Immunoassay Fabrication and Device Functionalization by Scanning Probe Lithography

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Summary

In this chapter, we describe scanning probe lithography (SPL) techniques (a diverse set of techniques, including dip-pen nanolithography (DPN), polymer pen lithography (PPL), microchannel cantilever spotting (μCS), and microcapillary printing (MCP), among others) with a special focus on applications involving deposition of antibodies. Generally, these methods utilize a probe or an array of probes scanned over a surface to deliver materials ("inks") or introduce energy for material modification. They typically employ an atomic force microscopy (AFM)-like setup with nanometer-scale positioning precision. Unlike conventional lithographic techniques such as photolithography or electron beam lithography, SPL operates under mild conditions, avoiding vacuum environments and harsh chemical treatments. This makes SPL particularly suitable for patterning delicate biomaterials while enabling mask-free, multiplexed, and direct-write surface functionalization. These features position SPL as a powerful tool particularly also for applications such as immunoarray fabrication

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and biomaterial deposition on sensor devices. Future advancements will likely involve integrating SPL with traditional lithographic techniques, leveraging the advantages of both to create hybrid systems. This approach can enable precise functionalization of semiconductor structures with biomolecules, facilitating highly localized and on-demand modifications. The synergy between SPL and conventional lithography presents a promising avenue for advancing biosensors, biomedical devices, and biofunctionalized surfaces.

Abbreviations

AB	Antibody
AFM	Atomic Force Microscopy
AFP	Alpha-fetoprotein
BPL	Beam Pen Lithography
DISC1	Disrupted-in Schizophrenia 1
DMD	Digital Mirror Device
DMSO	Dimethyl Sulfoxide
DPN	Dip-pen Nanolithography
EIS	Electrochemical Impedance Spectroscopy
EpCAM	Epithelial Cell Adhesion Molecule
EV	Extracellular Vesicle
FluidFM	Fluidic Force Microscopy
HIV	Human Immunodeficiency Virus
IgG	Immunoglobulin G
MCP	Microcapillary Printing
MHA	Mercaptohexanoic Acid
MPTMS	(3-Mercaptopropyl) trimethoxysilane
NFP	Nano-fountain Pen
NHS	N-Hydroxysuccinimide
PEG	Polyethylene Glycol
PPL	Polymer Pen Lithography
SAM	Self-assembled Monolayer
SLE	Systemic Lupus Erythematosus
SLM	Supported Lipid Membrane
SPL	Scanning Probe Lithography
TNF-α	Tumor Necrosis Factor-α
μCS	Microchannel Cantilever Spotting

29.1 Introduction

Scanning probe lithography (SPL) is an umbrella term for different techniques such as dip-pen nanolithography (DPN), polymer pen lithography (PPL), microchannel cantilever spotting (μCS), microcapillary printing (MCP), to name a view in particular relevant to biomaterials, and many more derivatives of these methods (Fan et al. 2022). SPL methods are a very diverse set of techniques, with the common feature of using a probe (the "tip" or "pen") or array of probes that is scanned over the surface on which patterns are to be introduced and deliver either materials ("inks") directly or introduce energy (mechanical, thermal, or electrical) to the surface to remove material or induce modifications or chemical reactions. SPL methods usually utilize an atomic force microscopy (AFM)-like setup to gain high precision down to the nanometer range in probe positioning and control the contact of the probe with the surface. In addition to the high resolution and precise location control, SPL methods offer mild process parameters (no vacuum, ambient temperature, aqueous solvents) in comparison to standard lithography approaches such as photolithography or electron beam lithography, which makes them in particular interesting for the deposition of delicate biological materials. They are usually additive and mask-free, offering direct-write, digital surface patterning, allowing for rapid change in desired patterns, without the need for manufacturing of expensive masks or templates. Finally, they offer multiplexing (i.e., the deposition of more than one ink within a micro/nanopattern) without the need for repeated coating of the sample with resist or passivation layers, avoiding the risk of destroying prior functionalizations by applying coatings or lift-off processes. This is again in particular advantageous when processing sensitive biomaterials as proteins, which can be easily denatured or lose functionality in many conventional lithography approaches. All these features make them a promising route for the fabrication of immunoarrays and for the functionalization of devices with antibodies.

