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Photopatterned materials in bioanalytical microfluidic technology

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Abstract

Microfluidic technologies are playing an increasingly important role in biological inquiry. Sophisticated approaches to the microanalysis of biological specimens rely, in part, on the fine fluid and material control offered by microtechnology, as well as a sufficient capacity for systems integration. A suite of techniques that utilize photopatterning of polymers on fluidic surfaces, within fluidic volumes, and as primary device structures underpins recent technological innovation in bioanalysis. Well-characterized photopatterning approaches enable previously fabricated or commercially fabricated devices to be customized by the user in a straight-forward manner, making the tools accessible to laboratories that do not focus on microfabrication technology innovation. In this review of recent advances, we summarize reported microfluidic devices with photopatterned structures and regions as platforms for a diverse set of biological measurements and assays.

1. Introduction

Significant technological gaps exist in the repertoire of tools for biological inquiry. While genomics technology has afforded tremendous leaps in measurement throughput and quantitation, biological experiments for biochemical study of proteins or cells are still generally labor intensive and slow. For example in proteomics, the powerful and frequently used two-dimensional electrophoresis assay, as well as the Western blotting assay, can take tens of hours to complete and requires user intervention at several steps (Wu *et al* 2007). To address pressing present-day challenges in biology and biomedicine, the development of quantitative, automated, and sophisticated biochemical assays is needed. Consequently, major research foci for chemistry, materials, and engineering innovators are in analytical systems. We first motivate the reasons supporting the use of microfluidics and photopatterning in the development of bioanalytical platforms. We then proceed to summarize key publications that use photopatterned materials in microfluidic devices for biological assays and measurements.

Microfluidic technology as a basis for analytical tools

Microanalytical platforms offer a capacity for integration of multiple steps, rapid fluid and materials transport, low dead volume, and programmable control (i.e., voltage control, pressure programs) among other performance attributes. The capability to conduct rapid, programmable assays in integrated workflows overcomes the low-throughput, non-quantitative limitations of many current benchtop (macroscale) biomolecule measurement techniques. In particular, automated protein quantitation for numerous experimental conditions would aid in advancing and broadly realizing systems biology frameworks. Such biochemical assays are presently arduous and labor intensive. Unified biochemical assays would also improve quantitation capability by minimizing variable user interventions. Furthermore, due to low volume consumption and near-lossless material handling,

microfluidic technology is better suited than benchtop approaches for the handling and analysis of precious and/or expensive samples and reagents. Taken together, microfluidic technologies offer compelling advantages for conducting biochemical study of living systems, either through down-scaling of conventional assays or through invention of new assays that are feasible only on the micro- and nanoscale.

Although microfluidic technology is beginning to drive advances in bioanalytical methods, there are still challenges to widespread adoption that must be addressed. Chiefly, the fabrication of microfluidic devices still requires a high level of expertise in micro and nanofabrication in addition to the availability of dedicated and expensive equipment. Furthermore, due to the nature of microfabrication, device structures are often fabricated in a layered fashion. Device features have to be created by depositing or removing portions of layers on a 2D substrate. Bonding to another substrate is then necessary to seal the microchannels. Devices with different functional regions are necessary for the integration of the multiple experimental steps needed for automated assays. Fluids can be used for chemical modifications of channel surfaces and volumes, but without photopatterning, the development of spatially-separate regions with different properties is extremely challenging.

1.1 The role photopatterning in microfluidics

The spatially controlled creation of device features using light comprises a microfabrication approach known as photolithography. Photolithographic processes result in the patterning of a design onto a substrate. Typically, the patterning of features is controlled using a photomask, which only allows certain regions on the material to be exposed to light. Subsequently, exposed or unexposed regions can be selectively removed. Photolithography has also been used extensively for the fabrication of microfluidic devices. Soft lithography, in particular, has gained popularity as a process for rapid prototyping of microfluidic devices (Duffy *et al* 1998; Kim *et al* 2008). These processes yield devices constructed from elastomeric polymers such as polydimethylsiloxane (PDMS). Previously fabricated, solid molds are used to imprint channels on the elastomeric polymer. Subsequently, the imprinted structure is bonded to glass to close the channels. Photolithography is frequently used to fabricate molds from SU-8 photoresist on silicon wafers. SU-8 is an epoxy-based, near-UV, negative photoresist (regions unexposed to UV light can be removed using a solvent). As excellent reviews are already available regarding soft lithography for biological applications (Kane *et al* 1999; McDonald *et al* 2000; Whitesides *et al* 2001; Sia and Whitesides 2003), the present review will focus on a suite of burgeoning fabrication approaches that rely on photopatterning in existing microfluidic devices to impart specific functionality or to integrate multiple functions on devices used for bioassays.

Photopatterning enables the creation of unique, discrete, and separate structures inside previously fabricated microfluidic devices. Photo-reactive chemicals can be loaded into existing devices and exposed in a spatially controlled manner using lasers or photomasks. In this manner, light exposure allows for the grafting of biomolecules or chemistries to device surfaces in specific regions and yield channels or chambers filled with polyacrylamide (PA) gels or porous polymer monoliths (PPM). Photopatterning thus enables the formation of structures and regions with a high spatial resolution inside previously fabricated channels made from light-permeable materials such as glass or plastics (Figure 1).

2. Modifications of channel surfaces

As channel cross sections get smaller, surface-area-to-volume ratios increase, making surfaces increasingly important in microfluidic devices. Photopatterning enables the modification of channel surfaces with a high degree of spatial precision, through the use of photografting and photolinker chemistries. Photografting is the formation of a covalent bond

between a molecule and a substrate following chemical modification using light. As summarized in Table 1 and discussed in this section, photografting has been employed for a variety of substrate modifications by attaching biomolecules and chemicals to impart different functionalities and properties to a surface. Similarly, covalent bonds can also be broken using light. Spatially controlled removal of a surface coating is used for the creation of functionally distinct regions in microfluidic devices.

