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Biochemical surface patterning in microfluidic devices

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The capacity to pattern biomolecules within microfluidic devices expands the scope of microfluidic technologies. In such patterned systems, surface-bound components remain localized, while the microfluidic network supplies reagents and removes waste products. This approach has enabled continuous protein expression from patterned DNA, chemical synthesis from immobilized enzymes, and cell capture assays. Here, we review methods to pattern surfaces within microfluidic devices. Patterns may be printed before or after the device is assembled; pre-bonding methods are compatible with well-established open-surface patterning protocols but present challenges for device bonding and alignment. Conversely, post-bonding methods are compatible with standard bonding procedures but rely on less established, sequential patterning protocols. Future progress will require consistent reporting of pattern signal and noise relative to controls.

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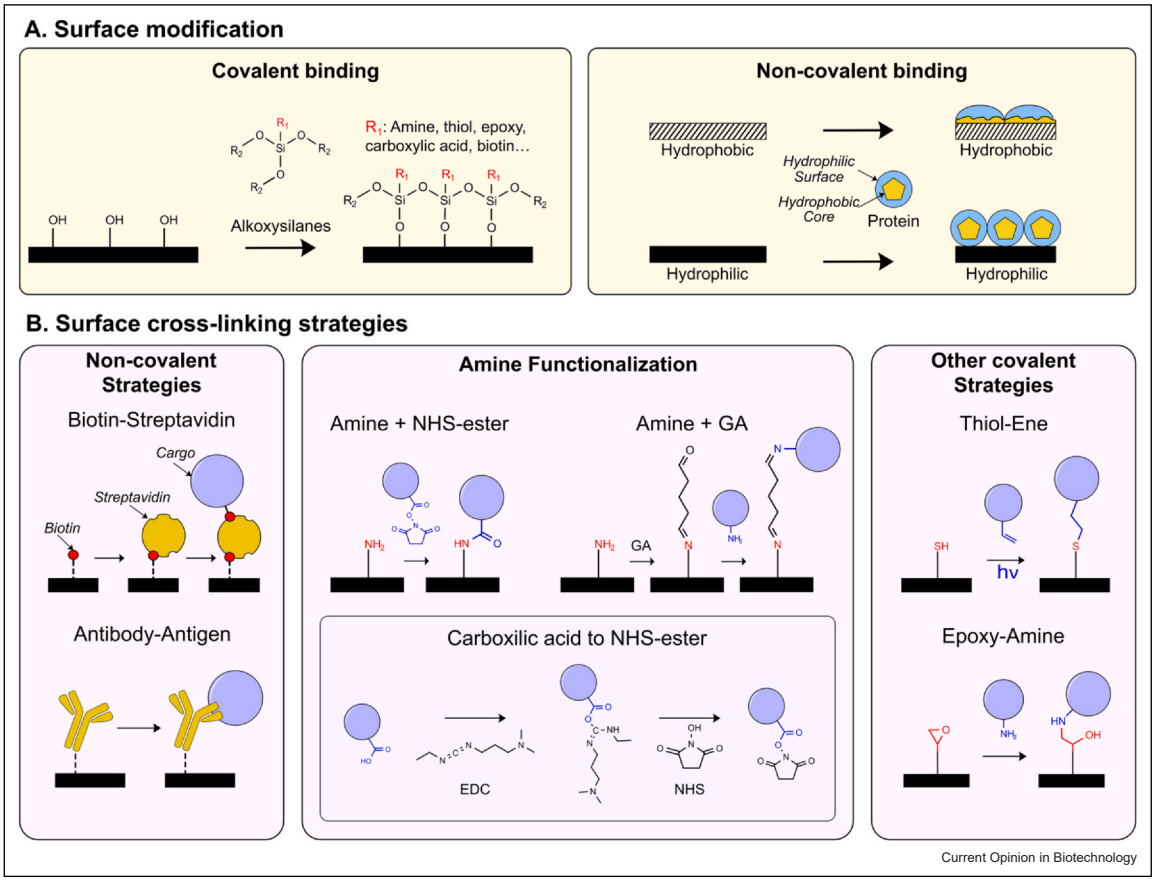
Introduction

