Advances in monoliths and related porous materials for microfluidics

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In recent years, the use of monolithic porous polymers has seen significant growth. These materials present a highly useful support for various analytical and biochemical applications. Since their introduction, various approaches have been introduced to produce monoliths in a broad range of materials. Simple preparation has enabled their easy implementation in microchannels, extending the range of applications where microfluidics can be successfully utilized. This review summarizes progress regarding monoliths and related porous materials in the field of microfluidics between 2010 and 2015. Recent developments in monolith preparation, solid-phase extraction, separations, and catalysis are critically discussed. Finally, a brief overview of the use of these porous materials for analysis of subcellular and larger structures is given. Published by AIP Publishing. [<http://dx.doi.org/10.1063/1.4948507>]

I. INTRODUCTION

Porous monoliths have been explored intensively for more than two decades, resulting in development of various materials possessing unique properties along with distinct morphologies.¹ Extending the surface area is a key aspect in many applications like separations,² extraction,³ purification of both small molecules and macromolecules,⁴ filtration of bioparticles,⁵ and immobilization of enzymes, antibodies or cells.⁶

With the advent of microfluidics, monoliths were implemented in microchannels, providing an easily fabricated and versatile support needed in many research areas. Initial publications showed potential for use in solid-phase extraction (SPE) and electrochromatography,⁷ pressure-driven systems,⁸ micromixers,⁹ catalysis,¹⁰ and in a variety of biological applications.¹¹ The versatility of microfluidics provides the potential to integrate processes often necessary to study biological samples, reducing complex sample treatment. Significant effort has been put not only into miniaturization, but also into the development of automated systems in biochemical, proteomic, pharmaceutical, and medical applications.¹²

One of the main advantages of monoliths lies in their ease of preparation. A wide range of methods have been developed;¹³ the most common approach is where one or more monomers and a crosslinker are mixed with porogenic solvents and an initiator. Reactive precursors are soluble in porogens, but the formed polymer becomes insoluble during its growth, and as a consequence, a porous structure is obtained. Careful selection of conditions leads to formation of materials with different porosities that suit the desired use. Mostly, *in-situ* preparation using UV-photoinitiated radical polymerization is employed as it only requires common laboratory equipment. This approach further enables spatial placement of the monolith and better control over polymerization parameters than thermally initiated polymerization. However, the obtained porous structures can differ from those formed by bulk polymerization due to confinement in microchannels.¹⁴ Compared to particle-packed columns, monoliths do not need frits because the formed polymer is anchored to the microfluidic channel wall. An interconnected network of pores provides columns with low backpressure and mass transfer dominated by convection,

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resulting in faster analysis with enhanced chromatographic performance for large biomolecules with low diffusivity.¹⁵

Using different precursors, various surface functional groups can be obtained or subsequently tailored for a desired application. A broad range of organic monomers were used for preparation of structures with very high porosity and providing biocompatible supports.¹¹ On the other hand, inorganic monoliths present an alternative with different properties, and silica monoliths have been explored widely in separation science due to their high surface area.¹⁶

The microfluidic device material plays an important role in attaching the prepared monolith. Poly(methylmethacrylate) (PMMA) is often used with methacrylate monomers, providing enough surface reactive sites to anchor the formed polymer.¹⁷ Silica monoliths were successfully made in polydimethylsiloxane (PDMS) microfluidic channels.¹⁸ Silanization of glass chips is performed in order to introduce reactive groups, similar to treatment of silica surfaces.¹⁴ Poorly reactive substrates like cyclic olefin copolymer (COC) require surface activation with hydrogen abstracting initiators as benzophenone.¹⁹

This article details progress in the field of microfluidics involving monolithic stationary phases. Articles between 2010 and 2015 were considered; earlier work was summarized in an excellent review.⁷ Articles involving only channel wall coatings, packed columns or pillar structures are not included here, as other recent reviews cover these areas.^{20–22} Advances in monolith material development, and use for SPE, separations, and reactors are reviewed, as are selected papers on the use of soft porous materials in analyzing for subcellular and larger biostructures.