2.1 Attaching biologically active functionalities on channel surfaces

Creating regions with different functionalities enables devices that can perform sequential operations on the same sample in specific channel regions. Patterning of well-defined patches of molecules was achieved by photobleaching of dye-biotin species (Holden *et al* 2004). The photobleaching of the fluorophore moieties in the dye created highly reactive species. After bleaching, reactive species attached to a protein coated channel surface via a singlet oxygen-dependent mechanism. Different enzymes linked to streptavidin or avidin were then attached to the biotinylated patches. This approach was demonstrated for the sequentially patterning of avidin-linked glucose oxidase and streptavidin-linked horseradish peroxidase on the biotinylated channel surfaces. Product formed at the glucose oxidase patch became the substrate for the downstream-patterned horseradish peroxidase. The fluorogenic substrate turnover rate was then recorded via epifluorescence microscopy. Using a different approach, DNA and proteins were attached on fused-silica capillary walls for hybridization, immunoassays, and protein interaction assays (Balakirev *et al* 2005). The capillary surface was modified with a photoactive benzophenone derivative. Upon UV illumination, benzophenone was excited into a highly-reactive triplet state that forms a stable covalent bond with a target molecule. Region specific and sequential UV illumination steps allowed the desired solute molecules to become covalently attached to the activated capillary surface regions. Proof-of-concept protein binding and oligonucleotide hybridization experiments were demonstrated on this platform suggesting the use of capillaries for these types of assays as an alternative to low-density 2D arrays. Compared to the previous multi-step process, this one-approach patterns and immobilizes biomolecules in a single step. A one-step process decreases the total fabrication time especially when multiple species are being patterned. Nevertheless, the biomolecules being patterned are exposed to UV radiation and chemically reactive species, which might lead to damage.

Another approach for the creation of well defined patches of specified surface chemistry inside microchannels involves deep UV lithography (Shi *et al* 2008). UV exposure is used to selectively remove patches (~90×90µm) of a uniform bovine serum albumin protein layer on the inner surface of a glass coverslip bonded to a PDMS microchannel device. Using this non-uniform surface characteristic, supported phospholipid layers were formed on the exposed patches of glass. Through flow patterning, various proteins were subsequently patterned on the lipid patches. This technology was developed to study the interaction of ganglioside M1 (GM1) with the subunits of cholera toxin B (CTB). Different concentrations of GM1 were patterned on the lipid layers along the length of a microfluidic channel. Different concentrations of CTB subunits were flowed through several channels in parallel. Using total internal reflection fluorescence microscopy, the interaction between GM1 and CTB subunits was quantified to obtain multiple binding parameters for different ligand concentrations concurrently. Equilibrium dissociation constants were measured to be 0.20, 0.29, and 0.35 nM for supported phospholipid layers containing 0.2, 0.5, and 2.0mol % GM1 respectively. The ability of this platform to immobilize proteins onto a surface suggests that it can be adapted to measure binding kinetics between antibody-antigen pairs and measure binding in a label-free manner using other parameters other than fluorescence.

2.2 Chemical surface modifications

To form the basis for an analytical platform for on-chip cell culture, cell expression, and cell interaction studies, photopatterning was employed to localize cells on specific regions of microchannel walls (Jang *et al* 2009; Jang *et al* 2010). Channel surfaces were modified using a 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer combined with a photolabile linker. An amine terminated silanized glass surface was coated with MPC to decrease non-specific absorption. MPC was then selectively removed by UV illumination revealing a surface for cell attachment. High spatial resolution photo-removal allowed the authors to vary the spatial distribution and number of cells in a given channel. As a proof-of-concept experiment demonstrating the potential for cell interaction studies in this device, Piccells (cells with genetically-encoded molecular indicators) and vascular endothelial cells were cultured separately for 14 days in downstream and upstream regions of the device (Figure 2). Upon adenosine triphosphate stimulation, the endothelial cells release nitrous oxide. The dynamics of nitrous oxide were measured using Förster resonance energy transfer (FRET) in the Piccells, which are cell-based nitrous oxide sensors. Combining cell culture and analysis on the same device could prove to be a powerful tool for high-throughput and reliable measurements in cell interaction studies.

In another application of photocleaving, the process has been used for the creation of virtual channels and pressure-sensitive gates. A self assembled monolayer (SAM) of 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-1-octyl-4-(11-trichlorosilyl-1-oxoundecyloxymethyl)-3-nitrobenzoate (FSAM) was first formed inside microfluidic channels. Photopatterning was used to strip the SAM in specific channel regions. The surfaces where the *o*-nitrobenzyl-oxygen bond of FSAM was cleaved upon UV exposure become hydrophilic, allowing fluid to flow preferentially in those regions. These pressure-sensitive gates have been demonstrated to respond to pressure differences of 10mm H₂O. (Zhao *et al* 2002; Zhao *et al* 2003)

3. Formation and modification of structures in channel volumes

Modifications to the volumes of microfluidic channels have led to the development of devices with capabilities including integrated sample preparation and multi-dimensional separations of biomolecules. Versatile photopatterned materials including PA gels, polymer membranes, and PPM have been fabricated inside microfluidic channels to imbue devices with multiple functions. With PA gels, photopatterning enables spatial control of gel properties such as placement, gel density (mean pore size), and the functionalization of gels with different molecules. Channel volumes are also modified by the polymerization of PPM. One of the main functional distinctions between PA gels and PPM is that while gels prevent hydrodynamic flow, monoliths allow pressure-driven fluid to pass through them. Monolith placement and surface modifications can be spatially controlled by photopatterning. Several comprehensive reviews on the wide assortment of PPM preparation techniques are available (Walsh *et al* 2008; Nischang *et al* 2010; Svec 2010; Walsh *et al* 2010). Specific advances on the morphological aspects of PPM on chips and capillaries have also been presented in detail (Nischang *et al* 2010). The surfaces of PPM have been modified using photografting to impart different functionalities and properties. These materials provide versatile functions, spanning from crude sample manipulation to analytical characterization.

