Surface patterning of bonded microfluidic channels

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Microfluidic channels in which multiple chemical and biological processes can be integrated into a single chip have provided a suitable platform for high throughput screening, chemical synthesis, detection, and alike. These microchips generally exhibit a homogeneous surface chemistry, which limits their functionality. Localized surface modification of microchannels can be challenging due to the nonplanar geometries involved. However, chip bonding remains the main hurdle, with many methods involving thermal or plasma treatment that, in most cases, neutralizes the desired chemical functionality. Postbonding modification of microchannels is subject to many limitations, some of which have been recently overcome. Novel techniques include solution-based modification using laminar or capillary flow, while conventional techniques such as photolithography remain popular. Nonetheless, new methods, including localized microplasma treatment, are emerging as effective postbonding alternatives. This Review focuses on postbonding methods for surface patterning of microchannels. © 2010 American Institute of Physics.

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I. INTRODUCTION

The ability to locally modify the chemistry of surfaces has facilitated many biomedical and chemical applications. Reagents and samples can be precisely arranged on planar surfaces using methods such as inkjet printing, photolithography, and plasma treatments. However, microchannels are far less amenable to the patterning methods employed for planar surfaces. Channel geometries and designs vary greatly and can be extremely complex, incorporating a variety of structures from junctions for droplet formation^{1,2} to multichannel networks^{3,4} with tailored surface properties.^{5–8} The next generation of microchips will incorporate more diverse surface functionalities in already complex geometries, requiring advanced methods for patterning the internal surfaces of the microchannels. Conventional surface modification methods which require close proximity to the substrate are restrictive when applied to microchannels due to the requirement to seal the microchip after patterning. To overcome these bonding restrictions, postbonding surface modification techniques are being developed, and are the focus of this Review.

Prebonding modification is effective for selected systems; however, in many cases bonding conditions damage the surface treatment, e.g., thermal and plasma bonding. An alternative is pressure sealing, where the upper and lower components of the chip are sandwiched in a holder (e.g., Refs. 8–11). As this method of channel sealing does not require surface preparation and is carried out at ambient conditions, this method is suitable for any type of surface modification, provided a high pressure seal is not required. Various adhesives may also be carefully introduced as a thin film between the plates and cured, providing a strong bond.^{12–14} In other cases, contact bonding with careful surface preparation has been used to bond PyrexTM glass and quartz microchips;^{15–18} however, these procedures risk damage to sensitive surface chemistries. Thus, the

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FIG. 1. Alternative fabrication pathways to chemically or biologically patterned microchannels. The prebonding pathway faces challenges maintaining the desired functionality during bonding and aging (where microchips are stored prior to application, e.g., commercial chips). The postbonding pathway offers the noted advantages, provided postbonding methods are available, efficient, and effective.

push toward bonding under milder conditions is driven by the acute understanding that existing bonding techniques can limit the potential diversity of surface chemistry and patterning and, therefore, chip functionality.

Postbonding and prebonding surface modifications represent two alternative pathways for chip fabrication, as outlined in Fig. 1. In principle, postbonding modification of microchannels has a number of favorable characteristics over prebonding methods. The first has been alluded to already and is the ability to use existing, highly effective bonding methods, especially for glass^{14,16–20} and poly(dimethylsiloxane) (PDMS).²¹ In many cases, postbonding modification can be carried out immediately prior to microchip use, as a value-adding customization step, avoiding aging of the surface chemistry in the time between fabrication and application. This is particularly relevant to PDMS chips, which tend to lose their functionality with storage time, depending on ambient conditions and the specific modification method.²² Postbonding modification also circumvents potential alignment problems, where chemical patterns are required at specific locations within the microchannel network. In addition, some postbonding methods permit all four microchannel walls to have the same chemistry, which is important in some applications, e.g., for Laplace pressure valves.^{23,24} Postbonding techniques also offer the reuse of microfluidic devices that are expensive or difficult to fabricate, where internal surface functionality can be chemically stripped from the channel walls under flow and retreated accordingly.^{10,11}

Modification methods often require access to the surfaces that are to be patterned and, while open channels can be modified using a host of methods, the treatment of bonded channels is not straightforward. For example, high resolution UV-photolithography requires contact or close proximity between the mask and the surface to be patterned or a highly collimated UV source. A range of alternative methods has now been developed to modify internal channel surfaces after bonding, which ensures that the treatment maintains its functionality for the desired application. Most of these methods draw on knowledge from conventional techniques of surface modification, with

some degree of adaptation to microscale confinement, e.g., photolithography^{25–27} or microplasmas,^{28–31} while other methods are emerging which take advantage of specific physical behavior in microchannels, e.g., laminar and capillary flow.^{26,27,32,33}

This Review discusses prominent examples of postbonding channel patterning that are, or have the potential to become, routine tools for the microfluidics community. The discussion is organized as follows. Section II briefly describes the nature of microchannels as substrates for surface modification to provide a general context. In particular, common microchip bonding methods and how they can dictate microchannel surface chemistry is highlighted. Section III considers in more detail a range of methods now available for postbonding surface patterning, namely, laminar flow and capillarity (Sec. III A), photolithography (Sec. III B), microplasmas (Sec. III C), and electrochemical biolithography (Sec. III D). Finally, a summary of these approaches and a perspective on potential developments and future challenges is presented.

II. MICROCHANNEL SUBSTRATES

The existing surface properties of microchannels play an important role in determining appropriate patterning methods. The sheer number of microchip materials and surface chemistries prohibits an exhaustive account of homogeneous surface modification here, and is adequately dealt with elsewhere.^{5,7} Instead, the following briefly outlines the context for the patterning techniques described in Sec. III.