II. MATERIAL DEVELOPMENT

A. Organic porous polymers

Typical monomers for preparation of organic monoliths are based on methacrylates,²³ acrylates,¹⁹ or styrenes.²⁴ The latter, however, are difficult to use with photoinitiated polymerization due to the UV absorbance of the monomer, preventing penetration of light into the polymerization mixture. Methacrylate-based monomers modified with multiple functional groups are widely available, and formulations for many applications have been studied and optimized.¹

Reproducibility of flow properties in monoliths based on 2-hydroxyethyl methacrylate and ethylene dimethacrylate (EDMA) using 1-octanol as the porogenic solvent was found to be better compared to previously developed formulations.²³ The authors introduced a technique for characterization of flow based on photobleaching of plugs of a fluorescent dye, and these plugs were then detected downstream, which allowed easy determination of the linear flow rate. Interday repeatability of flow rates was found to be lower than 2% RSD, and batch-to-batch variation was 5% RSD, both achieved in a narrow range of composition of the polymerization mixture. The authors suggested that flow stability was largely affected by the amount of the crosslinking monomer in the polymerization mixture, providing higher mechanical strength.

Templating with a sacrificial polystyrene colloidal crystal was exploited for fabrication of a polyaniline-based structure that was grown electrochemically around the sacrificial material in a glass chip.²⁵ Various monolith morphologies were obtained by varying the electropolymerization time, providing extended surface area that could be utilized in many lab-on-a-chip applications taking advantage of this conducting polymer, a non-traditional material in microfluidics.

Recently, several groups have developed strategies for fabrication of organic porous polymers in PDMS channels. The properties of PDMS are not favorable for anchoring of monoliths due to its swelling in organic solvents, absorbing hydrophobic compounds (i.e., initiator) into the bulk material, and permeability to oxygen that can act as a radical inhibitor. The usual procedure for modification of poorly reactive surfaces involves grafting using benzophenone that diffuses into the bulk material and upon irradiation produces free radicals via hydrogen abstraction. Chan *et al.*²⁶ prepared a monolith with glycidyl methacrylate (GMA) using thermally initiated polymerization with azobisisobutyronitrile in a benzophenone-treated channel. This monolith was then modified with diethylamine to provide a weak anion-exchange column, and experimental conditions were investigated for binding of bovine serum albumin (BSA) and

ovalbumin. Photografting approaches for monolith anchoring on PDMS are often irreproducible, leaving gaps between the channel surface and monolith. Burke and Smela developed a procedure that in the first step modifies the surface of a $200\ \mu\text{m} \times 70\ \mu\text{m}$ channel with a polymer that is entangled within PDMS as shown in Figure 1(a).¹⁸ In the second step, a monolith is polymerized within the surface-modified channel (Figure 1(b)), providing anchoring that withstands pressure exceeding the bonding strength of PDMS. 2,2-Dimethoxy-2-phenylacetophenone in chloroform was used to swell PDMS and adsorb the photoinitiator that triggered polymerization of monomers only within the PDMS. This monolith was used to concentrate and mechanically lyse B lymphocytes. However, such a procedure could not be readily used in much smaller channels as they could be blocked during swelling. A single-step approach for monolith anchoring was introduced by Araya-Farias *et al.*²⁷ who studied several photoinitiators for preparing a monolith from a novel monomer, ethylene glycol methacrylate phosphate. It was found that a high concentration of photoinitiator, in this case 2-methyl-4'-(methylthio)-2-morpholino-propionophenone, provided efficient hydrogen abstraction from the channel surface resulting in a robust anchoring of the monolith to the PDMS wall.

B. Silica monoliths

The traditional procedure for preparation of silica monoliths consists of hydrolysis and condensation of silicon alkoxides.¹⁶ The requirement of thermal treatment associated with silica monoliths makes it difficult to transfer capillary methods directly into the microfluidic chip format since the stationary phase needs to be selectively localized. In an initial demonstration of overcoming this difficulty, Jindal and Cramer²⁸ developed a method for selective filling in which a reversibly bonded PDMS slab was attached to a part of the microfluidic channel into which a suspension of silica particles in a sol-gel solution containing tetraethoxysilane (TEOS) was introduced. After gelation, the PDMS slab was peeled off, and the chip was thermally treated and sealed.