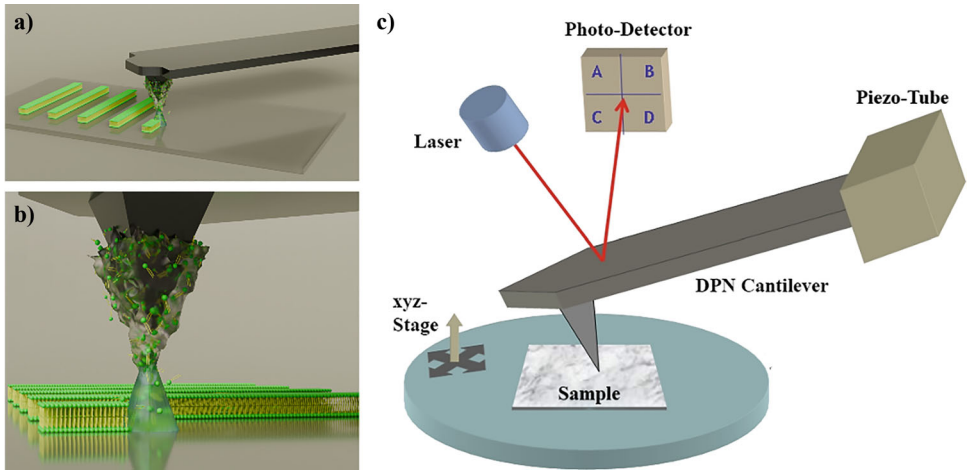

Fig. 29.1 Schematic of DPN. (**a**) Schematic of a DPN cantilever depositing a line pattern onto a substrate. (**b**) Close-up on the DPN tip, where a molecular ink is transferring to the substrate over a water meniscus. (**c**) Scheme of an AFM-based SPL setup. The probe (in this case, a DPN cantilever) is mounted in an AFM setup comprising precise positioning control of the sample by an xyz-stage and/or a piezo tube that can move the probe. If a precise force control is desired, a laser-based deflection control of the cantilever can be used. (Caption (**c**) adapted from "The Opensource Handbook of Nanoscience and Nanotechnology" (https://en.wikibooks.org/wiki/Nanotechnology) under CC-BY-2.5.)

29.2 Scanning Probe Lithography (SPL) Methods

In the following, we will briefly introduce the most common SPL methods with high relevance to printing biological materials such as proteins and in particular antibodies.

29.2.1 Dip-pen Nanolithography (DPN)

DPN was introduced in 1999 by the Mirkin group as a tool for generating nanoscale features of thiol self-assembled monolayers (SAMs) on gold surfaces (Piner et al. 1999, Li et al. 2021, Liu et al. 2020a, b). The working principle of DPN can be likened to writing with a feather-quill on paper, only that a much finer writing tool is used here, in the form of the tip of an AFM, which has a very sharp apex (on the order of 10 nm). The tip can also be much more precisely moved over a substrate because of the high positioning control offered in typical AFM setups (Fig. 29.1).

The DPN tip is first coated with the desired "ink" (either by dip-coating or gas phase deposition, depending on the molecules used), then mounted to the setup and brought into controlled contact with the substrate to write on. When the tip approaches the substrate, a microscopic water meniscus will build in between the tip and the substrate, over which the molecular ink can transfer onto the substrate and build structured SAMs. As the evolution of such SAMs follows a 2D difussion from a point source (the DPN tip), not only the humidity (responsible for the general size of the transfer meniscus) but also the dwell time (how long a tip is held in stationary contact with the substrate) and speed of tip movement (when writing lines) influence the resulting feature size and line width.

This basic principle was quickly extended to more types of inks and substrates, in particular also viscous inks that—rather than traveling over a water transfer meniscus—are transferring in bulk, resulting in a more complicated transfer process, now governed mainly by hydrodynamic flows and the balance between surface energy of the substrate and the surface tension of the ink,

defining the contact angle and spreading behavior of the transferred liquid on the surface. The use of viscous inks enable DPN to deposit many more materials as molecules or particles, that would not travel over a water meniscus on its own. These can be either solved in an appropriate viscous ink, or carried along in a viscous ink as matrix. In particular relevant for biological inks is the use of water-based mixtures containing hygroscopic admixings like polyethylene glycol (PEG), DMSO or glycerol to avoid drying during the DPN process and allow to deposit proteins (including antibodies) via DPN without destroying their functionality. Another interesting ink in regard to biomaterials are phospholipids, that combine aspects of molecular and viscous inks, as they self-assemble on surfaces into stacks of lipid membranes (Lenhert et al. 2007, Urtizberea & Hirtz 2015). At the same time, the overall height and structure of the resulting lipid stacks is governed by the surface energy of the surface, and the meniscus between tip and surface is built from the lipid ink itself. To raise throughput during the writing process, in DPN often probe arrays (1D or even 2D arrays of multiple writing tips) are utilized. While generally all tips in such arrays write the same pattern in parallel, the ink on each tip can be individually delivered during the inking process by microfluidic networks, allowing the creation of multi-ink (multiplexed) patterned arrays.

29.2.2 Polymer Pen Lithography (PPL)

PPL was developed as a means for highly parallelized, large area patterning of surfaces with micro-/nanostructures (Huo et al. 2008). This is achieved by using an elastomeric stamp carrying 2D arrays of several thousand pyramidal tips per square centimeter. These stamps can be readily produced by pouring a polymer into a silicon master mold featuring pyramidal holes produced by anisotropic wet etching. The molds can be reused many times to produce stamps, making PPL a very cost-effective way of large-area nanopatterning. While the pyramidal pen's apex is slightly bigger than in DPN (~50 nm), still sub-100 nm features can be achieved. Like in DPN, the PPL stamp is mounted in a setup that allows for controlled movement of the stamp with regard to the surface to be printed on. In this regard, PPL could be seen as high-precision microcontact printing; however, the added control allows also for arbitrary (dot matrix) patterns and multiplexed patterning (Brinkmann et al. 2013) by bringing the PPL stamp into contact with the surface several times. PPL stamps are usually inked by spin-coating or soaking of the whole stamp, and ink transfer generally follows the behavior of DPN tips (2D surface diffusion for molecular inks and viscous flow for viscous inks) and thus can also be controlled by contact time and humidity. Furthermore, the elastic nature of the polymeric pyramidal pens allows for an additional mode of control in comparison to DPN. When the stamp is pressed into the surface further after initial contact, the pyramidal pens get (reversibly) deformed into squares; thus, square-shaped features of contact-force-dependent size can be produced, yielding contact force as an additional process control parameter (Fig. 29.2).