The many materials employed in chip fabrication provide a diverse range of native chemistries for anchoring surface modification. PDMS persists as the most popular choice of chip material in the literature due to its low cost, ease of fabrication, rapid prototyping, transparency, and other benefits such as oxygen permeability.²² However, for some applications PDMS is far from ideal. The material exhibits an inherent hydrophobicity,³⁴ is swelled/leached by organic liquids,³⁵ and is not suited to high pressure applications. Therefore, glass chips are often employed for applications where the channel's physical and chemical robustness is crucial.

PDMS microchips are typically bonded by pretreating with oxygen plasma before contact,²¹ while glass is bonded at high temperature (up to 650 °C for Pyrex^{19,36} and 1080 °C for quartz²⁰). Pre-existing surface chemistry based on binding organic species at the channel walls (e.g., self-assembled monolayers) is oxidized or entirely removed during these bonding processes. Thus, regardless of the choice between the most common chip materials (glass or PDMS), the chemistry of the microchannel walls after bonding is dominated by hydrophilic silanol groups (Si–OH), although aging of PDMS to its inherent hydrophobicity has been well-documented.²²

Aging of PDMS surface chemistry is usually problematic, with exceptions for particular patterning techniques,³⁷ and cases where a hydrophobic channel is required and solvent swelling can be averted, e.g., using fluorinated oils.³⁸ To render PDMS hydrophilic, Abate *et al.*³⁹ used a sol-gel method in which microchannel walls were sealed by a silica layer. The authors demonstrated good resistance to swelling by organic solvents and diffusion of dye (rhodamine B) into the PDMS. Where other inorganic chemistries are preferred, sol-gel chemistry can also deposit transition metal oxides (titania, zirconia, and vanadia) on PDMS microchannels.⁴⁰ Metal oxides are also potential candidates for patterning channels prior to thermal bonding of glass substrates, based on experiments on planar substrates.⁴¹

For postbonding modification, the silanol groups exposed at the PDMS or glass surface can be used to covalently bind alternative chemistries at the interface. For example, an organic phase containing a chlorosilane can be introduced either by pressure driven flow or capillarity. The result is a self-assembled monolayer (SAM) at the interface, with an alkyl chain (with the desired functional group) exposed at the microchannel walls.^{23,42,43} Depending on their functionality, SAMs and other thin organic layers provide resistance to biofouling,^{44–46} a wettability contrast,^{26,32} or intermediates for surface patterning.^{25,26}

The silanol groups present at glass and oxidized PDMS surfaces lead to a native negative surface potential in aqueous phases, except at very low pH (the point of zero charge for silica is $pH \sim 2-4$).⁴⁷ The surface potential of microchannels is important for electrokinetic phenomena and, therefore, controlled modification of the surface chemistry is very necessary.^{5,48} Polyelectro-

lyte adsorption from aqueous solution is an effective means to alter the surface potential of a channel.⁴⁹ Inorganic materials (metal oxides) are also good candidates for altering the surface potential as the point of zero charge differs depending on the oxide present. Furthermore, inorganic materials can provide a combination of physical and chemical modifications via deposition of particles from solution on the microchannel walls.^{50,51}

Modification of surfaces that exhibit functional groups typically found on microchannel walls is relatively straightforward for plates and open structures. However, the nature of bonded microchannels demands a more complex approach to channel patterning. Future microfluidics applications will require much greater control over bioactivity, wettability, roughness/structure, surface potential, and stimuli response. In many cases, these channels will be functionalized postbonding to preserve sensitive biomaterials and chemistries. Section III highlights existing approaches to postbonding patterning of channels. Some are well established, while others are emerging techniques that are good candidates for further development.

III. PATTERNING BONDED CHANNELS

Surface patterning of bonded microchannels presents a number of key challenges, depending on the desired application. For some applications, not all walls within the three-dimensional microchannel must be patterned, e.g., specific binding sites for protein detection,⁵² while other applications require all surfaces to be modified, e.g., a Laplace valve.^{23,24} Furthermore, the connectivity of the surface patterns is an important consideration. For example, laminar flow can be directed using a continuous stripe, which is preferentially wet by the liquid of interest,²⁶ whereas a discontinuous hydrophilic patch can be used for fusion of water droplets.³⁷ Thus, a suite of techniques is now available for engineering regions of surface functionality in microfluidic chips, each with key characteristics that should be carefully considered. The following reviews four approaches: laminar flow and capillarity, photolithography, microplasmas, and electrochemical biolithography. The discussion is limited to surface *patterning of bonded or sealed* microchannels.

A. Laminar flow and capillarity

For wet chemical treatments of bonded microchannels, the introduction of reagents must be via flow through the channels themselves. Homogeneous modification is therefore straightforward; liquid is introduced by capillarity (Laplace pressure) or external pressure (pumping) and allowed to react or adsorb at the channel walls. However, exclusive modification of discrete regions within the channel is more challenging.

Laminar flow surface patterning by wet chemistry has been used to prepare parallel features along microchannels.^{26,27,33,53} The process takes advantage of the lack of mixing (by diffusion) over the short time-scales that a liquid resides in the channels. Kenis *et al.* demonstrated in-channel surface patterning as small as 5 μm using selective etching, surface reactions, and precipitation controlled by “fabrication using laminar flow”, which the authors called FLO.⁵³ The FLO method is suitable for a wide range of chemical treatments, including polyelectrolyte adsorption,^{54,55} various organic SAMs,²⁶ and inorganics.³³ Zhao *et al.* used streams of octadecyltrichlorosilane (OTS) in an organic solvent and OTS-free streams to pattern glass microchannels with hydrophobic stripes, as shown in Fig. 2. By configuring the channels and streams in different ways, various patterns could be prepared to guide liquids in parallel flow. The strength of the wettability contrast between the OTS treated and untreated regions determines the maximum pressure drop across the liquid-liquid interface (Laplace pressure) that can be handled before liquid escapes the surface guides.