3.1 Sample preparation

Integrated sample preparation is critical when minimal user intervention and the processing of complex samples such as serum is required. Membranes allow for the integration of sample preparation steps into chips with other photopatterned structures. Polymerized membranes are particularly useful for sample preconcentration, which is an important step

for assays in which the analyte of interest is present in low concentrations. The creation of a thin (50 μ m) PA gel membrane adjacent to a sieving matrix allows for electrophoretically driven sample preconcentration integrated with a downstream electrophoretic separation. Using this approach to enrich analytes, 1000-fold increases in sample concentration were observed after only 5 minutes (Figure 3) (Hatch *et al* 2006). The approach has yielded a linear response with the voltage-time product for protein concentration increases up to the point of precipitation (Song and Singh 2006). The ability to integrate sample preparation with protein separations fills a current technological gap in diagnostic devices (Herr *et al* 2007). Examples of devices that use membranes and other photopatterned structures for sample preparation are summarized in Table 2.

Other important sample preparation steps, such as dialysis and fractionation have been demonstrated on-chip using laser-polymerized membranes (Song *et al* 2003; Song *et al* 2004). Adjacent channels separated by the membrane are arranged in a counterflow configuration. Water and a solution containing the sample flow in opposite direction; diffusion leads to the dilution of species in the sample below the molecular weight cutoff of the membrane. Protein desalting and fractionation are possible by designing membranes with different molecular weight cutoffs. A 58% reduction of free dye concentration was achieved in 5mm-long channels.

Spatial control of monolith location allows for creation of periodic discrete monolith segments in the channels of injection molded cyclic olefin copolymer (COC) chips (Mair *et al* 2009). Photopatterning of 100 μ m wide monoliths in regularly spaced intervals leads to enhanced reaction and mixing efficiency. To quantitatively determine mixing efficiency, the fluorescence intensity of the product resulting from a fluorescamine-lysine reaction was measured. Solutions containing lysine and fluorescamine were introduced into the mixing channel through separate arms of a tee-junction by pressure-driven flow. Laser-induced fluorescence intensity was measured 1cm from the tee-junction. Mixing-reaction efficiency of on-chip labeling reactions with the periodic monolith arrangement was found to be 22% more effective compared to open channel or continuous monolith (Figure 4).

Photopatterned monoliths have been demonstrated as particularly useful for sample preparation when decorated with affinity molecules such as antibodies. As a powerful example, affinity integrated monoliths have been used for protein immunoextraction of specific species from complex samples. In one incarnation, monoliths with epoxy groups for antibody immobilization were fabricated inside poly(methyl methacrylate) (PMMA) chips (Sun *et al* 2008). Elution efficiencies of 92% were obtained for the immunoextraction of fluorescein isothiocyanate tagged human serum albumin from complex sample. The extracted protein was then separated using capillary electrophoresis. This platform demonstrates integrated sample pre-treatment and separation on the same device. The same approach has also been demonstrated not only for immunoextraction, but also for protein preconcentration (Yang *et al* 2008). The enrichment capability of the affinity integrated monoliths was evaluated by purifying fluorescently tagged amino acids from a heterogeneous protein mixture containing green fluorescent protein (GFP). A capillary electrophoresis separation revealed that preconcentration using the affinity integrated monoliths resulted in 20-fold enrichments and 90% recoveries of the amino acids and in a reduction of GFP concentration by 4 orders of magnitude.

In addition to protein capture regions, oligonucleotide capture regions have also been demonstrated inside microfluidic devices. A capture region at the head of a separation channel was created using a PA gel that was copolymerized with acrydite-modified oligo capture probes. (Thaitrong *et al* 2009) Capture of polymerase chain reaction (PCR) products allows for the removal of PCR primers to achieve higher signal-to-noise ratios. The device

performs sensitive and high-throughput (75min assay time) PCR on 250nl reactors. Photopatterning provides this approach with several advantages over previous approaches that loaded a capture gel through a sidearm (Paegel *et al* 2000; Paegel *et al* 2002; Toriello *et al* 2007). Firstly, photopatterning of the oligonucleotide capture region avoids meticulous gel loading protocols needed for liquid gels. Secondly, the in-line PA capture region does not extend into the separation channel by thermal expansion during the release step. This expansion is observed in the sidearm design) and results in significant band broadening and distortion (inline design bands are 2000 μ m narrower Thirdly, the inline capture and separation region avoids biases inherent to cross-injection geometries. These advantages provide a 25-fold increase in separation efficiency yielding efficient multiplexed assays. Oligonucleotide capture regions have also been created using PA gels copolymerized with streptavidin (Yeung *et al* 2009). Subsequent to PA gel polymerization, biotinylated oligonucleotides were immobilized via streptavidin in the gel regions, thus avoiding the need to copolymerize oligonucleotides directly into the gel matrix.