In 1990, Manz and co-workers envisioned using micrometer-scale devices to perform laboratory workflows on a chip [1]. Since then, microfluidics has grown into a multidisciplinary field spanning medicine, life sciences, and chemical synthesis. The advantages of microfluidic technologies lie in miniaturization: at the micro-scale, viscous forces dominate over inertial forces, leading to controllable, laminar fluid flow. In addition, microfluidic devices are typically made of optically transparent materials such as glass and poly(dimethylsiloxane) (PDMS), enabling *in situ* microscope imaging. Miniaturization has another consequence: as channel sizes decrease, the surface-area-to-volume ratio (SA/V) increases, and with it, the influence of surface interactions. Microfluidic functions can therefore be engineered by spatially patterning microchannel surface reactivity [2].

Spatial organization in microfluidic devices is relevant to biosynthesis, biosensing, and cell-based assays. In natural biosynthetic pathways, enzymatic reactions are often compartmentalized in organelles, condensates, or multienzyme assemblies [3]. Enzyme compartmentalization is also useful for *in vitro* enzymatic cascades — for example, if enzymes have different optimal conditions, or to avoid cross-inhibition effects [4]. In some cases, enzyme localization also determines reaction output. For example, glycan synthesis in the Golgi apparatus is governed by the sequential addition (or removal) of monosaccharides; the spatial localization of only a few enzymes underlies the remarkable diversity of glycans [5]. From the perspective of biosensing, microfluidic devices containing patterned micro-arrays have been used to detect DNA, proteins, and cells. The high SA/V of microfluidic environments enables fast surface binding assays with low sample volumes [6–8]. Microfluidic devices containing cell patterns have been applied to support cell-based assays monitoring cell differentiation, cell–cell interactions in co-cultures, and responses to drug exposure [8–10]. Surfaces within microfluidic devices patterned with chemical gradients (including chemoattractants) have been used to study cell growth and migration, for example, by guiding the growth of neuron axons [11,12].

Here, we review methods to produce biomolecular patterns within microfluidic devices, emphasizing challenges unique to microfluidics and strategies developed to mitigate them. Fundamentally, surface patterning depends on creating a chemical reactivity contrast between patterned and non-patterned regions. Once this contrast has been established, downstream surface functionalization is modular: commercially available cross-linkers can be employed to tune pattern reactivity — for example, to convert an amine pattern into a biotin pattern. For this reason, we focus on *how* contrasts in surface chemistry are established, rather than their downstream pattern functionalization with biomolecules. Common microfluidic surface functionalization strategies are summarized in Figure 1 and reviewed extensively elsewhere [2]. Surface patterning within microfluidic devices has also been applied to control channel hydrophobicity. Although hydrophobic patterning lies outside the scope of this review, selected methods are included for their underlying patterning strategies. Lastly, the majority of the methods discussed concern devices fabricated from glass

Figure 1



Common microfluidic surface functionalization methods that can be applied to a patterned surface. **(a)** Left: Covalent modification of hydroxylated surfaces by silanization. Hydroxylated surfaces include glass, as well as plasma-treated silicon or polydimethylsiloxane (PDMS). Right: Noncovalent protein adsorption. Proteins adsorb strongly on hydrophobic surfaces through hydrophobic interactions, which may result in protein denaturation as tertiary structures are disrupted. In contrast, protein adsorption on hydrophilic surfaces is weaker and primarily mediated by hydrogen bonds and electrostatic interactions. **(b)** Common surface-to-cargo cross-linking strategies. Left: Noncovalent cross-linking strategies include biotin/streptavidin and antibody/antigen interactions. Center: Covalent functionalization of aminated surfaces. Cargoes modified with N-hydroxysuccinimide (NHS)-esters readily react with amine groups in slightly basic aqueous environments. Carboxylic acids (e.g. from proteins) are commonly reacted with 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and NHS to produce NHS-esters, which then covalently bind aminated surfaces. Alternatively, aminated cargoes can be cross-linked with homobifunctional linkers such as glutaraldehyde (GA). Right: Other covalent cross-linking strategies include photo-induced thiol-ene click reaction and epoxide reactions. Cargoes (purple circles) may represent DNA, proteins, vesicles, or cells, depending on the application. Some reactants and products are omitted for clarity.

and PDMS — this bias reflects the centrality of those materials in microfluidics.