FIG. 1. Scanning electron micrographs of porous polymers in PDMS microfluidic channels. (a) Modified PDMS surface with a thin layer of methyl methacrylate and EDMA. (b) BMA-EDMA monolith successfully anchored to the PDMS channel wall. Adapted with permission from Biomicrofluidics 6, 016506 (2012). Copyright 2012 AIP Publishing LLC. (c) Silica monolith in PDMS microfluidic channel. (d) Detail of silica monolith. Adapted with permission from Levy *et al.* Chromatographia 76, 993 (2013). Copyright 2013 Springer.

A thermally activated silica-based monolith made from potassium silicate solution was prepared by Shaw *et al.*²⁹ To ensure that the monolith was prepared only at a desired location, the rest of the glass microfluidic device was filled with glycerol, which was removed after thermal treatment. Alzahrani and Welham³⁰ prepared a monolith made of tetramethylorthosilicate outside a microfluidic device, and then a disk of the monolith material was placed inside a chamber, providing increased surface area for immobilization of tris(2-carboxyethyl)phosphine hydrochloride, which was used to reduce disulfide bonds in proteins.

Levy *et al.*³¹ photopolymerized silica sol-gel monoliths in PDMS-based microfluidic devices using 3-trimethoxysilylpropylmethacrylate and glycidyoxypropyl trimethoxysilane. The best results were obtained using Irgacure 1800 as photoinitiator and ethylene glycol diglycidyl ether as porogen in a ratio of 28% monomer to 72% porogen. Calcium chloride was added as a phase separation additive in order to incorporate higher amounts of the epoxide containing silane. Electron micrographs of a prepared monolith are shown in Figures 1(c) and 1(d).

C. New preparation approaches

In-situ preparation is one of the most commonly mentioned advantages of monolithic stationary phases, allowing fast and easy formation in only a certain part of a microfluidic channel. Kendall *et al.*³² reported *ex-situ* formation of methacrylate-based monoliths in a microfabricated mold followed by integration of a modified monolith during COC chip assembly utilizing solvent-assisted bonding (Figures 2(a) and 2(b)). This procedure allowed easy trimming and control of size and shape. The authors reported that they achieved good uniformity together with good adhesion to the channel wall. Parallel preparation would enable batch fabrication of

FIG. 2. Novel monolith preparation approaches. (a) *Ex-situ* prepared GMA-co-ethoxylated trimethylolpropane triacrylate monolith. (b) Scanning electron micrograph of monolith incorporated into COC microfluidic channel. (c) Two monoliths integrated into the same channel; (left) attached rhodamine, (right) attached FITC-IgG. Adapted with permission from Kendall *et al.*, Sens. Actuators, B **202**, 866 (2014). Copyright 2014 Elsevier. (d) Preparation of a porous polymer monolith *in-situ* in a DMF device. A droplet from the monomer reservoir is moved to a desired position, where it is photopolymerized. The prepared monolith is used on the same device for SPE and concentrating sample into a smaller volume. Adapted with permission from Yang *et al.*, Anal. Chem. **83**, 3824 (2011). Copyright 2011 American Chemical Society.

monoliths that could be easily modified off-chip, also allowing different chemistries to be used on a single device. This capability was demonstrated by attaching rhodamine- and fluorescein isothiocyanate (FITC)-labeled immunoglobulin in a single microfluidic channel as shown in Figure 2(c).

Shape anchoring was exploited by Nordman *et al.*³³ who used 355 nm laser-induced polymerization for maskless patterning and precise definition of a monolith zone in an SU-8 chip. The laser allowed the polymerization to be finished in 7 min, and the monolith formed at the injection cross did not require any chip surface treatment for anchoring, as it was held in position by its shape. This modified microfluidic chip was coupled to mass spectrometry (MS) for SPE of pharmaceuticals.

Overlap of the chip substrate optical transparency with the absorption maximum of the photoinitiator needs to be maintained for successful preparation of monoliths. Because polyimide lacks transparency in the UV region in which most radical initiators are used, a novel initiator system was developed by Walsh *et al.*³⁴ using 660 nm light emitting diodes. Methacrylate monoliths were prepared and used for separation of a model protein mixture.