3.2 Single-stage biochemical assays

Single-stage biomolecule separations were some of the first modalities demonstrated using lab-on-a-chip approaches. (Harrison *et al* 1993) Mobility-based separations are frequently used for biomolecule identification. Performing on-chip separations allows for the application of higher electric fields thus yielding high resolving power. Compared to slab gels, in which separations can take hours to complete, protein separations in cross-linked uniform PA gels fabricated inside microfluidic chips occur on the order of seconds to minutes (Herr and Singh 2004). Microfabricated nanostructures have also been employed as sieving matrices for biomolecule separations (Fu *et al* 2007). The pore size in these microfabricated matrices can be controlled more precisely than in PA gels. PA gels have the advantage of being cheaper and much easier to fabricate since no dedicated microfabrication equipment is necessary.

Gradual transitions in gel pore-size along a microchannel can also be obtained using photopatterning of PA gels. The fabrication of gradient pore-size PA gels in separation channels includes a combination of multi-step patterning and diffusion to form the gel gradient. First, a high density gel plug is photopolymerized specifically at the end of the separation channel via photomasking. This gel plug eliminates hydrodynamic flow in the separation channel which allows for the introduction of low percentage acrylamide precursor solution to different regions. Acrylamide monomer diffusion then forms a percentage gradient that is photopolymerized after a specified time interval. Gel density gradients along the length of separation channels have been used to size proteins within a wide range of molecular weights (20 to 116kda) in short lengths of only 0.3cm. The wide range of proteins that can be separated means heterogeneous biological samples can be processed in the same assay. The short separations lengths ensure low electrical potential requirements (less than 300V) for the creation of the high electric fields necessary for the fast separations (Lo *et al* 2008). Devices that utilize photopatterned structures in channel volumes for separations and other single-stage assays are summarized in Table 3.

A special class of mobility-based separations (called homogeneous immunoassays) enables identification and quantitation of specific biomolecules by combining antibody-based immune-complex formation and electrophoretic separations. To conduct a homogeneous electrophoretic immunoassay, fluorescently tagged antibody is incubated with a sample and if the antigen is present in the sample, a complex is formed. During subsequent electrophoretic analysis of the sample mixture, complex formation can be detected due to mobility differences between the complex and remaining free (unbound) antibody. Homogeneous electrophoretic immunoassays in PA sieving gels (integrated with sample

enrichment at a PA gel membrane) have been demonstrated as a platform for rapid assessment of protein biomarkers in human saliva (Herr *et al* 2007).

High gel density gradients have also been used for pore-limit electrophoresis (PLE), whereby proteins of different sizes are serially pseudo-immobilized along the axis of separation as each species reaches a gel pore size that prevents its further migration. PLE has been used for sample fractionation and enrichment (Sommer *et al* 2009). Optimized size exclusion of particular proteins has led to fractionation of complex biological samples. Additionally, the decreasing pore size leads to sample stacking and concentration increases of 3 orders of magnitude as samples migrate through the gradient gel.

Photopatterning allows for the creation of discontinuous gel density distributions within single or multiple channels and chambers. For microfluidic electrophoretic separations, a lower gel density in chip regions used for loading of samples is ideal, as this design offers fast loading of most protein species. Transition from a low to high density gel in a separation channel used for electrophoresis offers higher separation resolution. Such gel density discontinuities result in stacking of sample due to the decrease in mobility of the species upon entering the higher density region. The optimization of these discontinuous interfaces has further reduced separation times (<10s) and separation lengths (as low as 350µm). Assay sensitivity using fluorescence detection has been demonstrated in the 10ng/ml range (Hou and Herr 2010). Decreasing device footprints (owing to ultra short separation lengths) further increases device design flexibility and decreases power requirements potentially fostering transition of electrophoresis to point-of-care settings.

Photopolymerization of PA has been used to control the spatial distribution of different monomers along the gel. Acrylamido buffers (Immobilines) of different pK enable the creation of immobilized pH gradients along a gel. The linear distribution of each Immobiline forms a particular pH gradient if the proper boundary conditions are present. The pH gradients are first established inside a microchannel using diffusion. These pH gradients are then immobilized by incorporation of these Immobilines responsible for the pH gradient into the gel structure. Immobilized pH gradients have been used to perform on-chip isoelectric focusing of different pH markers in less than 20 minutes (Sommer *et al* 2008). On-chip IEF occurs faster compared to macroscale IEF due to the shorter length along which focusing occurs. Performing isoelectric focusing (IEF) inside a gel structure ensures transport is purely electrokinetic since the gel prevents hydrodynamic bulk flows. Performing isoelectric focusing on-chip enables fast separations that can be integrated into multi-step assays.

Biomolecules such as enzymes have been specifically immobilized on spatially separate regions on monoliths (Logan *et al* 2007). Specific 750 µm regions are patterned in sequential steps (Figure 5). Three different enzymes were immobilized in separate patches on monolith surfaces following spatially controlled photo activation of the monolith. Directional synthesis of product was observed only when these enzymes were placed in the correct order within the channel. Having separate reactions allows for measuring the activity of different enzymes on the same device and can be applied toward the creation of synthetic multiple-enzyme synthesis pathways. The immobilization of enzymes on monoliths has been specifically discussed in another review (Krenkova and Svec 2009).

Another application of affinity integrated monoliths is immunosensing (Liu *et al* 2010a). The porous morphology of the monoliths allows for sufficient mass transport and provides increased surface area for antibody-antigen interactions when compared to the channel surface. Using immunoglobulin G (IgG) and Anti-IgG as a model immunosensing system, the authors demonstrate that the monolith sensor exhibits a linear response within the 0.1 – 50.0ng concentration ranges with 5 minute assays times and has a sensitivity of 700aM. The

authors used a reaction process including thiol treatment, acid hydrolysis, and heterobifunctional crosslinker grafting for the antibody immobilization, thus avoiding the commonly used diamine-dialdehyde linking process immobilization that can lead to problems such as protein denaturing and multimer formation.