The FLO technique is not limited to continuous structures, despite the continuity of the treatment streams. Sequential surface treatment of channels using laminar streams also yields discontinuous features, as shown by Kenis *et al.*⁵³ Figure 3 shows their multistep laminar flow method for creating discontinuous surface features in a microchannel. In this case the surfaces were patterned with gold and silver electrodes. A gold electrode (deposited prebonding) was selectively etched by a central stream of etch solution, flanked by streams of pure water. Deposi-

FIG. 2. *Left*: two schemes for laminar flow patterning using octadecyltrichlorosilane solution and silane-free solvent to form chemically patterned microchannels. *Right*: corresponding flow configurations of water (containing rhodamine B dye). The aqueous phase follows the untreated (hydrophilic) paths through the microchannel. Reprinted with permission from B. Zhao *et al.*, Science, 291, 1023, 2001. AAAS.

tion of the reference electrode was then carried out by parallel flow of the two components of an electroless silver plating solution. Where the streams met, a silver wire formed which was shown to be electrically conductive.

Where existing channel geometries are able to pin the contact line or direct liquids via Laplace pressure difference, capillarity can be used to spatially control surface modification.^{24,32} Hibara *et al.* used silane chemistry and capillarity to selectively hydrophobize one channel of a multichannel gas-liquid extraction chip.³² The authors employed a step in the channel geometry to guide liquid containing a hydrophobizing agent (OTS) along only one of the channels during capillary filling. Hydrophobicity of the channel (for the gas phase) ensured stability of the liquid-gas interface due to the Laplace pressure that opposes entry of the aqueous phase (leak pressures were several kilopascals). However, the use of capillarity to chemically pattern channels in this way is usually accompanied by pinning sites that guide the progress of the liquid front during patterning and, furthermore, is limited to patterns that are connected to at least one inlet.

Where capillary and laminar flow modification of microchannels is possible, the method is extremely simple, requiring standard pumping systems (or none at all) and wet chemistry; however, the complexity of the surface patterns is quite limited, as noted by Zhao *et al.*²⁷ Other techniques have therefore been developed to complement capillary and laminar flow patterning, which are discussed in Sections B to D.

B. Photolithography

Photolithography is a routine tool for creating microscopic features (physical and chemical) on, usually, planar substrates such as silicon wafers. The method is also suited to microchannel modification. The process occurs via at least three basic steps: application of a photosensitive layer, masking a pattern, and UV irradiation through the mask. Depending on the type of photosensitive layer, development may be required to realize the pattern, e.g., in photoresist.

As homogeneous photosensitive layers are easily applied to channels via capillary or laminar flow and microchips are generally transparent to long-wavelength UV, photolithography is well-suited to microchannel patterning. Zhao *et al.*²⁷ employed silane SAMs with the photocleavable *o*-nitrobenzyl functionality to pattern bonded microchannels. UV irradiation (320 nm) was applied through various masks to cleave the nitrobenzyl-oxygen bond, yielding regions of hydrophilic carboxylic acid groups at the microchannel surface. Liquid could then be directed by the “virtual

FIG. 3. A sequence of surface treatments (etching and deposition) to generate gold and silver electrodes in a bonded microfluidic channel. The step-wise patterning method is illustrated to the left, with the outcome shown right. Reprinted with permission from P. J. A. Kenis *et al.*, Science, 285, 83, 1999. AAAS.

walls” of the wettability pattern within the confinement of the channel. The complexity of these patterns is only limited by the mask design and the resolution of the lithography technique, cf. Fig. 4. Furthermore, the ability to precisely control immiscible liquid phases in this way has enabled the preparation of polymer membranes at microfluidic junctions.⁵⁶

The photolithographic approach to channel patterning is not limited to photocleavage. Photo-initiated grafting and polymerization have also been demonstrated in the confinement of a microchannel.^{25,57–60} Photografting to thiol-terminated SAMs was used by Besson *et al.*²⁵ to reduce the required exposure time for patterning wettability gradients in channels. One advantage of grafting is the ability to graft different functionalities without changing the primary surface treatment, as shown by Hu *et al.*⁵⁸ Elsewhere, photoinitiated grafting of poly(acrylamide) was used to activate PDMS microchannels for bioconjugation of protein (casein) to selected regions of

FIG. 4. [(a) and (b)] Patterning of photocleavable SAMs (i.e., containing the photocleavable 2-nitrobenzyl group) using UV irradiation. Cleaved regions are rendered hydrophilic in contrast to the uncleaved hydrophobic SAM. [(c) and (d)] Pattern complexity is only limited by the mask design and the resolution of the technique, allowing the unusual designs shown. Reprinted with permission from B. Zhao *et al.*, Science, 291, 1023, 2001. AAAS.

the channel.⁶⁰ Coupling this method with laminar flow exhibiting a lateral concentration gradient of two labeled proteins (casein and collagen), the authors demonstrated the preparation of a protein gradient across the microchannel.

Although it is often the case, photoresponsive layers need not always be self-assembled organics for photopatterning. Inorganic materials such as titanium dioxide are good candidates for their photocatalytic properties. Photocatalytic decomposition of thin organic films deposited (covalently or otherwise) on TiO₂ is well-known.^{61,62} Titanium dioxide may be sputtered on PDMS microchannels prior to plasma bonding; however, Tuantranont *et al.*⁶³ showed that the resulting layer exhibits cracking and the PDMS surface still ages to its hydrophobic state. For other materials, prebonding modification by TiO₂ is suitable but requires careful alignment of the two halves of the microchip.⁶⁴ Therefore, alternative approaches have been developed for bonded microchannels. The introduction of TiO₂ nanoparticle dispersions into a silica microchannel can be used to prepare a photocatalytic TiO₂ layer.²³ This layer can then be treated with an alkylsilane, e.g., octadecyltrichlorosilane,²³ to render the surface hydrophobic. Subsequent UV irradiation oxidizes unmasked regions of the hydrophobic organic layer, rendering the exposed regions hydrophilic. The roughness created by the TiO₂ particles at the interface further enhances the wettability contrast, allowing the channel wettability to be tuned from superhydrophobic to superhydrophilic, e.g., for a highly effective Laplace valve.²³ Furthermore, a reversible flow switch also based on photocatalysis at the TiO₂ surface has been presented.⁶⁵