PPL also saw a rapid development into more advanced methods, e.g., by including aspects of photolithography into the patterning process. In this beam pen lithography (BPL), individual pens of a PPL stamp array can be addressed with light by a digital mirror device (DMD), allowing focus of light onto the surface, allowing for sub-wavelength feature sizes in photolithographic resist or light-triggered chemistry on confined spots that allow selective binding of compounds from the liquid phase.

29.2.3 Microchannel Cantilever Spotting (μCS)

In μCS, minute volumes (fL to pL) of liquid inks can be deposited on surfaces. For this, a microchannelled cantilever attached to a reservoir filled with a μL volume of desired ink is attached to an AFM/DPN-like setup (Xu et al. 2004, Xu et al. 2006). When the tip is brought into contact

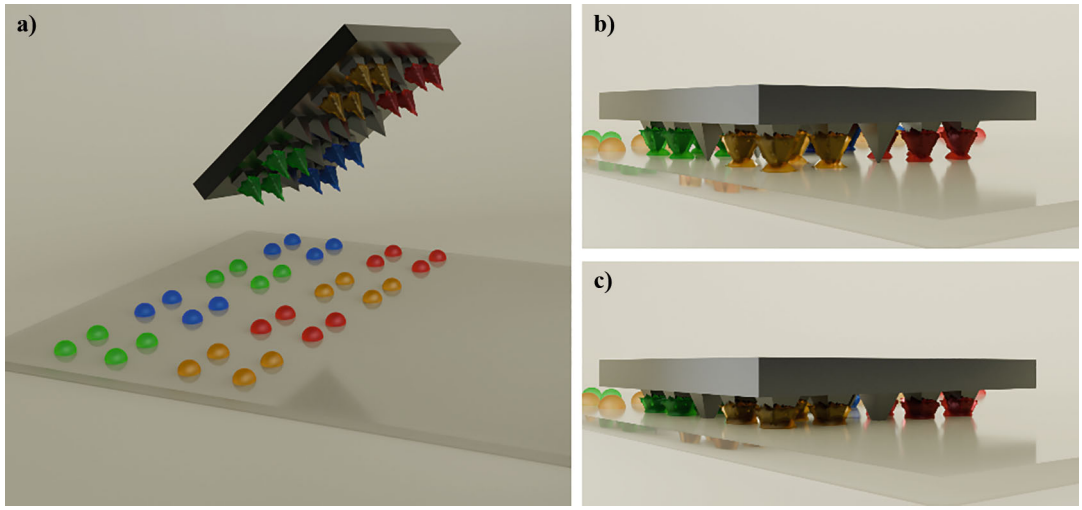

Fig. 29.2 Schematic of PPL. (**a**) Schematic of a PPL stamp with four differently inked segments hovering over a printed array. (**b**) DPN-like ink transfer mode: the PPL pens barely touch the surface, and ink can transfer to the surface by capillary forces. (**c**) Microcontact-printing-like mode: the PPL stamp is pressed into the surface, deforming the elastic pens, stamping ink onto the surface

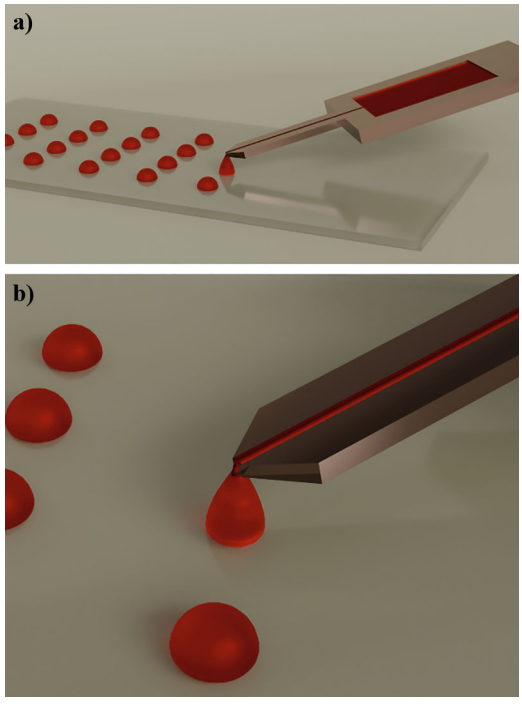

Fig. 29.3 Schematic of μCS. (**a**) Scheme of a microchannel cantilever with ink-filled reservoir spotting an array. (**b**) Close-up scheme of droplet deposition. The ink flows down the microchannel on the cantilever and deposits on the surface by capillary forces