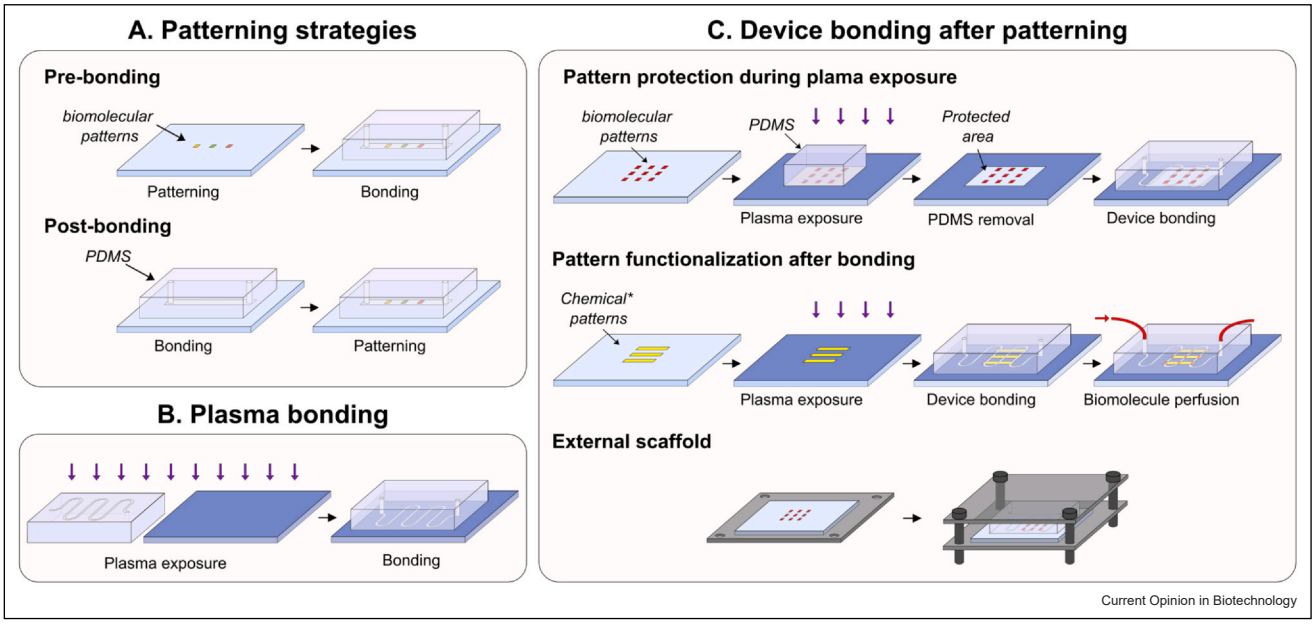
Patterning strategies requiring direct surface access

Microfluidic surface patterning strategies can be classified as pre- or post-bonding methods (Figure 2a). While pre-bonding strategies are compatible with patterning methods requiring direct surface contact, device assembly after patterning requires careful consideration.

Device bonding and alignment considerations

Microfluidic devices are typically assembled by exposing surfaces to an air or oxygen plasma in order to promote adhesion (Figure 2b). Unfortunately, plasma also degrades DNA and proteins [9,13]. Consequently, when surfaces are patterned before device bonding, strategies must be implemented to protect patterns. For instance, patterns can be protected by placing PDMS slabs (sometimes hollowed out) on top of patterns during plasma exposure (Figure 2c), although this method can lead to leaks in poorly-bound protected regions [8,14]. Alternatively, surfaces can be patterned with plasma-resistant reagents (such as silanes), which are then functionalized with biomolecules after the device is bound (Figure 2c) [15]. Lastly, plasma can be eliminated by assembling devices using external scaffolds, chemical adhesives, or lamination (Figure 2c) [13,16–18]. Another