Titanium dioxide films may also be used to write metal nanoparticles on a microchannel wall after bonding, as shown by Castellana *et al.*⁵² A glass slide was coated with a 6 nm TiO₂ layer using chemical vapor deposition and bonded to a PDMS plate with 300 μm wide microchannels. When irradiated with UV (365 nm) through a mask in the presence of silver nitrate solution (0.1

FIG. 5. (a) Silver patterning with UV irradiation of 300 μm wide TiO_2 -coated microchannels in silver nitrate solution. The pattern shown is generated through a photomask. Adapted with permission from E. T. Castellana *et al.*, *Anal. Chem.*, 78, 107, 2006. © 2006, American Chemical Society. (b) Regions patterned with silver nanoparticles, as shown in (a), can be used to achieve selective binding of proteins. Channels with regions of biotin-PEG and DNP-PEG modified particles are shown, where PEG and DNP refer to polyethylene glycol and 2,4-dinitrophenyl functionalities, respectively. Channels from left to right: addition of pure streptavidin (red), a mixture of streptavidin/anti-DNP (red/green), and pure anti-DNP (green) to three microchannels (oriented vertically) resulted in selective binding of the fluorescently labeled proteins. Reprinted with permission from E. T. Castellana *et al.*, *Anal. Chem.*, 78, 107, 2006. © 2006, American Chemical Society.

M, pH 5), the authors demonstrated patterning of silver metal at specific locations in the channels, Fig. 5(a). The deposited regions can be derivatized with enzymes to achieve specific detection of proteins at different locations along the microchannel, cf. Fig. 5(b).⁵²

To date, many of the reported feature sizes for the photolithography approach have been relatively large, e.g., $>100\ \mu\text{m}$,^{25–27,56,60} with modest edge resolution of $\sim 20\ \mu\text{m}$.²⁷ Edge resolution is quite limited due to diffraction²⁵ or imperfect collimation.²⁷ These effects can be resolved for flat plates by reduced mask-sample separation, but this is not straightforward for bonded chips that are several hundred micrometers thick. However, by reducing the thickness of at least one plate of the bonded microchip, features as small as 6 μm have been demonstrated.⁵² The photolithographic approach to patterning bonded channels is also dependent on the quality of the outer surface of the microchannel. Scattering or absorption of incident UV by defects in the microchip's outer surface may limit the resolution and reproducibility of the patterned regions.

C. Microplasmas

Plasma surface treatment is well-known to the microfluidics community for its extensive application to PDMS chip bonding and chemical modification. Plasmas are highly excited gases, containing reactive species including ions, radicals, electrons, and radiation.⁶⁶ These reactive species may be used for surface etching or, alternatively, deposition of various surface layers.^{67–70} While plasma chemistry can be very complex, due to the many different species present, there are significant practical advantages. Plasma surface modification is rapid, solvent-free, can be spatially controlled (e.g., as a jet), and can deposit a wide variety of chemistries. The technique is now extensively employed for chemical modification of structured and planar surfaces, including microfluidic channels. Recently, plasma patterning of microfluidic channels has received significant attention.^{28–31,46,71,72} In most cases, microchannel patterning was achieved prebonding using appropriate masks or directed jets; however, only a few examples of postbonding treatment have been reported.^{28–31,72,73}

Postbonding plasma modification of polystyrene microchannels has been demonstrated by Evju *et al.*²⁸ Platinum electrodes were engineered in the microchannel to generate an electric field along the length of the channel, Fig. 6(a). The gases used to generate the confined plasma included helium (He), water-saturated He, and 2 vol % hexafluoroethane in He, which result in different surface wettability. For ignition potentials of up to 15 kV and operating potentials of 1.5 kV, confined microplasmas were generated along the length of the microchannel, cf. Fig. 6(a). For

FIG. 6. (a) Helium microplasma generated by Evju *et al.* (Ref. 28) along the length of a microchannel. The tips of the platinum electrodes were positioned at either end of the plasma emission, embedded in the microchip. Reprinted with permission from J. K. Evju *et al.*, Appl. Phys. Lett., 84, 1668, 2004. © 2004, American Institute of Physics. (b) A schematic of the experiment design by Klages *et al.* (Ref. 30). Electrodes were positioned outside of the microchip, covering specific regions of the channel (as small as 2 mm). The shaded region of the microchannel (between the electrodes) is selectively modified using plasma polymerization. Adapted with permission from C.-P. Klages *et al.*, Plasma Processes Polym., 4, 208, © Wiley-VCH Verlag GmbH & Co. KGaA.

water and hexafluoroethane containing He, the channels were hydrophilic and hydrophobic, with x-ray photoelectron spectroscopy revealing the presence of oxygen and fluorine peaks, respectively.

For plasma patterning microchannels the electrode geometry is crucial. In the method described above,²⁸ and elsewhere,⁷² the plasma completely fills the channel between the electrodes. This approach is therefore limited to plasma treatment of a continuous region of the channel, with little flexibility in the electrode design. In a step toward more complex patterns, Dixon and Takayama³¹ off-set the electrodes with respect to the channel to generate a plasma that was guided along a single side of a 1.5 cm long, 2 mm wide channel. Thus, a plasma-generated hydrophilic stripe (~ 50 μm wide) was created along one side of the PDMS channel. The method is rapid, 5 s treatment, but requires very high applied potentials, i.e., up to 50 kV. Treatment of the channel surface by a Pluronic F127 layer (resistant to cell adhesion) prior to selective plasma modification led to preferential protein (fibronectin) and cell adhesion. Although effective, the authors conceded that the method is limited in precision, and cautioned that uniformity of surface modification using plasmas is highly dependent on the nature of the experimental design. With regard to electrode alignment, their study demonstrated this point very clearly.

