic biological study of cell spreading and growth on patterned surfaces, and this has remained a popular and productive research field. However, several other application fields, notably biosensors and protein microarrays for diagnostics, also have seen advances due to the development of the bio-patterning techniques. Common to all these developments is the need to understand, predict and engineer the interactions between proteins and patterned surfaces.

Before going deeper into the discussion, a caveat may be in place: It should be noted that, in the majority of cases, the protein adsorption that takes place locally on a patterned surface is not different from the protein adsorption that would take place on a non-patterned surface with the same surface chemistry. Provided that the pattern dimensions are large compared to the dimensions of the adsorbing species (which has traditionally been the case) the same fundamental interactions can be expected to govern both processes. A special case, which has attracted considerable interest in recent years and which will be discussed briefly in this Opinion (see Section 4), is when the pattern dimensions start to approach or even become smaller than those of the proteins.

1.1. Applications of bio-patterning

One of the most important aspects of any patterning process is the size scale selected for the patterns. In microelectronics, the motivation for making increasingly smaller patterns is typically to be able to fit a greater number of electronic components onto a single chip. In the biomedical field, the development of patterned surfaces for analytical applications, e.g. protein microarrays, may have similar goals. However, in many other applications, the reasons for creating small

* Corresponding author.
E-mail address: bolie@ifm.liu.se (B. Liedberg).

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patterns are not always as straightforward. Simply making the patterns as small as possible is not likely to be the most productive route in all applications. Instead, the pattern dimensions should be carefully engineered with regard to the biological system which will interact with the patterned surface. Therefore, relatively large patterns with dimensions of hundreds of micrometers or even millimeters may be appropriate in some cases, while nanoscale patterns are preferred in other applications.

One major biological application of patterned surfaces is as substrates used in fundamental studies aiming to explore specific aspects of cell biology, particularly cell adhesion and spreading. A living cell exists in an environment where it is in contact with other cells and with the extracellular matrix. This three-dimensional network, with its specific spatial structure, provides cues which direct the development and fate of the cell [2]. Many attempts have been made to emulate these conditions for *in vitro* cell culture, typically using two-dimensional systems and selected cell lines, providing a system with greatly reduced complexity compared with the natural biological environment. However, some of these studies have still been able to provide answers to important biological questions. Some examples are the investigations of the geometric control of cell apoptosis conducted by Chen et al. [3] in the 1990s, and the more recent studies of how the strength of cell adhesion is controlled by the spacing of adhesion receptors [4] (see Fig. 1 for an example of this type of work). Several excellent reviews have been dedicated to this research field [5,6].

Another important application for biologically active patterns can be found in the field of bioanalytical devices, such as biosensors and protein biochips. These techniques are used for detection and quantification of biomedically relevant analytes, so-called biomarkers. A common strategy is to immobilize bio-recognition elements (e.g. antibodies) on a surface, which is then allowed to interact with a biological sample, giving a detectable response upon analyte binding. Parallel detection of several analytes generally demands that the different surface–analyte interactions take place in separate and specifically addressed positions on the surface. Patterns, in this context, are the resulting discrete spots of different bio-recognition elements, surrounded by an inert and non-reactive background chemistry. The work in this field has largely been devoted to the development of techniques which allow dense and specific distribution of bio-recognition elements with preserved biological activity. This application will be described in more detail below (Section 3).