Figure 2



Microfluidic device patterning and bonding considerations. **(a)** Comparison of pre- and post-bonding patterning strategies. **(b)** Plasma bonding: plasma-activated surfaces (purple arrows) form covalent bonds upon conformal contact, irreversibly sealing microfluidic channels. **(c)** Common strategies for binding microfluidic channels to patterned surfaces. Top: Use of a reversibly bound PDMS blocker to protect biomolecular patterns during plasma exposure. Middle: A chemical pattern (e.g. alkoxysilanes) withstanding plasma exposure is first patterned, and then functionalized with biomolecules after the device is sealed. Bottom: External, bespoke scaffolding systems can be used to bypass plasma bonding altogether. Plasma-activated surfaces are shown in dark blue.

difficulty in patterning before bonding is alignment, that is, aligning patterned features (typically transparent) to microfluidic architectures. To achieve this, stereoscopes have been used to align channels to pre-existing alignment marks on the patterned surface [19].

Droplet printing

Droplet printing is commonly used to pattern arrays on open surfaces. Liquid volumes as low as 10 pL are dispensed in arrays, typically on surfaces pre-functionalized with reactive groups (such as amine, epoxide, or aldehydes) (Figure 3a) [8,17,18]. Droplet printing is readily automated and well-suited for multipatterning, that is, patterning different biomolecules in different patterns. For example, droplet printing was used for on-chip DNA arrays and protein–protein interaction screens [13,20]. The resolution of droplet-printed patterns is typically in the range of 100 µm.