For greater flexibility and patterning, Klages *et al.*^{29,30} demonstrated plasma treatment of bonded channels using an electric field perpendicular to the direction of the microchannel, cf. Fig. 6(b). By adjusting the electrode design, regions of the channel could be treated selectively. For channel patterning, electrodes (2 mm wide) were well-separated (20 mm apart) along the length of the channel. The design facilitated localized deposition of hydrophobic layers generated by hexamethyldisiloxane plasma, corresponding to the electrode positions. Reflectivity analysis revealed no appreciable deposition outside of the electrodes; however, the thickness of the deposited film at the downstream electrode was slightly greater. This was attributed to flow effects, where plasma species generated an upstream deposit on downstream plasma activated surfaces (i.e., at the electrodes). Klages *et al.* stated that regions as small as 1 mm can be modified using refined electrode and gas configurations,³⁰ although an absolute limit was not determined in their study.

D. Electrochemical biolithography

In some cases, prebonding patterning with inorganic materials, e.g., metals or metal oxides, can facilitate postbonding patterning of less robust materials, e.g., organic SAMs, proteins, DNA, and alike. Embedding metal or metal oxide electrodes in microfluidic devices can be achieved using standard photolithography,^{74–76} providing a suitable platform for postbonding surface modification by electrochemical means. However, to date, only a few examples of postbonding channel patterning using this method have been published. The potential of this technique to create complex biochips for a range of applications warrants further discussion here.

FIG. 7. (a) *In situ* patterning of microchannels by electrochemical deposition of chitosan (Ref. 10). (i) Electrochemically deposited chitosan layer (unlabelled). (ii) Microchannel filled with NHS/PBS solution (fluorescently labeled, green). (iii) NHS-labeled chitosan layer. (iv) Negative control, i.e., no deposition of chitosan. Reproduced from J. J. Park *et al.*, Lab Chip, 6, 1315, 2006, with permission of the The Royal Society of Chemistry. (b) Microchannel patterning with different proteins using step-wise electrochemical treatments from Ref. 79. Electrochemical generation of Br_2 and, subsequently HBrO , in a microfluidic channel is used to locally remove (by oxidation) an adsorbed polyethyleneimine-heparin layer prior to adsorption of protein A. A three stage patterning process is shown (top to bottom). Top: electrochemical patterning with protein A, followed by binding of mouse IgG-Cy3 (left region). Middle: electrochemical patterning with protein A, followed by binding of human IgG-Cy2 (center region). Bottom: electrochemical patterning with protein A, followed by binding of mouse IgG-Cy3 (right region). Reprinted with permission from H. Kaji *et al.*, Anal. Chem., 78, 5469, 2006. © 2006, American Chemical Society.

Patterned assembly of biomaterials, or biolithography, can be achieved using electrochemical techniques. Recently, electrochemical biolithography has been adapted for immobilization of biomaterials in the confinement of a sealed microfluidic channel.^{10,11,77} Park *et al.*¹⁰ used the pH sensitivity of chitosan (an aminopolysaccharide) to deposit a hydrogel network on a negatively biased gold electrode, cf. Fig. 7(a). The chitosan hydrogel is exclusively deposited at the electrode ($1\text{ mm} \times 0.5\text{ mm}$), as revealed by fluorescent tagging. In the same paper, localized chitosan hydrogel (untagged) was then used to successfully capture carboxyfluorescein succinimidyl ester (NHS-fluorescein) under flow. No apparent adsorption of NHS-fluorescein was observed elsewhere in the channel. The authors also demonstrated selective protein binding of green fluorescent protein at the chitosan layer. The layer maintains its fluorescence after binding, indicating that its protein structure is preserved. Later, Luo *et al.*¹¹ developed a one-step deposition of an enzyme-chitosan conjugate at the gold electrode, which was used to demonstrate an enzymatic reaction (cleavage of *S*-adenosylhomocysteine to form *S*-ribosylhomocysteine and adenine). Key features of the electrochemical patterning technique include enzyme assembly in aqueous phase (no dry processing is required) and the ability to reuse the microchip after straightforward cleaning with hydrochloric acid.^{11,78}

Using a slightly different electrochemical approach, Kaji *et al.*^{79,80} individually addressed specific regions of a bonded microchannel for protein binding. Generation of Br_2 , and subsequently HBrO , under stopped flow conditions at electrodes deposited on one microchannel wall was used for oxidative removal of a polyethyleneimine-heparin layer from the adjacent wall.⁷⁹ Subsequently, protein A was locally adsorbed and, where the electrochemical patterning process is repeated in a step-wise manner at different locations along the channel, stripes of different antibodies can be generated along a single channel. Figure 7(b) shows alternating stripes of two

TABLE I. Comparison of postbonding approaches to surface patterning of microchannels.

Approach	Feature size ^a	Pattern type ^b	Processing conditions	Dry/Wet ^c
Laminar flow	5 μm ^d	Continuous	Reagent in solvent	Wet
Capillarity	170 μm ^e	Continuous	Reagent in solvent	Wet
Photolithography	6 μm ^f ~1 mm ^h	Continuous patches/regions complex	Photosensitive layer, irradiation, masking, development	Dry/wet ^g
Microplasmas	~50 μm ⁱ ~1 mm ^{j,k}	Continuous patches/regions	High electric field, electrode alignment	Dry
Electrochemical biolithography	~300 μm ^l >500 μm ^m	Continuous patches/regions	Patterned electrodes, applied potential	Wet

^aThese values are typical feature sizes demonstrated in the cited literature, rather than any technical limitation.