Post-polymerization modifications of monoliths allow for the creation of pH independent monolith structures as well as ensuring that the desired functionalities are fully available on the monolith surface (Krenkova *et al* 2009a). The modification of monoliths via the UV initiated photografting of 2-acrylamido-2-methyl-1-propanesulfonic acid or acrylic acid allows monoliths to be used for cation exchange chromatography in both strong and weak modes respectively. Simple separations of proteins and protein digest have been performed as proof-of-concept experiments in capillaries.

Fast separations on modified monolithic polymer layers have been demonstrated (Woodward *et al* 2010). Electrophoresis separations on unmodified generic monolithic layers were shown to be possible in one minute. The generic hydrophobic monolith is modified by photografting of a charged layer consisted of 2-acrylamido-2-methyl-1-propanesulfonic acid and 2-hydroxyethyl methacrylate. This modification enabled the monoliths to be used in pressurized electrochromatography and to perform separations in this mode in two minutes.

3.2 Multi-stage biochemical assays

Single-step assays can identify target biomolecules by one characteristic such as charge, size, affinity, or electrophoretic mobility. The ability to sequentially assay multiple characteristics in one sample allows for the identification and separation of more biological species, as is especially relevant for complex samples and/or analytes with similar characteristics. Multi-stage separations also allow for more information to be extracted about a single target of interest. For example, the combination of molecular sizing with charge-state information from IEF allows for more specific separations of protein species, allowing for the detection of specific isoforms and post-translational modifications. These multidimensional separations are possible by using photopatterned gels to create regions inside chips with different buffers (Yang *et al* 2009). The creation of gels in only certain regions of the channels allows for removal of hydrodynamic flow, as well as reagent storage and metering. Discrete gel plugs have been used as reagent container anolyte, catholyte, and sample media for IEF. Such control allows for the creation of platforms that are able to perform isoelectric focusing and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on the same device. This approach and others that utilize photopatterned structures to enable multi-stage assays are summarized in Table 4.

As discussed previously, PA gel density gradients and PLE have been used for molecule sizing. This technology has also been applied to zymography, allowing on-chip specific enzyme identification without the use of antibodies and the measurement of enzyme activity (Hughes and Herr 2010). Enzymes are loaded into a single channel with a gradient PA gel. Resolved species are then pseudo-immobilized due to size exclusion. Subsequently, substrates are loaded on the same channel. Enzyme activity is measured through the detection of the fluorescent product created as the substrate arrives to the pseudo-immobilized enzymes. This assay allows for the measurement of enzyme activity in a fully quantitative manner, and allows for substrate detection with sensitivity to low zeptomole amounts of enzyme present. The pseudo-immobilization of enzymes as opposed to chemical immobilization does not greatly affect the catalytic activity of the enzymes being used.

In addition to being able to spatially control gel-density, spatially non-uniform functionalization of different molecules on the gel is also possible, thus enabling integration

of separations with heterogeneous assays (i.e., immobilized antibodies). Automated protein blotting assays have been demonstrated using protein blotting regions adjacent to PA separation gels for protein electrophoresis. Blotting regions are created by covalently linking streptavidin to the PA gels during the polymerization process. Subsequently, biotinylated antibodies were immobilized to the streptavidin-functionalized gel regions. Use of a 2D chamber format allows for separation of species based on mobility difference and transfer of the resolved species to the blotting region (Figure 6). Analogous bench top approaches such as Western blotting are an extremely powerful and widely-used experimental assay. The microfluidic approach overcomes several of the limitations of the slab gel modality by automating the otherwise laborious workflow, thereby reducing human intervention, antibody requirement (1ug vs. 10ug), and assay time (from days to minutes) (He and Herr 2009; He and Herr 2010).

The ability to perform pressure-driven and electrokinetic assays on one device offers a versatile analytical tool set (Liu *et al* 2010b). For example, such capability allows integration of pressure flow driven reversed-phase liquid chromatography (RPLC) with the equilibrium electrophoresis technique, IEF. In this work polymethacrylate monolith frits were employed as physical retention systems to localize silica particles to specific regions of microchannels for assays including RPLC, as monoliths allow hydrodynamic flows while exhibiting size exclusion capabilities.

Methacrylate monoliths have been developed for solid-phase extraction (SPE), which is important for sample pretreatment steps like enrichment and clean-up (Liu *et al* 2009). SPE has been coupled to photopatterned polymethacrylate monoliths that serve as separation columns for liquid chromatography. Furthering the concept of integration, solvent splitting was also performed on-chip, enabling the use of a standard liquid chromatography pump for the generation of solvent gradients. Demonstrated on cyclic olefin polymer chips (COP), the HPLC system was shown to withstand high pressures (>20MPa) and performed efficient high-performance liquid chromatography (HPLC) separations. The online integration of SPE with HPLC enabled removal of free dye and enrichment of labeled protein which lead to a 150-fold increase in sensitivity.

Integration of planar monoliths with different properties has also been demonstrated (Han *et al* 2010). In one compelling application, ion exchange chromatography (IEC) was performed in a virtual hydrophilic channel followed by reverse phase chromatography in the transverse direction on a planar hydrophilic region. The virtual channel was photopatterned on a super hydrophobic 50 um thick monolith layer. The hydrophilic region was formed by wetting of the monolith with a mixture containing 2-acrylamido-2-methyl-1-propanesulfonic acid, 2-hydroxyethyl methacrylate, and benzophenone followed by UV exposure through a photomask. The creation of the virtual channel enables two-dimensional thin layer chromatography for the separation of peptides. Proof-of-concept separations for peptides were undertaken and demonstrated.