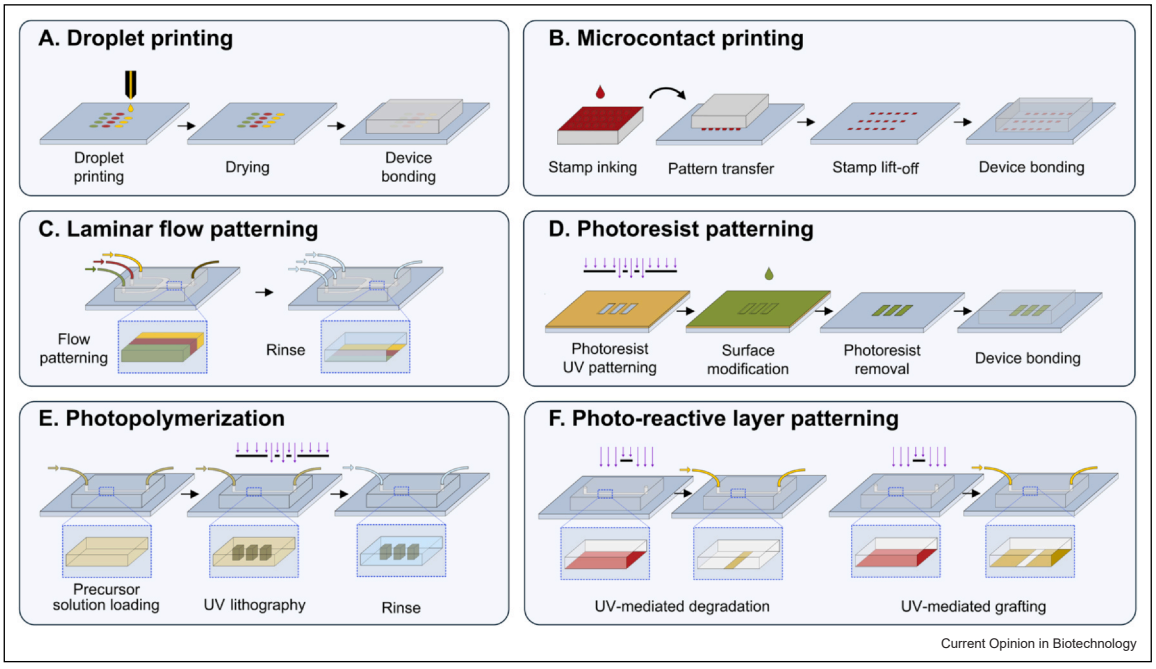
Microcontact printing

In microcontact printing (µCP), stamps with micron-sized features are inked, dried, and placed on surfaces to transfer patterns of molecules (Figure 3b) [21]. While accessible, µCP is incompatible with proteins prone to denaturation during the drying step. Instead, surfaces are often first patterned with chemical cross-linkers (e.g. EDC/NHS, alkoxysilanes), bound to devices, and proteins immobilized inside sealed channels [7,22,23]. When using µCP, the stamp can be used to protect patterns during plasma exposure [7,24]. µCP pattern resolution is limited by stamp microfabrication: ~200 nm patterns were printed with a stamp manufactured using electron beam lithography (Figure 4a,b) [7]. µCP is not well-suited for multipatterning, as each stamp delivers a single ink and is manually aligned to surfaces. This limitation can be overcome by combining µCP with other methods, such as laminar flow patterning (LFP) [23].

Plasma micropatterning

Plasma micropatterning (PmP) is similar to µCP in that a PDMS slab with micron-sized features is reversibly placed on a surface to transfer patterns. The difference lies in the function of the PDMS chip: in PmP, the PDMS partially protects the surface during plasma exposure. Plasma treatment removes any organic residues and introduces reactive groups on surfaces. PmP methods can be classified as constructive or destructive. In constructive methods, molecules such as alkoxysilanes selectively bind plasma-treated areas [25]. In destructive methods, plasma removes organic molecules outside patterns — for example, layers of poly (ethylene glycol) (PEG)-polyacrylamide or cyclodextrin [9,26]. As with µCP, PmP pattern resolution is limited by PDMS microfabrication, with patterns typically in the 10–100 µm range.

Figure 3



Summary of microfluidic surface patterning methods. **(a)** Droplet printing: a liquid dispenser prints surface arrays, which are then enclosed within microfluidic channels. **(b)** Microcontact printing (μCP): a PDMS stamp transfers an ink to the surface, which is integrated into a device. **(c)** Laminar flow patterning (LFP): different inlets supply surface-reactive reagents, coating predefined areas defined by the flow. **(d)** Photoresist patterning: a photoresist is patterned by standard lithography to selectively protect the surface from a secondary treatment (green). The photoresist is then removed to reveal the final patterns. **(e)** Photopolymerization: a precursor solution containing photo-reactive monomers is loaded into the device; UV exposure polymerizes the monomers into solids or gels, which are retained as the unreacted precursor solution is rinsed out. **(f)** Photo-reactive surface layers (red): surfaces are patterned via UV-mediated degradation (left) or binding (right), followed by loading of secondary reagents (yellow) to coat unexposed (left) or exposed (right) regions.

Patterning using microfluidic device features

Patterning surfaces within sealed devices offers a means to bypass bonding and alignment issues. In particular, flow-based patterning methods rely on microfluidic components themselves.

Laminar flow patterning

In microfluidic devices, fluid flow is predominantly laminar, so flow rates and device geometries determine fluid streamlines. Surfaces can thus be patterned by transporting surface-binding reagents to specific regions of the microfluidic device (Figure 3c). For example, fluid streams converging in a channel have been used to deposit parallel lanes of bovine serum albumin (BSA) (Figure 4c,d). Similarly, rectangles have been produced by sequentially patterning lanes in two directions using 'crossed' devices (Figure 4e,f) [27]. LFP is accessible and versatile: proteins, alkoxysilanes, and collagen hydrogels have been patterned using this method [28]. Although more complex devices have been proposed (with eight parallel streams or 5×5 crossed lanes), patterns are limited to simple shapes, with dimensions in the 10–100 μm range. [8,10]. Another drawback is reagent diffusion between streams, producing gradients at pattern edges instead of sharp boundaries [27]. Interestingly, the combination of LFP with gradient generator modules has been used to produce continuous surface gradients [29,30]. For applications requiring sharp boundaries, diffusion can be reduced with surface passivation or buffer-only streamlines between patterned lanes [10].