^bContinuous: unbroken features (usually along the length of the channel). Patches/regions: discrete areas of surface modification. Complex: discrete areas with fine details or unusual geometries.

^cProcess conditions, wet chemical/solvent processing, and/or dry (gas or vapor phase) processing.

^dReference 53.

^eReference 32.

^fReference 52.

^gTypically dry processing.

^hReference 26.

ⁱReference 31.

^jReference 29.

^kReference 30.

^lReference 79.

^mReference 78.

antibodies indicated by red (mouse IgG-Cy3) and green (human IgG-C) fluorescence signals. The demonstrated feature size (~300 μm) would be suited to many applications; however, the minimum feature size is a potential limitation of this method due to the reliance on diffusion of HBrO across the channel to the adjacent microchannel wall. Nonetheless, this method has significant potential to develop microsensor arrays along microfluidic channels using aqueous process conditions, which are favored for biomaterials.

IV. SUMMARY AND OUTLOOK

Surface modification of microchannels is becoming increasingly complex as new microfluidic applications emerge. Microchannel walls are no longer simply guides for fluid flow but rather are important instruments in the functionality of microchips. This diversity of applications translates to diversity in chemistry and biology at the channel walls. Patterned microchannels are now employed for the integration of multiple, highly selective detectors in a single microfluidic stream. To date, the vast majority of channel patterning has been carried out prior to bonding the microchip. However, postbonding techniques have significant advantages in a variety of applications, especially where the surface chemistry/biochemistry is sensitive to the traditionally harsh bonding conditions. The realization that postbonding surface treatments offer new potential for sensitive surfaces has led to a new wave of patterning methods.

Table I summarizes some key details of the postbonding patterning methods considered in this Review. The minimum feature sizes stated in the table are not technical limitations of the respective methods, but rather representative of the status quo in the literature. While laminar flow and capillarity are generally restricted to simple (continuous) surface patterns, the method is also an enabling tool for other postbonding techniques. For example, photosensitive SAMs deposited from laminar stream patterns could later be segmented using the photolithographic approach. To date, photolithography has provided the most flexibility in pattern design, although photosensitivity of unexposed regions of the final microchip may not be desirable for some applications due to reduced chip life-times. Microplasmas and electrochemical biolithography are two emerging

methods that have potential to open new possibilities for postbonding patterning. Further developments in microplasma technologies may offer the potential to pattern bonded channels with a wide range of chemistries at scales competing with photolithography (this has already been realized on open surfaces⁶⁹). On the other hand, electrochemical biolithography provides the important advantage of aqueous phase patterning, which preserves the functionality of deposited biomaterials.

Despite more than ten years of research and development, postbonding patterning of microchannels is still quite limited. Realized surface patterns are generally relatively large compared to the microchannel's dimensions and, with the exception of photolithography, patterns are quite simple in design. One major challenge for emerging techniques will be the need for greater spatial control over surface modification to realize more complex, smaller designs. For example, using microplasmas to create precise microscopic surface features (now restricted to the millimeter scale) without diffuse boundaries will be particularly challenging. Even where spatially well-defined microscopic features can be generated on conventional chip materials (discussed in this Review), the push toward cheaper fabrication methods and new chip applications will require postbonding patterning of alternative microchannel substrates, e.g., inexpensive polymers for disposable biochips or metals for process intensification in industry. Opaque materials, for example, would disqualify well-established photolithography methods.

The rate at which emerging postbonding surface modification develops will depend on many factors, including the success of competing prebonding patterning methods and alternative mild bonding techniques. It is therefore clear that not one but, rather, all of the discussed approaches (along with new ones) will be utilized in the fabrication of next generation microchips. In many cases, robust prebonding patterning will provide a platform for adding more sensitive functionalities using one or more postbonding techniques. This concerted approach is already evident in the literature,^{10,53} and is likely to become increasingly important in the realization of the next generation of functionalized microfluidic chips. At present, it remains unclear whether postbonding patterning of microchannels will drive or be driven by new applications in the microfluidics field, although they will certainly play a key role in realizing them.