A digital microfluidic (DMF) approach was utilized for *in-situ* preparation and use of porous polymer discs as shown in Figure 2(d).³⁵ A droplet of the polymerization mixture was dispensed and manipulated to a central position for UV-initiated polymerization. Next, monolith was washed by actuating a solvent across the disc and was then used for SPE of angiotensin II, with extraction efficiency comparable to other methods. Such an approach may be especially attractive for automated renewal of the extraction phase for sample cleanup and concentration.

Microfluidics enables precise manipulation of liquids, which was exploited for synthesis of porous methacrylate particles.³⁶ Co-flow of monomers and initiator in decyl alcohol and a solution of polyvinyl alcohol and Triton X-100 in water through a droplet generator was used to form droplets that were cured by UV light while travelling through the tubing. These macroporous particles possess higher surface area than traditionally used non-porous particles, allowing more efficient immobilization of antibodies for immunosensing. By using various monomers and adjusting the viscosity of the mixture, synthesis of diverse structures including beads, rods, or hollow capsules was feasible in a different study.³⁷

III. ON-CHIP SOLID PHASE EXTRACTION AND SEPARATION

To realize the unique potential of microfluidic technology, sample preparation is an important step, which includes sample extraction, purification, pre-concentration, and labeling, among many other processes.^{38–41} On-chip polymerized monolith-based SPE has been evaluated considerably for this purpose, primarily due to strong synergy with the mature liquid chromatography field.^{38,39} Porous polymer monolith stationary phases form the basis of this sample preparation approach. Typically, a solid support is immobilized inside the microfluidic channels, which can then be used to extract, retain, and thus purify the captured analytes. Moreover, separations can be done based on differential analyte retention on monolithic stationary phases. Depending on the application, reverse-phase, ionic, and affinity interactions are commonly used to extract analytes from a complex sample matrix, as discussed in Secs. III A–III C. Sample loading is an important aspect of monolith operation, and samples can be loaded either using voltage or pressure with each approach having nuances that are discussed in detail below. A brief overview of the interaction methods, analytes processed, and monolith and device materials used, is provided in Table I.

A. Reverse-phase monoliths

Reversed-phase chromatography retains sample based on hydrophobic interactions with the polymer monolith, such that the retained analyte can subsequently be eluted by introducing a solvent that disrupts this interaction. Depending on the sample loading method used, monoliths may need surface modifications, which are discussed in Secs. III A 1 and III A 2.

TABLE I. Summary of stationary phases, analytes, monoliths, and device materials used in microfluidic SPE techniques. Acronyms are: OMA: octyl methacrylate; HFBA: heptafluorobutyl acrylate; MAA: methacrylic acid; EGDMA: ethylene glycol dimethacrylate; MPTMOS: 3-trimethoxysilylpropyl methacrylate; EPMA: 2,3-epoxypropyl methacrylate; and G-CSF: granulocyte colony stimulating factor protein.

Stationary phase	Analyte	Monolith or porous material	Device material	References
<i>Reverse phase</i>	Peptides, HSP90, BSA, ferritin	BMA, HFBA, OMA, LMA	Glass, COC, silica, PDMS-COC	42, 43, 45–47, 50, and 53
	DNA	Silica	Glass	29 and 49
	Hydrocarbons	LMA	COC	44
	Dopamine, steroids, verapamil	EPMA, BMA, LMA, MAA-co-EGDMA	SU-8, glass, PDMS	33, 48, 51, and 52
<i>Ionic</i>	Peptides, HSP90, α -fetoprotein, albumin, ovalbumin, BSA	GMA, acrylamide	PMMA, glass, PDMS	26 and 54–56
	DNA	GMA	COC	58
	Catechin, 2 amino-4-chlorophenol	MAA-PEGDA, GMA	PDMA, PMMA	57 and 59
	Conalbumin, thrombin, G-CSF, IgG, H1N1, BSA	GMA, MPTMOS, PEGDA, Si nanowires	Glass, PDMS, PMMA, silicon	17, and 61–64
<i>Affinity</i>	Catecholamines	PEGDA	Glass	60

1. Electrokinetic sample loading

As the name suggests this method involves sample loading via electrical means including electroosmotic flow. Device fabrication is convenient with electrokinetic injection, and the simple instrumentation used to apply voltages further makes it an easy to use method; however, for some monolith types, surface modification may be needed to enable flow through the monolith itself. We first describe channel microfluidics for sample pre-concentration and/or separation, followed by a discussion of advances in on-chip labeling, and we conclude with a section on pre-concentration using DMF.