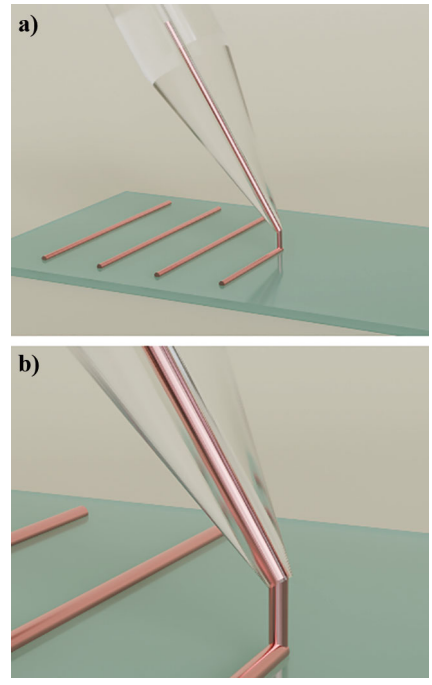

Fig. 29.4 Schematic of MCP. (**a**) Scheme of a microcapillary depositing a line pattern onto a substrate. (**b**) Close-up scheme of the microcapillary aperture during writing. The ink can either be deposited by capillary forces or actively ejected via pressurization by a microfluidic pump

with the surface, ink can flow by capillary force from the reservoir onto the substrate. The resulting droplet feature's size is governed by contact time (in particular for small feature sizes) and the balance between surface energy and ink surface tension (Fig. 29.3).

Droplet arrays deposited by μCS can act as microreactors for local surface chemistry, thus allowing efficient chemical coupling of ink components to the surface, e.g., by click reactions. Typical feature sizes in μCS range from 5 μm to 40 μm, making these in particular interesting for cell capture arrays due to their similar size range.

29.2.4 Microcapillary Printing (MCP)

The SPL methods described above all work with "open" ink reservoirs (either the tip/pen surface in DPN and PPL or as on-chip open reservoir in case of μCS) and rely on passive material transfer (e.g., by capillary forces or diffusive processes) to the surface. This can pose problems when the ink or carrier solution is prone to evaporation, and in cases where the ink adhesion force to the tip/pen is greater than the adhesion force between ink and the surface to print on (e.g., with an aqueous ink and a (super) hydrophobic substrate). A way to overcome these challenges is the use of microcapillaries, which allow for a closed line of delivery from a reservoir to the capillary apex that is used as a printing pen (Fig. 29.4). Furthermore, this setup allows active ink deposition by adding microfluidic pumps to eject ink via pressure. This also enables the processing of highly viscous inks and was, e.g., used in the deposition of liquid metals (Hussain et al. 2021).

Typical MCPs utilize capillaries with apex openings in the 1 to 100 μM range and are thus limited when high resolution (sub ~10 μm) is required. An early solution to raise printing resolution was the nano-fountain pen (NFP) technique (Lewis et al. 1999). This technique utilizes a nano capillary with an opening of ~100 nm mounted to an AFM tip, though no active ink delivery via microfluidics was incorporated, thus still relying on capillary forces for ink transfer. As an extension of the idea, fluidic force microscopy (FluidFM) offers an alternative (Grüter et al. 2013). Here, a hollow AFM tip with a small sub-micron range opening at the tip apex or side offers an alternative. These tips run in a regular AFM setup and comprise a closed ink reservoir that can be pressurized by a microfluidic controller for ink deposition. This allows also for the direct deposition of inks in a liquid environment.

29.3 Structured Deposition of Antibodies by SPL

All SPL methods described above were utilized for the generation of immunoarrays or deposition of antibodies to devices. In this chapter, the general concepts on how antibodies (and other proteins or complex biomolecules) are immobilized into structured surface patterns by SPL are introduced and selected examples for applications are showcased.

29.3.1 Direct-Write Approaches

As SPL methods can operate under ambient conditions and with aqueous solutions as inks, antibodies can be delivered straightforwardly in a direct-write fashion to their desired locations on a substrate. Insofar, this is the most straightforward method for delivering antibodies to specific locations on a surface while preserving their functionality. Here, usually antibodies are delivered in a buffered aqueous solution (e.g., PBS or commercial printing buffers), sometimes with the addition of a hygroscopic substance (e.g., glycerol or PEG), to avoid premature drying of the transfer ink on the probe or in reservoirs. In the easiest case, antibodies are delivered to a non-functionalized surface, and the immobilization of antibodies relies solely on physisorption by intrinsic interactions with the surface (e.g., van der Waals forces or surface charges). When a stronger or more controlled immobilization is desired, the surface is functionalized with chemical moieties that allow for specific binding of antibodies. This reduces loss of antibodies in