- ¹ S. L. Anna, N. Bontoux, and H. A. Stone, *Appl. Phys. Lett.* **82**, 364 (2003).
- ² C. Priest, S. Herminghaus, and R. Seemann, *Appl. Phys. Lett.* **88**, 024106 (2006).
- ³ S. K. W. Dertinger, D. T. Chiu, N. L. Jeon, and G. M. Whitesides, *Anal. Chem.* **73**, 1240 (2001).
- ⁴ H. Song, D. L. Chen, and R. F. Ismagilov, *Angew. Chem., Int. Ed.* **45**, 7336 (2006).
- ⁵ A. Pallandre, B. de Lambert, R. Attia, A. M. Jonas, and J. L. Viovy, *Electrophoresis* **27**, 584 (2006).
- ⁶ J. Atencia and D. J. Beebe, *Nature (London)* **437**, 648 (2005).
- ⁷ H. Makamba, J. H. Kim, K. Lim, N. Park, and J. H. Hahn, *Electrophoresis* **24**, 3607 (2003).
- ⁸ E. Delamarche, D. Juncker, and H. Schmid, *Adv. Mater. (Weinheim, Ger.)* **17**, 2911 (2005).
- ⁹ A. Hibara, M. Nonaka, H. Hisamoto, K. Uchiyama, Y. Kikutani, M. Tokeshi, and T. Kitamori, *Anal. Chem.* **74**, 1724 (2002).
- ¹⁰ J. J. Park, X. Luo, H. Yi, T. M. Valentine, G. F. Payne, W. E. Bentley, R. Ghodssi, and G. W. Rubloff, *Lab Chip* **6**, 1315 (2006).
- ¹¹ X. Luo, A. T. Lewandowski, H. Yi, G. F. Payne, R. Ghodssi, W. E. Bentley, and G. W. Rubloff, *Lab Chip* **8**, 420 (2008).
- ¹² S. Carroll, M. M. Crain, J. F. Naber, R. S. Keynton, K. M. Walsh, and R. P. Baldwin, *Lab Chip* **8**, 1564 (2008).
- ¹³ Y.-J. Pan and R.-J. Yang, *J. Micromech. Microeng.* **16**, 2666 (2006).
- ¹⁴ Z. Huang, J. C. Sanders, C. Dunsmor, H. Ahmadzadeh, and J. P. Landers, *Electrophoresis* **22**, 3924 (2001).
- ¹⁵ G. Zhuang, Q. Jin, J. Liu, H. Cong, K. Liu, J. Zhao, M. Yang, and H. Wang, *Biomed. Microdevices* **8**, 255 (2006).
- ¹⁶ L. Chen, G. Luo, K. Liu, J. Ma, B. Yao, Y. Yan, and Y. Wang, *Sens. Actuators B* **119**, 335 (2006).
- ¹⁷ Z.-J. Jia, Q. Fang, and Z.-L. Fang, *Anal. Chem.* **76**, 5597 (2004).
- ¹⁸ A. Iles, A. Oki, and N. Pamme, *Microfluid. Nanofluid.* **3**, 119 (2007).
- ¹⁹ Y. Akiyama, K. Morishima, A. Kogi, Y. Kikutani, M. Tokeshi, and T. Kitamori, *Electrophoresis* **28**, 994 (2007).
- ²⁰ B. Renberg, K. Sato, T. Tsukahara, K. Mawatari, and T. Kitamori, *Mikrochim. Acta* **166**, 177 (2009).
- ²¹ D. C. Duffy, J. C. McDonald, O. J. A. Schueller, and G. M. Whitesides, *Anal. Chem.* **70**, 4974 (1998).
- ²² J. Zhou, A. V. Ellis, and N. H. Voelcker, *Electrophoresis* **31**, 2 (2010).
- ²³ G. Takei, M. Nonogi, A. Hibara, T. Kitamori, and H.-B. Kim, *Lab Chip* **7**, 596 (2007).
- ²⁴ B. Zhao, J. S. Moore, and D. J. Beebe, *Langmuir* **19**, 1873 (2003).
- ²⁵ E. Besson, A.-M. Gue, J. Sudor, H. Korri-Yousseufi, N. Jaffrezic, and J. Tardy, *Langmuir* **22**, 8346 (2006).
- ²⁶ B. Zhao, J. S. Moore, and D. J. Beebe, *Science* **291**, 1023 (2001).
- ²⁷ B. Zhao, J. S. Moore, and D. J. Beebe, *Anal. Chem.* **74**, 4259 (2002).
- ²⁸ J. K. Evju, P. B. Howell, L. E. Locascio, M. J. Tarlov, and J. J. Hickman, *Appl. Phys. Lett.* **84**, 1668 (2004).
- ²⁹ C.-P. Klages, C. Berger, M. Eichler, and M. Thomas, *Contrib. Plasma Phys.* **47**, 49 (2007).
- ³⁰ C.-P. Klages, A. Hinze, K. Lachmann, C. Berger, J. Borris, M. Eichler, M. von Hausen, A. Zänker, and M. Thomas,