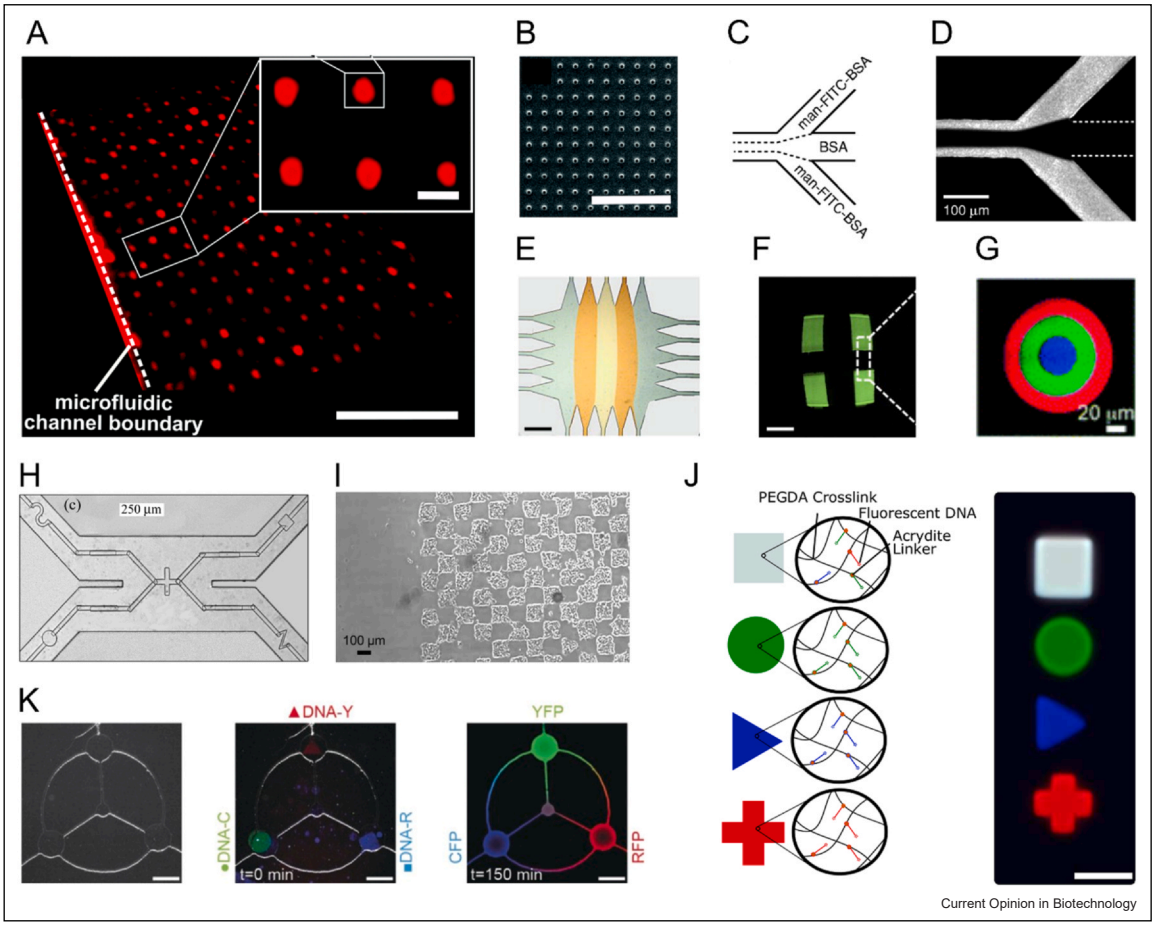
Button valves

Valves can be used for patterning by restricting reagent transport to patterned areas. In particular, circular patterns have been produced using PDMS button valves that deform and press down on channel surfaces when actuated [20]. Concentric protein rings were obtained by sequentially flowing in reagents and releasing the valves (Figure 4g) [31]. Compared to LFP, patterns can be produced in discrete locations, at the cost of more complex microfabrication to integrate valves. LFP pattern resolution is also in the 10–100 μm range.