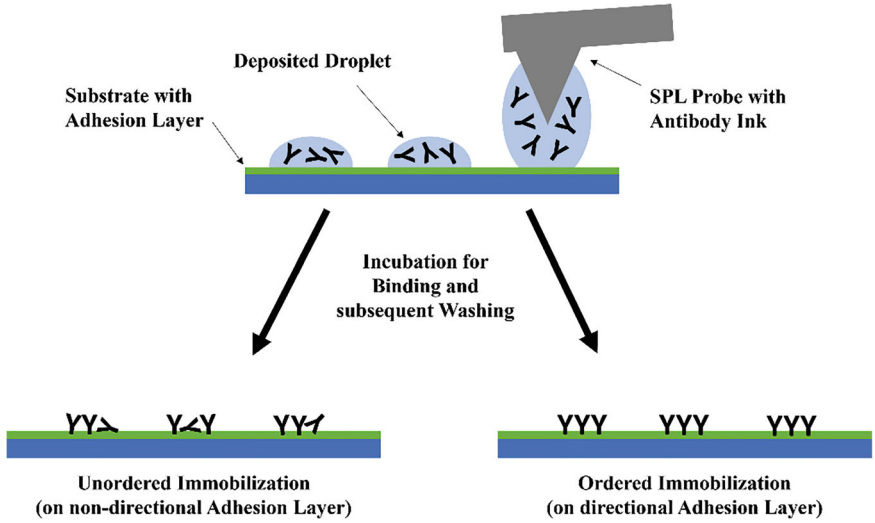

Fig. 29.5 Schematic of direct-write deposition of antibodies. In this approach, the ink used in the SPL process contains the antibodies desired for immobilization. The substrate is usually homogeneously coated by an adhesion layer, which allows for antibody binding, though also solely relying on physisorption, e.g., on untreated glass surfaces is possible. After some incubation time to allow for antibody binding, the surfaces can be washed to remove excess ink with non-bound antibodies. Depending on whether the adhesion layer offers directional binding sites, the resulting immobilized pattern comprises either unordered or ordered antibody alignment

Table 29.1 Literature examples for direct-write immobilization of antibodies via SPL methods

Publication	Method	Immobilization mechanism	Application
Bruckbauer et al. (2003)	Nanopipette	Specific capture Abs	Technology demonstration
Zheng et al. (2009)	PPL	NHS/commercial arraying slides	Technology demonstration
Tsarfati-BarAd et al. (2011)	NFP	Commercial arraying slides	Technology demonstration
Laing et al. (2013)	DPN	Physisorption on nitrocellulose slides	AFP immunoassay
Petersson et al. (2014a)	DPN	Commercial arraying slides	Serum protein screening
Petersson et al. (2014b)	DPN	Commercial arraying slides	Serum protein screening (SLE nephritis)
Liu et al. (2020a, b)	DPN/μCS	Biotinylated lipids/streptavidin/ biotinylated ABs	Technology demonstration
Lee et al. (2022)	FluidFM	NHS	Single-cell protein analysis (DISC1)
Mathew et al. (2024)	μCS	Physisorption on polymers	Functionalization of 3D structures

washing steps or during incubation of analytes. When the adhesion functionalization is implemented with specific binding sites (e.g., biotin- or histidine tags, or protein A/G) for sub-locations or tags on the antibody desired to be immobilized, ordered, directional antibody immobilization can be achieved, which can raise antibody activity in comparison to a random distribution when relying solely on physisorption or non-directional chemical binding (Fig. 29.5).

Selected examples of direct-write antibody delivery via SPL are given in Table 29.1. An early example of SPL for antibody arraying is given by Bruckbauer et al. 2003. Here, NFP was used to deliver specific IgGs (produced in rabbits) to a surface homogeneously coated with anti-

rabbit IgG that acted as a capture antibody to immobilize the printed IgGs. The authors present multiplexed IgG arrays with sub-micron dot features. An often-used chemical binding motif for antibody immobilization in direct-write approaches is n-hydroxysuccinimide (NHS). NHS binds to amine groups that are present in the antibodies, thus cannot control the direction of bound antibodies but will provide a strong and stable immobilization. Zheng et al. (2009) used this immobilization approach to create multiplexed immune arrays with three different antibodies and feature sizes as small as ~600 nm. Also, commercially available protein arraying slides that offer similar protein reactive surfaces for immobilization (often mediated by epoxy- or aldehyde coupling) were used and provide stable binding, demonstrated e.g., by Tsarfati-BarAd et al. 2011 during patterning via NFP, obtaining sub-micron feature size and Petersson et al. 2014a, b that utilized DPN for the fabrication of miniaturized immunoarrays for serum protein-profiling in the context of systemic lupus erythematosus (SLE) nephritis. Also pure physisorption can stabilize antibodies sufficiently, as exemplified by Laing et al. (2013) when spotting an AFP immunoassay by DPN on nitrocellulose slides. Physisorption was also relied on in the functionalization of 2PP-fabricated polymeric 3D microstructures via μCS (Mathew et al. 2024). Here, the stability of the antibodies on the microstructures was demonstrated by a secondary antibody staining. Also, streptavidin-biotin-mediated immobilization was utilized in antibody binding (Liu et al. 2020a, b), demonstrating that with direct delivery by μCS, different types of biotinylated antibodies can be bound into multiplexed arrays of biotinylated phospholipid membranes via a streptavidin linker. The utility and high sensitivity of miniaturized antibody spots were demonstrated by Lee et al. (2022) when using FluidFM printed capture antibody spots, enabling an amplification-free quantification of a neurological protein—disrupted-in schizophrenia 1 (DISC1), a schizophrenia-related biomarker of single cells.