Device design is a key consideration in microfluidic applications. Wang *et al.*⁴² developed an electrokinetic sheath flow assisted, 36-channel glass microfluidic device for the sequential collection, pre-concentration, and elution of model molecules, with minimal cross-contamination. The SPE process and flow inside the fractionation channel region were supported by a sulfonate-modified, hydrophobic butyl methacrylate (BMA) porous polymer monolith. With 2 min loading, 30-fold pre-concentration was observed for FITC-labeled BSA. Hua *et al.*⁴³ developed a glass microfluidic device for multiplex sample pre-concentration and sequential elution to a single exit channel for fluorescence detection using sheath flow assisted electrokinetic operation. To sustain reproducible fluid flow, the channels were coated with a cationic polymer, PolyE-323, and a mixed cationic [2-(methacryloyloxy)ethyl] trimethylammonium chloride and hydrophobic BMA-based porous polymer monolith was fabricated inside the channels for sample pre-concentration. Using these devices, sequential elutions gave a peak area reproducibility of 8%, and the run-to-run reproducibility from the same monolithic bed was <7% RSD. Utilizing the advantages of a COC substrate, Ladner *et al.*⁴⁴ developed a microfluidic device with a lauryl methacrylate (LMA) porous polymer monolith, for reversed-phase electrochromatography of a mixture containing five polycyclic aromatic hydrocarbons. The device provided 4% and 11% RSD for retention time and separation efficiency, respectively, and the chip-to-chip variation was similarly reported to be 5% and 8% RSD, respectively. In a different approach, Xu and Oleschuk⁴⁵ developed a glass microfluidic chip with a heptafluorobutyl acrylate-based porous polymer monolith photopolymerized inside a microchannel. Fluorine-fluorine interactions were used to separate a mixture containing fluorescent dye, fluorescently labeled peptides, and fluorine tagged fluorescently labeled peptides.

Nordman *et al.*³³ developed a new procedure to fabricate a methacrylate-based, cross-shaped, reversed-phase monolith inside an SU-8 microfluidic chip, that combined SPE with microchip electrophoresis (MCE) and electrospray ionization MS (Figure 3). The device provided 20-fold sample enrichment for verapamil with 3% RSD in migration time and 12% RSD for the peak height.

Genomic analysis is increasing in importance in healthcare, often needing to process a limited quantity of DNA sample available. A sample pre-treatment method such as SPE that can concentrate DNA is thus highly desirable. Shaw *et al.*²⁹ developed an automated, integrated glass microfluidic device for DNA sample preparation, including cell lysis, DNA extraction, and PCR amplification. A thermally polymerized silica monolith inside the microfluidic channel was used for DNA extraction, with on-chip sample movement controlled by electroosmotic pumping. A DNA extraction efficiency of 75% was achieved for manually inserted buccal cells that were lysed on-chip. Sample preparation reagents could be stored on-chip at 4 °C for 8 weeks, and this device combined with MCE had potential to be used as a miniaturized kit for forensic and clinical analysis.

Sample labeling is an important step in many bioanalyses, and on-chip labeling of pre-concentrated samples offers important benefits such as increased automation and reduced process time. Nge *et al.*⁴⁶ integrated on-chip pre-concentration and fluorescent labeling of proteins by fabricating a BMA monolith inside a COC microfluidic device. The microchip showed an enrichment efficiency of 10-fold for fluorescently labeled proteins, and the device was used for on-chip fluorescent labeling of proteins. In related work, Yang *et al.*⁴⁷ further developed COC microfluidic devices with integrated reversed-phase monoliths for pre-concentration and on-chip labeling applications. In this work, different monoliths were studied and conditions were optimized for on-chip labeling. Based on the monolith retention and elution properties, octyl methacrylate was chosen. The devices were used for on-chip fluorescent labeling of heat shock protein 90 (HSP90) with Alexa Fluor 488.