3.3 Post-analysis processing and interfacing

Mass spectrometry (MS) is a powerful and extensively-used technology for chemical verification and the identification of unknown substances. Performing sample preparation and separations is frequently necessary prior to MS. Devices that use photopatterned materials to interface with or process samples prior to MS are summarized in Table 5 and discussed in this section. Photopolymerizing a 5 mm lauryl acrylate-coethylene dimethacrylate monolith that caps the end of a separation channel has been demonstrated as a means to integrate on-chip separations with MS (Bedair and Oleschuk 2006). Spraying directly from the end of a chip eliminates dead volumes associated with inserted emitters or transfer lines. The monolith acts as an electrospray emitter and yielded as much as a 70-fold

increase in total ion current stability and sensitivity compared to spraying directly from an open channel. The porous nature of polymer monoliths was reported to further improve coupling performance, as the monolith emitter eliminated sample-related clogging encountered with traditional nanospray emitters. The monolith also enables important upstream sample preparation steps prior to MS (i.e., preconcentration and desalting).

Poly-(glycidyl methacrylate-co-ethylene dimethacrylate) PPM in capillaries have been modified for the immobilization of proteolytic enzymes used for sample digestion prior to MS (Krenkova *et al* 2009b). Using a two-step photografting protocol, two layered structures were fabricated on the monolith surface. The first layer was highly hydrophilic and used to prevent non-specific absorption of proteins. The second layer consisted of a reactive monomer that was used to immobilize the digestion enzymes (trypsin and endoproteinase LysC). The digests performed typically required shorter times and lower temperatures when compared to enzymes in free solution.

Other monolith surface modifications enable protein deglycosylation (Krenkova *et al* 2009c). Photografting of ethoxylated hydroxyethyl methacrylate was followed by photografting of 2-vinyl-4,4-dimethylazlactone and subsequent immobilization of peptide-N-glycosidase F allows for the independent separation and deglycosylation of proteins. The glycans released by deglycosylation were then analyzed by MS. The authors claim that their approach offers a support that does not contain charged groups and therefore eliminates interference by non-specific ionic interactions.

6. Microfluidic devices made from photopatterned materials

The ability to photopattern structures with a high degree of spatial precision can also be employed for the direct fabrication of microfluidic devices, features, and channels. Examples of fabrication approaches adapted to a variety of bioassay applications are summarized in Table 6.

Photopatternable silicone (PPS) has been used to fabricate microfluidic channels and combines advantages of SU-8 and PDMS devices (Desai, Taff *et al* 2008). Like SU-8 patterning, use of PPS enables creation of free standing structures. Free standing structures are useful for fluid processing steps such as valving. Like PDMS, PPS is an elastomeric material. Interfacing of elastomeric materials with pumps for pressure-driven flow is convenient and leak-free. The authors demonstrated biocompatibility and low autofluorescence of PPS, as well as the ability to fabricate substrate electrodes on the PPS devices (Figure 7). PPS possesses many useful properties as a device substrate for bioassays.

The creation of microfluidic channels is also possible using liquid-phase photopolymerization. In one example, isobornyl acrylate (PIBA) was introduced between a glass slide and a cover slip using capillary action (Mohanty *et al* 2006; Mohanty *et al* 2009). Using mask-based photopatterning, UV exposed regions are photopolymerized while masked PIBA regions are not. The unpolymerized PIBA inside the formed channel structure can be vacuumed out. The creation of channels using this approach is fast and does not require microfabrication equipment or a clean room. The technique has been applied for the creation of a device for the electrophoretic separation and collection of DNA of different molecular weights. Surface tension-driven flow (passive pumping) was used for the sample loading. Collection efficiencies up to 85% have been demonstrated. The simple device fabrication and fraction collection offer a potentially viable alternative to using slab gel formats in biological laboratories.

PDMS is one of the most commonly used materials used for microfluidics. PDMS devices are usually cast by soft lithography using SU-8 mastermolds. Recent advances have

improved the direct photopatterning of microchannels on PDMS (Bhagat *et al* 2007, Jothimuthu *et al* 2009). Adding benzophenone as a photoinitiator to PDMS makes PDMS susceptible to light UV light. This approach has been used for the creation of 100µm microchannels, free standing structures, and >30 µm thin films. Using benzophenone as the photoinitiator provides several advantages over the original photoPDMS patterning approaches (Lötters *et al* 1997). Namely, the PDMS is not susceptible to ambient light and oxygen. To ensure biocompatibility of these devices though, an extraction step is necessary to remove unreacted benzophenone from the PDMS. More recent approaches have demonstrated direct patterning of PDMS using direct UV lithography without the need for a reactive photoinitiator (Scharnweber *et al* 2011). By direct UV patterning followed by a wet etching step features 2–5µm features with 10µm depths can be achieved. This gain in resolution is attributed to exposing already cured PDMS which enables direct contact mode compared to the non-contact, liquid phase mode in used previous approaches. These photoPDMS approaches enable quicker prototyping of microfluidic devices because they bypass the need to fabricate a mastermold. PDMS might not be the ideal material for bioanalytical devices due to problems of material loss and absorption into the PDMS.