29.3.2 Self-Assembly on Pre-patterned Structures

An often-used alternative to direct writing of antibodies is the generation of pre-patterned substrates that comprise specific binding sites for self-assembly of the desired antibodies in an additional incubation step. While adding an additional step, this approach can be advantageous as of the usually longer shelf-life of the pre-patterned samples, as the chemistry of the binding sites is stable under dry conditions and without the need for cooling, while the more sensitive antibodies are only added just-in-time before an immunoassay or experiment is conducted. Furthermore, this approach adds flexibility to applications, as the specific antibodies can also be chosen just before use and do not need to be selected already during production. However, a drawback of this approach is that it complicates multiplexing. To enable multiplexed self-assembly, each antibody type requires a unique binding motif (e.g., biotin and histidine tags), which limits the total number of multiplexed antibodies in an array. In principle, oligonucleotide-based tags can alleviate this limitation but introduce new complications in the more complex synthesis and conjugation of such functionalized antibodies. A schematic of the self-assembly approach for patterned antibody immobilization is given in Fig. 29.6.

Table 29.2 presents selected publications, exemplifying antibody immobilization based on substrates pre-patterned by SPL methods. One of the first examples of antibody binding on DPN-generated prepatterns is Lee et al. (2004). Here, arrays of 16-mercaptohexanoic acid (MHA) SAM-spots were fabricated by DPN on gold surfaces. To avoid unwanted adhesion of antibodies outside of the binding spots, the remaining gold surface is passivated by incubation with a PEG-thiol, thus backfilling it with a protein-repelling SAM. Incubated antibodies can then selectively bind to the MHA spots by electrostatic interactions. In this demonstration, anti-p24 IgG was immobilized on the MHA to build an HIV-1 detecting immunoassay with

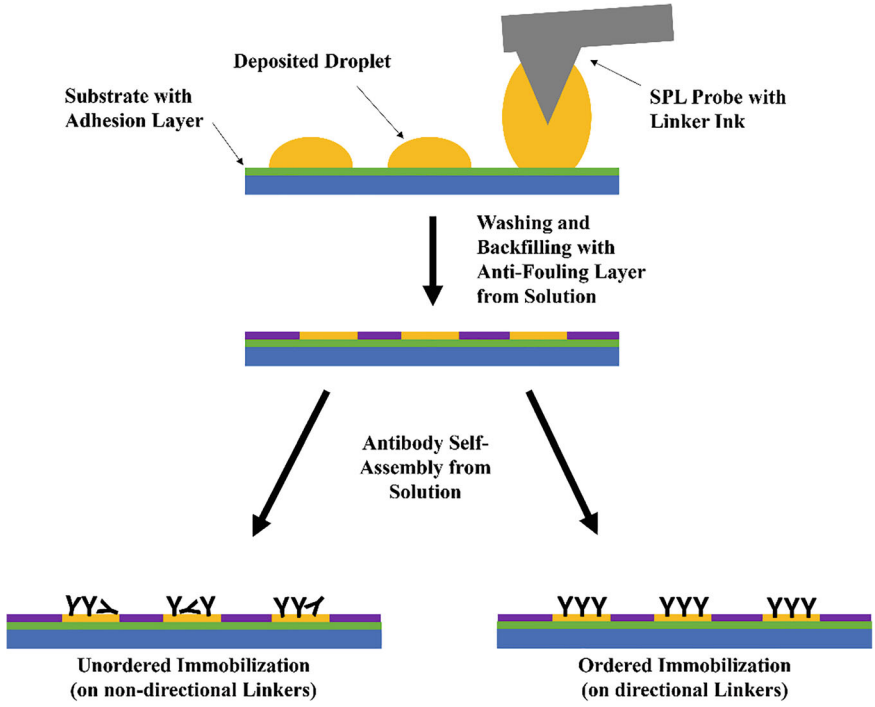

Fig. 29.6 Process scheme for immobilization of antibodies on SPL-generated pre-patterns. In the first step, SPL is utilized to deposit a linker ink onto a suitable functionalized substrate (adhesion layer that can chemically bind the deposited linker). Excess ink is washed away, and the areas between the linker spots are backfilled with a protein-repellent material from solution. Now, antibodies can self-assemble on the linker spots for immobilization. Depending on the used linker, either unordered or ordered immobilization will be achieved

Table 29.2 Literature examples for self-assembly of antibodies on pre-patterns fabricated via SPL methods

Publication	Method	Immobilization mechanism	Application
Lee et al. (2004)	DPN	MHA/PEG on gold	HIV detection
Lee et al. (2006)	DPN	NHS/PEG thiols on gold/protein A/G	Protein detection
Vega et al. (2006)	DPN	MHA/metal ions	Influenza A detection
Vega et al. (2007)	DPN	MHA/metal ions	Single-cell virus infectivity
Sekula et al. (2008)	DPN	Biotinylated lipids/streptavidin/biotinylated Abs	Technology demonstration
Lee et al. 2009	DPN	Biotin-silane/streptavidin/biotinylated Abs	Protein detection (TNF-α)
Liu et al. (2021)	DPN	Biotinylated lipids/streptavidin/biotinylated Abs	Capture of extracellular vesicles
Striebel et al. (2021)	μCS	Biotinylated polymer brush/streptavidin/biotinylated ABs	Macrophage sorting