Digital microfluidics is an attractive microanalysis tool that avoids some problems such as microchannel blockage that can occur in conventional microfluidics. Recently, Kim *et al.*⁴⁸ reported a DMF method for steroid hormone analysis in human breast tissue samples. This device

FIG. 3. SPE-MCE operation schematic and data. Squares and circles represent hydrophilic and hydrophobic components of the sample mixture, respectively. The hydrophilic cotinine is un-retained, eluted, and separated in steps 1 and 2, and hydrophobic verapamil is retained (steps 1 and 2), eluted (step 3), and separated before MS analysis (step 4). (Bottom) MS data from samples processed using SPE-MCE, with the table showing different process parameters. Adapted with permission from Nordman *et al.*, *Electrophoresis* 36, 428 (2015). Copyright 2015 Wiley.

performed on-chip extraction from the tissue sample, and the extracts were purified using BMA porous polymer monolith discs. Samples were transferred from the DMF device and then analyzed off-chip for estradiol, androstenedione, testosterone, and progesterone steroids.

2. Hydrodynamic sample loading

Fluid can also be manipulated in microfluidic devices through applied pressure. Advantages of pressure loading are that the forces are physical in nature and do not require surface modification to enable flow through a monolith; however, pressure flow can complicate device design and fabrication. We first discuss the use of monoliths addressed using external hardware, and then we describe work with on-chip pneumatic pumps and valves for flow through monoliths.

Kashkary *et al.*⁴⁹ developed a borosilicate glass microfluidic device with silica monoliths fabricated inside the channels. The device was used to pre-concentrate DNA samples (<15 ng) that were subsequently amplified using PCR. Monoliths are especially useful for separations. Pruijm *et al.*⁵⁰ studied BMA and LMA-based monoliths with different monomer concentrations in a fused silica microchip to optimize separation. For this gradient elution study, three monomer concentrations (30%, 35%, and 40%) and three elution times (5, 15, and 30 min) were used to separate a mixture containing 8 model peptides. Device performance was analyzed in terms of peak capacity, and the LMA monolith with 15 min gradient elution gave the highest peak capacity. Wang *et al.*⁵¹ developed a three-T shape glass microchip with a LMA-based monolith for separation with electrochemical detection. The three-T injection shape avoided sample leakage and dilution by improving focusing. The device separated 5-hydroxy-L-tryptophan, dopamine and 5-hydroxytryptamine in 25 min with <5% peak area RSD and a sub- μ M limit of detection.

Kang *et al.*⁵² manufactured a three-layer PDMS-glass microfluidic device with on-chip pneumatic controls and a monolith made of methacrylic acid and ethylene glycol dimethacrylate fabricated inside the microchannel. A hydrodynamically pumped dopamine sample was extracted, eluted, and collected in a different reservoir on chip. The 80-fold enriched sample was then analyzed using MCE and detected electrochemically. In very recent work, Kumar *et al.*⁵³ improved over this earlier study using a 4-layer integrated SPE-MCE system (Figure 4). The PDMS part of the device formed an on-chip peristaltic pump and pneumatic valves for hydrodynamic sample control, and a reverse-phase octyl methacrylate porous polymer monolith for SPE and sample pre-concentration was formed in COC. This device integrated the steps of sample loading, retention, elution to the injection intersection, plug capture and electrophoretic separation. Analysis was performed on a model peptide mixture and ferritin, a pre-term birth biomarker, with 50-fold sample enrichment achieved.

B. Ionic interaction

In another type of SPE method, molecules can be retained or passed through a porous structure based on electrostatic or polar interactions. Porous structures such as charge-selective membranes and charged polymers have been used for extraction and separation. Porous polymer monoliths with a permanent surface charge or one that depends on solution pH or ionic strength can also be utilized. We first discuss the use of ion-selective membranes or charged polymers for pre-concentration, and then focus on SPE and separations that involve polar or ionic interactions on porous polymer monoliths.