SU-8 is one of the most widely used photopatternable materials. While it is frequently used to create master molds for soft lithography, a lot of innovation has taken place for it to be also used for the creation of intricate microfluidic channels and features themselves. (Lin *et al* 2002, Carlier *et al* 2004, Kowpak *et al* 2010) SU-8 does not have all of the ideal properties for bioanalytical platforms. It is not optically transparent and its processing requires dedicated cleanroom equipment. The biggest advantage of SU-8 for bioanalytical microfluidic platforms is that it can be integrated directly with biosensors. SU-8 microchannels designed for continuous flows with pressure drops up to 1000kPa have been integrated with Mach-Zehnder Interferometer nanophotonic interferometric label free sensors (Blanco *et al* 2006). SU-8 photopatterning has also been employed to create oxygen sensor rings and cell trapping structures for the measurement of single cell oxygen consumption (Etzkorn *et al* 2010). Simple microfluidic devices made out paper are becoming increasingly popular. SU-8 soaked paper is photopatterned for the definition of hydrophobic channel boundaries (Martinez *et al* 2008). Colorimetric glucose and protein assays have been implemented in these devices. For more information on paper-based microfluidics please refer to a comprehensive review (Coltro *et al* 2010).

7. Conclusions

Microfluidics promises to provide the next generation of tools for quantitative biochemical assays relevant to life sciences and biomedicine. As a key enabling technology, photopatterning is maturing as a suite of techniques for the creation of structures and features inside fully enclosed microfluidic channels. In particular, photopolymerization allows spatially non-uniform fabrication of specific chemical, biological, and mechanical properties. Although the fabrication of microfluidic devices typically requires expertise and dedicated equipment; photopatterning of polymers has been introduced as an accessible technology for the fabrication of microfluidic channels and devices. Further, photopatterning of materials inside previously fabricated or commercially available devices allows end user device customization using standard laboratory equipment. Microfluidic devices with photopatterned structures are being employed for a diverse set of biological measurements and assays.

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Figure 1.

Typical photopatterning processes. (A) Modifications to channel surfaces by photopatterning. (i) Side view of previously fabricated microchannel made from light-permeable materials such as glass or transparent plastics. (ii) Channel is loaded with solution containing photoreactive species. (iii) Specific channel regions are exposed with UV light using photomasks. (iv) Photo-activated species react with channel walls and become immobilized. (v) Channel is washed to remove unreacted molecules. (vi) Previous steps can be repeated to pattern additional regions. (B) Creation of structures in channel volumes by photopatterning. (i) Side view of previously fabricated microchannel made from light-permeable materials such as glass or transparent plastics. (ii) Channel is loaded with solution containing photoreactive polymer precursors. (iii) Specific channel regions are exposed with UV light using photomasks. (iv) Photopolymerization occurs in regions exposed to UV light. (v) Channel is washed to remove unreacted precursor molecules. (vi) Previous steps can be repeated to pattern additional regions. (C) Formation of microfluidic channels by photopatterning. (i) Device cross section shown. Materials such as glass and silicon are typically used as substrates. (ii) Substrate is coated with photosensitive polymers, resins, etc. (iii) UV exposure of the photosensitive material through a photomask leads to a chemical reaction and the definition of features in the substrate. (iv) Exposed (or unexposed depending on material) material is removed with developing, washing, or etching steps. (v) The formed microchannels are closed by bonding a top layer.

Figure 2.

Spatially controlled photo-removal of MPC exposes discrete regions for cell attachment in microchannel. (A) Phase contrast images of MC-3T3 E1 cell aggregation in the microchannel after 2 h of incubation. (B) Patterned cells flown away after 24 h of incubation owing to cell aggregation. (C) Micro-patterned cells after 24 h of culture. (D) Phase-contrast images of micro-patterned endothelial cells after 24 h of culture, (E) 1 day of culture, and (F) 14 days of culture. (Jang *et al* 2010) - Reproduced by permission of the Royal Society of Chemistry.

Figure 3.

Photopatterned membrane allows for sample processing steps such as preconcentration prior to separation assay. (A) Fluorescence micrographs show the distribution of labeled protein at different times of the preconcentration process as they are driven against the size exclusion membrane. (B) The electric field across is reversed driving proteins away from the membrane. (C) Protein size-based separations by SDS-PAGE. Reprinted with permission from (Hatch *et al* 2006). Copyright (2006) American Chemical Society.

Figure 4.

PPM are photopatterned at regularly spaced intervals. Reaction-mixing efficiency is increased of on-chip labeling reaction compared to open channel and continuous monolith. (A) SEM images demonstrating periodic placement of monoliths in channel cross-section and 100 micrometer patterning resolution. (B) Fluorescence intensity and fluorescence ratio for the different channel configurations. (Mair *et al* 2009) - Reproduced by permission of the Royal Society of Chemistry.

Figure 5.

Sequential biomolecule photo immobilization steps enable serial enzyme linked regions for multi-stage on-chip synthesis. Consecutive patterning of GFP patches on a polymer monolith. The top images show the location of immobilized GFP and the bottom graph shows the fluorescence intensity along the monolith. Reprinted with permission from (Logan *et al* 2007). Copyright (2007) American Chemical Society.

Figure 6.

Different PA gel regions (loading, separation, blotting) are photopatterned in the device enabling automated separation and blotting. (A) Brightfield image of the glass device. (B) Magnified view of the chamber containing the different PA gel regions. The loading gel is shown in blue, the PAGE separation region in red, and the antibody functionalized blotting region in green. (C) Protocol steps overlaid on device micrographs. (D) Inverted grayscale image demonstrated band stacking at loading-separation interface. μ is the mobility and i indicates direction of electric current. Reprinted with permission from (He and Herr 2010) Copyright (2006) American Chemical Society.

Figure 7.

Microfluidic channels and free standing structures are fabricated on PPS. PPS also enables substrate electrode integration, is biocompatible, and has low-autofluorescence. (A–C) Schematics and bright field image of PPS structure aligned to an aluminum layer. Scale bar = 500 micrometers. (D–F) Bright field image, schematics, and fluorescent images showing alignment and operation of simple dielectrophoretic structure. Scale bar D = 200 micrometers. Scale bar E, F = 50 micrometers. Reprinted with permission from (Desai *et al* 2008). Copyright (2008) American Chemical Society.