- Plasma Processes Polym.* **4**, 208 (2007).
- ³¹ A. Dixon and S. Takayama, *Biomed. Microdevices* **12**, 769 (2010).
- ³² A. Hibara, S. Iwayama, S. Matsuoka, M. Ueno, Y. Kikutani, M. Tokeshi, and T. Kitamori, *Anal. Chem.* **77**, 943 (2005).
- ³³ P. J. A. Kenis, R. F. Ismagilov, S. Takayama, G. M. Whitesides, S. Li, and H. S. White, *Acc. Chem. Res.* **33**, 841 (2000).
- ³⁴ J. L. Fritz and M. J. Owen, *J. Adhes.* **54**, 33 (1995).
- ³⁵ J. N. Lee, C. Park, and G. M. Whitesides, *Anal. Chem.* **75**, 6544 (2003).
- ³⁶ D. J. Harrison, A. Manz, Z. Fan, H. Lüdi, and H. M. Widmer, *Anal. Chem.* **64**, 1926 (1992).
- ³⁷ L. M. Fidalgo, C. Abell, and W. T. S. Huck, *Lab Chip* **7**, 984 (2007).
- ³⁸ C. Holtze, A. C. Rowat, J. J. Agresti, J. B. Hutchison, F. E. Angilè, C. H. J. Schmitz, S. Köster, H. Duan, K. J. Humphry, R. A. Scanga, J. S. Johnson, D. Pisignano, and D. A. Weitz, *Lab Chip* **8**, 1632 (2008).
- ³⁹ A. R. Abate, D. Lee, T. Do, C. Holtze, and D. A. Weitz, *Lab Chip* **8**, 516 (2008).
- ⁴⁰ G. T. Roman and C. T. Culbertson, *Langmuir* **22**, 4445 (2006).
- ⁴¹ A. Kanta, R. Sedev, and J. Ralston, *Langmuir* **21**, 5790 (2005).
- ⁴² G. Sui, J. Wang, C.-C. Lee, W. Lu, S. P. Lee, J. V. Leyton, A. M. Wu, and H.-R. Tseng, *Anal. Chem.* **78**, 5543 (2006).
- ⁴³ E. Delamar, A. Bernard, H. Schmid, A. Bietsch, B. Michel, and H. Biebuyck, *J. Am. Chem. Soc.* **120**, 500 (1998).
- ⁴⁴ H. Bi, S. Meng, Y. Li, K. Guo, Y. Chen, J. Kong, P. Yang, W. Zhong, and B. Liu, *Lab Chip* **6**, 769 (2006).
- ⁴⁵ A.-J. Wang, J.-J. Xu, and H.-Y. Chen, *J. Chromatogr. A* **1147**, 120 (2007).
- ⁴⁶ M. Salim, G. Mishra, G. J. S. Fowler, B. O'Sullivan, P. C. Wright, and S. L. McArthur, *Lab Chip* **7**, 523 (2007).
- ⁴⁷ M. Kosmulski, *Colloids Surf., A* **222**, 113 (2003).
- ⁴⁸ D. Belder and M. Ludwig, *Electrophoresis* **24**, 3595 (2003).
- ⁴⁹ Z. Sui and J. B. Schlenoff, *Langmuir* **19**, 7829 (2003).
- ⁵⁰ H. Lindstrom, R. Wootton, and A. Iles, *AIChE J.* **53**, 695 (2007).
- ⁵¹ M. Pumera, J. Wang, E. Grushka, and R. Polsky, *Anal. Chem.* **73**, 5625 (2001).
- ⁵² E. T. Castellana, S. Kataoka, F. Albertorio, and P. S. Cremer, *Anal. Chem.* **78**, 107 (2006).
- ⁵³ P. J. A. Kenis, R. F. Ismagilov, and G. M. Whitesides, *Science* **285**, 83 (1999).
- ⁵⁴ D. G. Shchukin, D. S. Kommireddy, Y. Zhao, T. Cui, G. B. Sukhorukov, and Y. M. Lvov, *Adv. Mater. (Weinheim, Ger.)* **16**, 389 (2004).
- ⁵⁵ S. L. R. Barker, D. Ross, M. J. Tarlov, M. Gaitan, and L. E. Locascio, *Anal. Chem.* **72**, 5925 (2000).
- ⁵⁶ B. Zhao, N. O. L. Viernes, J. S. Moore, and D. J. Beebe, *J. Am. Chem. Soc.* **124**, 5284 (2002).
- ⁵⁷ T. Rohr, D. F. Ogletree, F. Svec, and J. M. J. Fréchet, *Adv. Mater. (Weinheim, Ger.)* **13**, 264 (2003).
- ⁵⁸ S. Hu, X. Ren, M. Bachman, C. E. Sims, G. P. Li, and N. L. Allbritton, *Anal. Chem.* **76**, 1865 (2004).
- ⁵⁹ A. R. Abate, A. T. Krummel, D. Lee, M. Marquez, C. Holtze, and D. A. Weitz, *Lab Chip* **8**, 2157 (2008).
- ⁶⁰ L. K. Fiddes, H. K. C. Chan, B. Lau, E. Kumacheva, and A. R. Wheeler, *Biomaterials* **31**, 315 (2010).
- ⁶¹ A. L. Linsebigler, G. Lu, and J. T. Yates, *Chem. Rev. (Washington, D.C.)* **95**, 735 (1995).
- ⁶² A. Fujishima, X. Zhang, and D. A. Tryk, *Surf. Sci. Rep.* **63**, 515 (2008).
- ⁶³ A. Tuantranont, T. Lomas, T. Matusos, A. Wisitsara-at, P. Thavarungkul, P. Kanatharana, W. Limbut, and S. Loyprasert, *NanoBiotechnology* **2**, 143 (2006).
- ⁶⁴ Y. Yamasaki, M. Goto, A. Kariyasaki, S. Morooka, Y. Yamaguchi, M. Miyazaki, and H. Maeda, *Korean J. Chem. Eng.* **26**, 1759 (2009).
- ⁶⁵ H. Nagai, T. Irie, J. Takahashi, and S. Wakida, *Biosens. Bioelectron.* **22**, 1968 (2007).
- ⁶⁶ R. Foest, M. Schmidt, and K. Becker, *Int. J. Mass. Spectrom.* **248**, 87 (2006).
- ⁶⁷ S. Kreitz, C. Penache, M. Thomas, and C.-P. Klages, *Surf. Coat. Technol.* **200**, 676 (2005).
- ⁶⁸ K. S. Siow, L. Britcher, S. Kumar, and H. J. Griesser, *Plasma Processes Polym.* **3**, 392 (2006).
- ⁶⁹ L. Dai, H. J. Griesser, and A. W. H. Mau, *J. Phys. Chem. B* **101**, 9548 (1997).
- ⁷⁰ H. Aizawa, T. Makisako, S. M. Reddy, K. Terashima, S. Kurosawa, and M. Yoshimoto, *J. Photopolym. Sci. Technol.* **20**, 215 (2007).
- ⁷¹ M. Salim, P. C. Wright, and S. L. McArthur, *Electrophoresis* **30**, 1877 (2009).
- ⁷² S. Thorslund and F. Nikolajeff, *J. Micromech. Microeng.* **17**, N16 (2007).
- ⁷³ M. Kadowaki, H. Yoshizawa, S. Mori, and M. Suzuki, *Thin Solid Films* **506–507**, 123 (2006).
- ⁷⁴ C. Priest, S. Herminghaus, and R. Seemann, *Appl. Phys. Lett.* **89**, 134101 (2006).
- ⁷⁵ J. G. Kralj, M. A. Schmidt, and K. F. Jensen, *Lab Chip* **5**, 531 (2005).
- ⁷⁶ W. Ebina, A. C. Rowat, and D. A. Weitz, *Biomicrofluidics* **3**, 034104 (2009).
- ⁷⁷ M. Hashimoto, H. Kaji, and M. Nishizawa, *Biosens. Bioelectron.* **24**, 2892 (2009).
- ⁷⁸ J. I. Park, A. Saffari, S. Kumar, A. Günther, and E. Kumacheva, *Annu. Rev. Mater. Res.* **40**, 415 (2010).
- ⁷⁹ H. Kaji, M. Hashimoto, and M. Nishizawa, *Anal. Chem.* **78**, 5469 (2006).
- ⁸⁰ M. Hashimoto, H. Kaji, M. E. Kempainen, and M. Nishizawa, *Sens. Actuators B* **128**, 545 (2008).