Nge *et al.*⁵⁴ photopolymerized an acrylamide, N,N-methylene-bisacrylamide and 2-(acrylamido)-2-methylpropanesulfonate porous hydrogel membrane on a PMMA microfluidic chip. This negative ion selective membrane was used for pre-concentration and electrophoretic separation of cancer biomarkers with 10-fold enrichment for α -fetoprotein and HSP90 in 10 min. Charge selectivity can also be used for electrostatic transmission or pre-concentration. Chun *et al.*⁵⁵ fabricated a negatively charged polymer inside a glass microfluidic device, which selectively transmitted cations from solution while generating an anion pre-concentration region in the near vicinity. The device was used for the pre-concentration of fluorescently tagged albumin and fluorescein.

FIG. 4. A 4-layer microfluidic device with integrated SPE and MCE modules. (a) Device photograph. (b) A micrograph showing the pneumatically actuated PDMS valves around the injector. (c) Electropherograms of on-chip enriched ferritin and peptides. Adapted with permission from Kumar *et al.*, *Analyst* **141**, 1660 (2016). Copyright 2016 Royal Society of Chemistry.

Monoliths can also be functionalized to create charged surfaces, some of whose properties depend on the solution conditions and ionic strength. Mudrik *et al.*⁵⁶ combined DMF with cation exchange SPE to purify proteins and peptides. Sulfonate-functionalized porous polymer monolith discs were prepared using GMA and were used for ion-exchange interactions; sample elution had comparable extraction efficiency to commercial methods ($\sim 30\%$). Lin *et al.*⁵⁷ developed a PDMS device with an ethylenediamine modified GMA-EDMA monolith for the extraction and chemiluminescence (CL) detection of catechin from green tea. In this elutionless study, catechin was pre-concentrated on the monolith with 20-fold enrichment efficiency, and was reacted on the monolith with CL reagent to generate a detection signal. Factors affecting device performance such as CL reagent concentration, solution pH, extraction time, and sample flow rate were optimized to get a limit of detection of 1 nM, with $>90\%$ catechin extraction efficiency from green tea. Using the pH-dependent charge-switching behavior of polysaccharides, Kendall *et al.*⁵⁸ developed a COC microfluidic chip with chitosan modified GMA monoliths for nucleic acid extraction. The device was used to process DNA with 100 ng loading and 55% extraction efficiency. Chan *et al.*²⁶ manufactured a PDMS device with a diethylamine-modified GMA monolith inside a microfluidic channel. The microchip device was used for anion exchange separation of FITC-labeled BSA and ovalbumin. Nanoparticle functionalization can be used to change the characteristics of a porous polymer monolith. Zhang *et al.*⁵⁹ fabricated an alumina nanoparticle modified methacrylic acid-co-poly(ethylene glycol) diacrylate (PEGDA) monolith in a PMMA microfluidic device. Using optimized conditions of solution pH, flow rate, and different times, the device was used for SPE of 2-amino-4-chlorophenol content in chlorzoxazone tablets.

C. Affinity extraction

Porous polymer monoliths can be designed with specific groups to enhance analyte selectivity through affinity interactions. Similar to other techniques, the extracted sample can then be eluted by disrupting these interactions through changing the solution pH or ionic strength, for example. We discuss methods involving boronic acid interactions, followed by bio-affinity and immunoaffinity techniques.

Cakal *et al.*⁶⁰ used a glass microfluidic chip with a vinyl phenylboronic acid-PEGDA copolymer monolith to extract cis-diol containing catecholamines. In this work, 100-fold sample enrichment was achieved using pH-assisted elution. Levy *et al.*⁶¹ fabricated boronic acid functionalized silica monoliths inside PDMS microfluidic devices. Utilizing affinity interactions with boronic acid, they selectively extracted and eluted conalbumin from a mixture containing conalbumin and BSA. The same device was also used for SPE of a peptide mixture obtained from a horseradish peroxidase digest.

Aptamers offer an alternative to antibodies for affinity interactions. Gao *et al.*¹⁷ fabricated a porous polymer monolith inside a PMMA chip to study extraction of thrombin using attached thrombin aptamer. The GMA-PEGDA monolith improved the affinity extraction by reducing non-specific protein adsorption. They used the microchip for extraction, elution, and fluorescent detection of thrombin in the presence of interfering human serum albumin and green fluorescent protein. Binding between carbohydrates and proteins is important in pharmaceutical applications. Liu *et al.*⁶² developed a glass microfluidic device with a heparin-modified GMA monolith for the affinity extraction of granulocyte colony-stimulating factor protein. Affinity-extracted protein was eluted using carbohydrate displacing reagents, and the critical elution concentration of carbohydrate provided the molecular binding strength.