Photopatterning

Contact-dependent patterning strategies are restricted to pre-bonding methods and flow-based methods to post-bonding methods. In contrast, photopatterning does not

Figure 4



Examples of surface patterning within microfluidic devices. **(a)** Confocal micrograph of µCP-patterned (3-Aminopropyl)triethoxysilane (APTES) spots functionalized with fluorophore-tagged IgG. Scale bar: 5 µm (500 nm in the insert) and **(b)** Scanning electron micrograph of the nano-imprint stamp used to pattern APTES [7]. Scale bar: 5 µm. **(c)** Laminar flow patterning microfluidic device used to pattern mannose-FITC-BSA and BSA in parallel lanes and **(d)** Fluorescence micrograph of the resulting mannose-FITC-BSA patterns. Dashed lines represent the microfluidic device boundary [27]. Copyright (1999) National Academy of Sciences, U.S.A. **(e)** LFP crossed patterning device. Vertical fluid flow is visualized using dyes, scale bar: 500 µm. **(f)** Fluorescence image of Huh7 cells tagged with green fluorescent protein patterned with the crossed LFP device; fibronectin was patterned in one direction, followed by Huh7 cells in the other. Scale bar: 100 µm. **(g)** Fluorescence micrograph of three fluorophore-tagged proteins patterned with a button membrane [31]. Scale bar: 20 µm. **(h)** Brightfield image of thin, 25 µm-wide PEG-DA hydrogel micro-structures photo-polymerized within a microfluidic device. **(i)** Phase contrast micrograph of HT-29 cancer cells immobilized on patterned poly(ethylene glycol) diacrylate (PEG-DA). **(j)** Left: Diagram of sequentially patterned PEG-DA hydrogels containing different fluorophore-tagged DNA strands. Right: Fluorescence microscopy composite image of the resulting structures [34]. Scale bar: 100 µm. (k) Photo-patterned fluorophore-tagged DNA and on-chip gene expression. Left: brightfield micrograph of the microfluidic device. Middle: Fluorescence overlay of DNA patterns in chambers. Right: gene expression from DNA brushes [19]. Scale bar: 300 µm.
(b) Used with permission of the Royal Society of Chemistry, from Ref. [7]. **(f)** Reprinted from Ref. [10], with the permission of AIP Publishing. **(h)** Used with permission of the Royal Society of Chemistry, from Ref. [32]. **(i)** Reprinted from [33], with the permission of AIP publishing.

require direct surface access or microfluidic features, and can therefore be performed before or after device bonding.

Light projection equipment

In photopatterning, patterns are transferred by projecting light (usually UV) onto photo-sensitive surfaces, typically with a resolution of 1–10 µm. To project patterns, mask-based and maskless methods have emerged. In mask-based methods, photomasks are placed between UV sources and substrates, for example, using commercial mask aligners [16,35]. Alternatively, masks can be placed in the optical path of microscopes (which can be equipped with a UV source), and the microscope stage used to localize projected patterns within microfluidic features [19,33,36,37]. Maskless methods are more flexible because different patterns do not require different photomasks. Microfluidic devices have been patterned using digital micromirror devices (DMD)

and direct laser writing [32,38]. These systems are often equipped with cameras, which can align patterns within microfluidic features. DMD systems can also be integrated with microscopes, enabling precise, automated alignment [34].

Photoresist patterning

Photoresists are photo-sensitive materials commonly used to pattern surfaces by photolithography. Photoresist patterns can protect selected areas from surface modification and can later be removed with solvents to reveal modified and unmodified regions (Figure 3d). Photoresists have been used to pattern poly-L-lysine, biotin-PEG, alkoxysilanes, and graphene oxide [15,16,39]. In contrast with the other photopatterning strategies, photoresist patterning has only been used in pre-bonding methods, perhaps because the solvents may interact with the PDMS.