nanoscopic feature-sizes. Similarly, also covalent immobilization can be induced by using an NHS-thiol pattern with PEG-thiol backfilling and subsequent binding of protein A/G to the NHS-thiol spots, which subsequently can selectively bind antibodies (Lee et al. 2006). Another option in this approach is to use the MHA spots as coordination sites for metal ions, which then can bind antibodies. This was first demonstrated by Vega et al. (2006), using DPN-generated MHA patterns to immobilize different antibodies via Zn^{II}-ions to detect influenza viruses. This

approach was also used to generate arrays of viral particles to study infectivity on a single-cell level (Vega et al. 2007). The approach was generalized to glass surfaces by silanization of the surfaces with (3-mercaptopropyl) trimethoxysilane (MPTMS) and subsequent fabrication of biotin arrays on the silane SAM via DPN (Lee et al. 2009). Then biotinylated antibodies could be bound over a streptavidin linker to selectively detect a cytokine (tumor necrosis factor-α (TNF-α)) on the array. Another way to open arbitrary surfaces for antibody functionalization via SPL is the use of biotinylated phospholipid membranes that allow for the immobilization of biotinylated antibodies over a streptavidin linker. These biomimetic phospholipid membranes can be created by DPN and allow even for multiplexed self-assembly by using nickel-chelating phospholipids as a second binding motif for histidine-tagged antibodies (Sekula et al. 2008). Alternatively, solely biotinylated phospholipids can be used, and the multiplexing realized by a direct-write approach via μCS (Liu et al. 2020a, b). In principle, this allows an arbitrary number of multiplexed antibodies without the need for additional orthogonal binding tags. The approach of supported lipid membrane (SLM)-based antibody arrays was successfully used in the selective capture of cancer-associated extracellular vesicles (EVs), which also showed that the phospholipid membrane-scaffolded antibodies show enhanced activity, probably because of directed presentation and remaining lateral mobility on the phospholipid membrane (Liu et al. 20,021). When excellent antifouling properties are required, SPL approaches can also be extended to more unconventional surfaces as polymer brushes. Striebel et al. (2021) demonstrated the selective capture of different macrophage subtypes on μCS-generated antibody arrays. As macrophages are extremely clingy to many surfaces, here the basis surface for the antibody microarray was a highly protein-repellant yet reactive polymer brush that allowed generation of biotin arrays via μCS on which the selective antibodies could be immobilized, avoiding unspecific adhesion of macrophages on the rest of the surface.

29.3.3 Device Functionalization

While there are very advanced lithography methods in the form of photolithography, e-beam lithography, and direct laser writing, which can produce 2D and 3D features in high resolution and build the basis for most modern electronic devices, these methods often struggle in terms of bio-functionalization, as of incompatible process steps that destroy delicate and fragile biological materials. Also, multiplexing can be challenging, because in conventional lithographic approaches, this often necessitates subsequent mask and lift-off processes, which again can compromise the functionality of previously applied materials. Another issue for post-functionalization of devices is the addressability of existing structures on a substrate, e.g., to apply an active material to a pre-fabricated sensor. A key feature of SPL methods is the high spatial control, allowing for highly localized deposition of inks to specific locations of a substrate and also to existing prestructures. This was used, e.g., to deliver phospholipid-based functionalizations to the rim of micro-goblet structures, which rendered them into whispering gallery mode (WGM) sensor devices for protein detection (Bog et al. 2013, Bog et al. 2014, Bog et al. 2015). The precision of DPN and PPL-like stamping approaches allowed to selectively deposit the functional ink to the rim sections of the micro-goblets and introduced biotin moieties that can be utilized for binding biotinylated antibodies and other proteins over a streptavidin linker. Another option to introduce biotin moieties is to generate needed microstructures already with intrinsic biotin functionalities, as, e.g., demonstrated by Berganza et al. (2022). Here, FluidFM was used to print polymeric microstructures (which can be extended to layer-by-layer 3D printing (Ventrici de Souza et al. 2018)) that bear biotin moieties on their surfaces by introducing biotinylated lipids into the polymeric ink, which align themselves at the interface of the printed structures and allow subsequent protein binding. Another example for direct-write delivery of antibodies and other