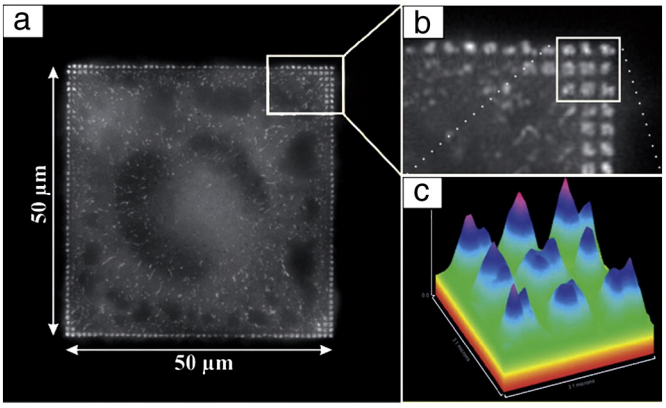

Fig. 1. Fluorescence microscopy images of a living cell adhering to a surface patterned both on the nanoscale (using nanometer-scale gold dots, not distinguishable in the image) and microscale (smaller squares 0.5 µm wide and large square 50 µm wide). The gold dots were coated with an adhesion promoting peptide and the surrounding substrate was coated with a chemistry selected to eliminate protein adsorption. The cell spreads on the micropattern, while the nanopattern can be used to investigate the distribution and function of adhesion receptors.
From reference [7] (http://dx.doi.org/10.1039/b815634d). Reproduced by permission of The Royal Society of Chemistry.

An emerging application of patterned surfaces, with particular relevance for protein adsorption issues, is the control of biofouling in marine and biomedical applications. The underlying idea is that a patterned surface, simultaneously presenting surface chemistries with very different physicochemical properties, should somehow confuse and discourage the organisms responsible for biofouling (e.g. bacteria, algae and barnacles) to attach and settle. The size scale of these patterns is usually adapted to the size of the organisms in question or their settlement organs/sensing systems [8], and hierarchical patterns may be considered for covering a range of different size scales. Protein adsorption is believed to be a prerequisite for organism adhesion to the surface, and is therefore very important in biofouling applications. However, as noted above, the mechanisms of protein adsorption will not be directly affected by the relatively large patterns used for these applications, only the distribution of the adsorbed proteins. A recent development in this field is the use of nanopatterned (or nanoheterogeneous) surfaces which may possibly provide more direct effects on the protein adsorption on the surface [9–11].

1.2. Overview of patterning methods

The surface patterning methods developed for biological applications may be broadly classified as either “direct” or “indirect”. The direct methods are based on active distribution of the bioactive substance in patterns on a (typically homogeneous) surface, while indirect methods rely on spontaneous and preferential adsorption of the bioactive substance from solution onto a pre-patterned surface (Fig. 2). Protein adsorption is clearly a central issue for either approach, both for creating stable protein patterns and for maintaining their integrity. In particular, it is an absolutely essential aspect of indirect patterning strategies, integrated in the patterning method itself. The simplicity of the indirect method, making it an attractive option ever since the advent of biological patterning, is primarily due to the fact that the patterns are formed by selective recruitment of the bioactive species to the pre-patterned surface. Direct patterning, which is a more recent development, is often more difficult to achieve but has a major advantage in allowing several different bioactive species to be distributed in discrete spots on the same surface; an outcome which is generally not possible through indirect patterning.

The actual pattern fabrication techniques, applicable to both direct and indirect methods, may be based on the use of elastomeric stamps [12,13], microfluidics [14], ink-jet printing [15], photolithography [16], or on different direct writing methods using pins or tips [17–19]. Indirect patterning may be a milder and therefore more suitable approach when patterning sensitive biological species, since dehydration could lead to protein denaturation and loss of biological activity (see Fig. 3). It is therefore somewhat surprising that the resulting activities of dry-stamped proteins have been found to be comparable to those of solution-adsorbed proteins, although some proteins are clearly more sensitive than others [20]. If possible, peptides, which are generally more stable than proteins, can be suitable alternatives in cases when the stability is an issue for direct patterning.

2. Micropatterns with binary composition

One of the most effective and simple patterning strategies involve the preparation of patterns composed of bioactive areas of a single type, such as a certain protein, surrounded by an inert background chemistry. Indirect patterning approaches are quite convenient for the preparation of such binary patterns. After a preliminary chemical patterning step, which can of course be a complicated procedure (for examples see Fig. 3), the whole surface is immersed in a solution of a certain protein, which adsorbs in a selective and “directed” fashion due to the pre-existing differences in surface chemistry. For this