Table 1

Modifications to channel surfaces

Function	Device materials	Modification by photopatterning	References
Single and multistep enzyme studies	PDMS/glass chip	Patches created by bleaching of biotin-dye species that attach to protein coated surface	(Holden <i>et al</i> 2004)
DNA hybridization, immunoassays, protein interaction assays	Fused-silica capillary, PDMS/glass	Benzophenone on surfaces forms covalent bonds with solute molecules upon photoactivation, deep UV photopatterning of protein layer(s)	(Balakirev <i>et al</i> 2005; Shi <i>et al</i> 2008)
Cell culture, expression studies	Mechanically etched glass	Selective photo- removal of MPC reveals areas for cell attachment	(Jang <i>et al</i> 2009; Jang <i>et al</i> 2010)
Virtual channels, pressure sensitive gates	Glass capillary, PDMS/glass	Selective photocleaving of bonds in FSAM reveals hydrophilic surface	(Zhao <i>et al</i> 2002; Zhao <i>et al</i> 2003)

Table 2

Formation and modification of structures in channel volumes for sample preparation

Function	Device materials	Modification by photopatterning	References
Preconcentration enrichment	Etched glass, hot-embossed PMMA	PA and polymer membranes, affinity integrated monoliths	(Hatch <i>et al</i> 2006; Song and Singh 2006; Herr <i>et al</i> 2007; Yang <i>et al</i> 2008)
Dialysis, desalting, washing, clean-up	Etched glass, PDMS/glass	PA membranes, PA capture gels	(Song <i>et al</i> 2003; Song <i>et al</i> 2004; Thaitrong <i>et al</i> 2009; Yeung <i>et al</i> 2009)
Immunoextraction	Hot-embossed PMMA		(Sun <i>et al</i> 2008)
Enhanced reaction, mixing for labeling	Injection molded COC	Photopatterned monoliths	(Mair <i>et al</i> 2009)

Table 3

Photopatterned structures for single-stage assays

Function	Device materials	Modification by photopatterning	References
PAGE for separations, homogeneous immunoassays, fractionations	Etched glass	PA gels with discontinuous density interfaces and decreasing density gradients	(Herr and Singh 2004; Herr <i>et al</i> 2007; Lo <i>et al</i> 2008; Sommer <i>et al</i> 2009; Hou and Herr 2010)
IEF	Etched glass	PA gels with immobilized pH gradients	(Sommer <i>et al</i> 2008)
Synthetic multi-enzyme synthesis	Fused-silica capillary	Photopolymerized monoliths later modified by photografting	(Logan <i>et al</i> 2007)
Immunosensing	Fused-silica capillary	Affinity-integrated monoliths	(Liu <i>et al</i> 2010a)
Chromatography	Monoliths in fused-silica capillaries, thin monolith layers on glass plates	Surface modifications of monoliths by photografting	(Krenkova <i>et al</i> 2009a; Woodward <i>et al</i> 2010)

Table 4

Photopatterned structures in channel volumes enabling multi-stage assays

Function	Device materials	Modification by photopatterning	References
IEF and SDS-PAGE	Micromilled PMMA	PA gels with discontinuous buffers	(Yang <i>et al</i> 2009)
PLE and enzyme assay	Etched glass	Gradient PA gels	(Hughes and Herr 2010)
SDS-PAGE and immunoblotting	Etched glass	Functionalized PA gels	(He and Herr 2009; He and Herr 2010)
IEF and RPLC	Micromilled COP	Photopolymerized monolith frits to hold silica-particles	(Liu <i>et al</i> 2010b)
SPE and HPLC	Hot-embossed COP	Surface modifications of monoliths by photografting	(Liu <i>et al</i> 2009)
IEC and RPLC	Thin monolith layers on glass plate	Surface modifications of monoliths by photografting	(Han <i>et al</i> 2010)

Table 5

Photopatterned structures for processing samples and interfacing with MS

Function	Device materials	Modification by photopatterning	References
Electrospray emission directly from end of microchannel	PDMS, COC, PMMA	Photopolymerized monolith at end of channel	(Bedair and Oleschuk 2006)
Enzyme digestion prior to MS	Fused-silica capillary	Surface modifications of monoliths by photografting	(Krenkova <i>et al</i> 2009b)
Deglycosylation of proteins for MS analysis of released glycans	Fused-silica capillary	Surface modifications of monoliths by photografting	(Krenkova <i>et al</i> 2009c)

Table 6

Devices

Function	Device materials	Modification	References
Elastomeric, biocompatible microfluidic devices with free-standing structures, electrodes	Photopatternable Silicone	Direct photopatterning of structures and channels on PPS	(Desai <i>et al</i> 2008)
DNA sizing and collection	Isobornyl acrylate between glass slide and coverslip	Liquid-phase polymerization of PIBA	(Mohanty <i>et al</i> 2006; Mohanty <i>et al</i> 2009)
Elastomeric, multilayer, inexpensive microfluidic devices for rapid prototyping	PDMS/glass	Direct photopatterning of PDMS	(Bhagat <i>et al</i> 2007, Jothimuthu <i>et al</i> 2009, Scharnweber <i>et al</i> 2011)
Inferometric label free sensing	SU-8/silicon in PMMA housing	SU-8 photolithography	(Blanco <i>et al</i> 2006)
Single cell oxygen consumption sensing	SU-8/glass	Photopatterned oxygen sensor rings and photopatterned SU-8 traps	(Etzkorn <i>et al</i> 2010)
Glucose and protein assays	Paper, tape, SU-8	SU-8 soaked onto paper is patterned to define channels	(Martinez <i>et al</i> 2008)