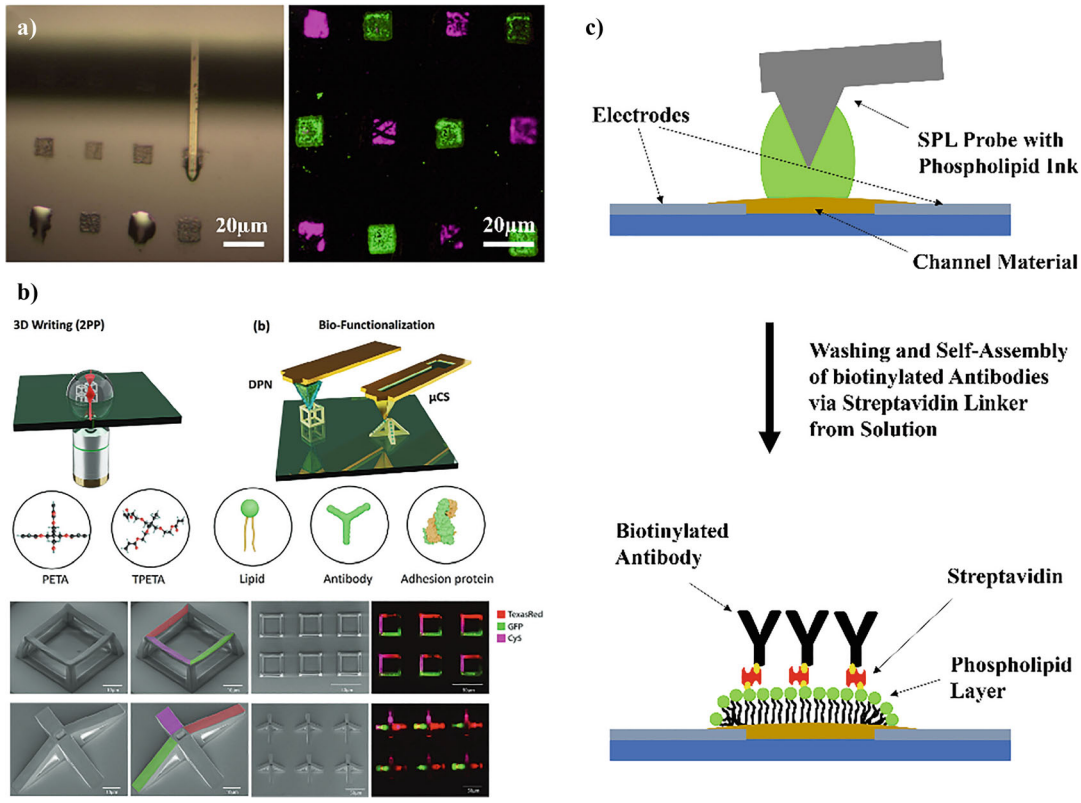

Fig. 29.7 Device functionalization with SPL methods. (**a**) Multiplexed deposition of fluorescently labeled antibodies via µCS on previously DPN-generated phospholipid patches. The left side of the panel shows an in situ image of the µCS deposition process, with the phospholipid squares in the background and the µCS tip in contact with the upper right phospholipid feature. On the bottom row, two already deposited droplets of antibody solution are visible. On the right side of the panel, a fluorescence microscopy image is given, which shows the immobilized antibodies (EpCAM antibody (green) and Annexin A1 antibody (purple)) on the phospholipid squares after washing away the excess solution (Adapted from Liu et al. 2020 under CC BY 4.0.). (**b**) Scheme of bio-functionalization of 2PP-written microstructures via DPN and µCS (upper panel), SEM images and fluorescence microscopy images of polymeric microstructures with multiplexed functionalization (Reproduced from Mathew et al. 2024 under CC BY 4.0.). (**c**) Scheme of fabrication of a biohybrid sensing device via SPL. Here, DPN was used to deposit a phospholipid membrane onto the channel material of a two-electrode device. Biotinylated antibodies can selectively self-assemble onto the lipid membrane from solution over a streptavidin linker. The resulting device can now sense binding of target analytes to the antibodies via electronic read-out by EIS

biomaterials to pre-fabricated 3D microstructures is given by Mathew et al. (2024). Here, DPN and µCS were used to deliver the biomaterials to 3D polymeric microstructures prior written by 2-photon polymerization (2PP), successfully bringing multiplexed functionality of DNA, phospholipids, adhesion proteins, and antibodies, respectively, onto the structures, and demonstrating their retained functionality in targeted protein binding and selective cell adhesion (Fig. 29.7).

The abilities of SPL methods to process sensitive biomaterials and to deposit them in multiplexed patterns render them suitable for the fabrication of hybrid bioelectronic devices (containing functional biological and electronical

components). A current example from our group is the functionalization of a printed biosensor for protein detection via electrochemical impedance spectroscopy (EIS). Here, an inkjet-printed two-electrode device with an indium oxide channel is functionalized with a biomimetic phospholipid layer via DPN. As described above, the phospholipid membrane comprises biotinylated binding sites that allow for the immobilization of antibodies over a streptavidin linker that can act as recognition elements. The bound target protein can be sensitively measured by EIS. Arrays of such devices can selectively be functionalized with phospholipid layers of different composition and with different antibodies to target several analytes in parallel, opening up new possibilities for miniaturized, low-analyte, and multiplexed diagnostic devices and applications.

29.4 Conclusions

SPL methods offer attractive properties for surface patterning and device functionalization with biomaterials. Their general mild process parameters are compatible with delicate and sensitive biological compounds such as proteins, including antibodies. Furthermore, the high spatial control and resolution in SPL allows for miniaturized immunoarrays and highly localized deposition of biomaterials on active sites of devices to endow them with desired functions, as, e.g., selective capture of analytes in sensor devices. A very promising route for future application will be the use of SPL in tandem or as a complementary technique to standard lithography approaches. This approach can achieve a best-of-both-worlds situation, where the precise anorganic and semiconductor material structures and devices generated by technologies such as photo- or electron beam lithography can be functionalized with biomaterials on demand and highly precise to the needed sites.

Conflict of Interest The authors do not declare any conflicts of interest.

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