Photopolymerization

Microfluidic channels can be patterned through UV-induced polymerization of acrylic acid or acrylamide into solid poly(acrylamide) (pAM) or poly(acrylic acid) (pAA). To do so, PDMS channels are first coated with the photoinitiator benzophenone, then loaded with precursor monomer solutions, exposed to UV, and rinsed to remove unpolymerized monomers (Figure 3e) [40]. The resulting patterns contain carboxylic acids (for pAM) or amine groups (for pAA), which can bind biomolecules, for example, by EDC/NHS cross-linking [30,41]. Polymerization is inhibited by O_2, so unless reactions are carried out under an inert atmosphere, pattern boundaries form gradients instead of sharp edges [41,42].

Similarly, hydrogels can be patterned in microfluidic devices through the photo-induced polymerization of PEG diacrylate (PEG-DA) (Figure 4h,i). PEG-DA precursor solutions typically contain water-soluble photoinitiators such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), but can also contain enzymes, which become trapped in hydrogels after polymerization [35]. To avoid diffusion out of hydrogels, biomolecules can be covalently bound to precursor monomers. For example, proteins have been bound to monomers by disulfide coupling [43]. In addition, DNA modified with acrylate groups can covalently bind hydrogels during polymerization [34]. Silane-acrylate has also been used to covalently bind PEG-DA hydrogels to channel surfaces [32]. PEG-DA patterning with LAP is O_2-tolerant and occurs in aqueous buffers, making it well-suited for precise, sequential multipatterning within microfluidic devices (Figure 4j) [34].

Monolayer photopatterning

Biomolecules can be patterned in microfluidic devices with photo-reactive monolayers. In some cases, light exposure triggers surface binding (Figure 3f). For example, a surface-bound monolayer containing benzophenone was synthesised to bind aminated DNA upon UV exposure [6]. Photo-induced binding is typically carried out using thiol-ene click chemistry, with surface-bound thiolated alkoxysilanes binding ene-containing reagents upon UV exposure [44]. Another grafting method relies on visible light: fluorophores are bleached using microscope lasers, producing reactive groups that then mediate surface binding, for example, to pattern fluorescein-4-biotin in BSA-coated channels [37,45].

In contrast with photo-induced binding, UV light has also been used to remove reagents (Figure 3f). In some cases, high UV doses are used to non-specifically degrade proteins or organosilanes in exposed areas [46,47]. In other cases, lower UV doses are used to remove photocleavable groups (such as 2-nitrobenzyl derivatives). Photocleavable groups can be used to pattern specific chemical groups. For example, 2-nitrobenzyl-protected carboxylate groups were patterned to render the microfluidic surface partially hydrophilic [48]. Similarly, patterned Nvoc-protected amine groups could selectively bind NHS-functionalized linkers [49]. Lastly, DNA containing photocleavable linkers could selectively capture DNA molecules on surfaces (Figure 4k) [19].

Conclusion

Pre-bonding patterning facilitates loading and rinsing steps [50] and is well-suited for multipatterning (in particular using droplet printing). It is, however, difficult to precisely align the resulting patterns with microfluidic features when sealing devices. Post-bonding patterning, in contrast, enables precise pattern positioning within microfluidic channels, at the cost of direct surface access, which hinders rinsing steps and restricts multipatterning to biocompatible and orthogonal reagents that are sequentially loaded, patterned, and rinsed.

Characterizing surfaces within sealed microfluidic devices is challenging: common surface analysis methods such as X-ray photoelectron spectroscopy, atomic force microscopy, or time-of-flight secondary ion mass spectrometry require direct surface access. While surface-enhanced Raman spectroscopy does not require direct access, it requires metal substrates and complex data analysis [51]. Patterns within microfluidic devices are instead typically characterized by fluorescence microscopy. Fluorescent probes, however, rarely verify the surface chemistry, and we note that fluorescence values are rarely reported, calibrated, or compared to appropriate controls. Key estimations — including biomolecule density inside patterned areas, nonspecific binding outside patterns, and pattern stability — are also underreported. Future efforts in standardizing measurements would enable more systematic protocol comparisons.

Author contributions

Kate Collins: Conceptualization, Writing. **Claire E Stanley:** Supervision, Editing. **Thomas E Ouldridge:** Supervision, Editing.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare no conflict of interest.

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