Immunoassay technology is commonly used to detect protein targets, requiring immobilized antibody. Kang *et al.*⁶³ developed a PDMS microchip with a GMA-based monolith that had antibodies immobilized for a microfluidic immunoassay. The device was used to perform a direct immunoassay with model compounds and a sandwich immunoassay for the H1N1 virus, resulting in 4 ng/ml and 10 pg/ml limits of detection for IgG and H1N1, respectively. Nanoscale materials have several similarities with monoliths. Krivitsky *et al.*⁶⁴ fabricated a 3D porous structure of silicon nanowires on a silicon chip for the capture and on-chip sensing of proteins in whole blood and urine samples. Antibody-modified silicon nanowires were used to extract proteins, and the silicon nanowire field-effect transistors provided biosensing for a whole blood analysis in 10 min.

IV. MICROREACTORS

Microfluidics has advanced significantly in the past few years, and there has been increasing interest in the development of on-chip microreactors. These microreactors offer a powerful alternative to current techniques, with more efficient and faster reactions, automation, reduced reagent needs and ability to be integrated with multiple processes. A recent review by Yao *et al.*⁶⁵ outlines various applications of microreactors. Monolithic materials provide important advantages as supports for microreactors as they can be easily polymerized *in situ*, offer high surface area and yield low back pressure. Reactors containing immobilized catalyst on a stationary support are also gaining attention due to catalyst stability and the ability to recover products without catalyst contamination. Microreactors in microfluidics can be broadly classified into two categories: enzymatic and non-enzymatic.⁷ This section focuses on the application of monoliths and other porous materials as solid supports for catalyst immobilization for such reactors in microfluidics.

A. Enzymatic microreactors

Immobilized enzyme reactors have received growing interest in the past few years as they have shown great efficiency, reproducibility, stability, and compatibility with a variety of downstream processes. Several reviews have been published recently focusing on the advancement of enzyme-based microreactors and their applications in microfluidics.^{7,65,66} Additionally, Kim and Herr⁶ reviewed various materials and strategies for protein immobilization in microfluidics. A key advantage of monolithic supports is the wide range of available surface modifications for protein immobilization in enzymatic reactors. In Secs. IV A 1–IV A 3, recent developments and innovations in enzymatic microreactors are discussed. Table II also summarizes the materials, enzymes, analytes, and reaction times for various microfluidic enzymatic reactors described in the last five years.

TABLE II. Summary of critical information about recently developed enzymatic microreactors. Acronyms: NAS: N-acryloxysuccinimide; APTES: 3-amino-propyltriethoxysilane; TMOS: tetramethoxysilane; MTMOS: methyltrimethoxysilane; PEO: polyethylene oxide; EGMS: ethylene glycol modified silane; PEI: polyethylenimine; PIPA: poly(N-isopropylacrylamide); CHO: choline oxidase; G6PD: glucose 6-phosphate dehydrogenase; CMC: carboxymethyl cellulose; AchE: acetylcholinesterase; and G6P: glucose 6-phosphate.

Enzyme reactor	Material	Enzyme	Immobilization technique	Analytes	Reaction time	References
<i>Monolithic</i>	NAS-PEGDA	Trypsin	Covalent	Myoglobin, cytochrome C, BSA	12–71 s	67
	BMA-EDMA	Trypsin	Hydrophobic adsorption	Cytochrome C, BSA	30 min	68
	TEOS-APTES	Trypsin	Covalent	Myoglobin, cytochrome C, BSA	0.3–5 min	69
	TMOS-MTMOS	GOD, CHO	Electrostatic	Glucose	1 h	70
	TMOS-MTMOS	AchE, CHO	Electrostatic	Acetylcholine chloride	15–60 min	71
	TEOS-PEO, EGMS-PEO	G6PD, cellulase	Sol-gel based encapsulation	G6P, CMC	1–6 h	72
	PMMA-PEI	GOD	Covalent	Glucose	30 s	73
	PIPA	GOD	Hydrophobic adsorption	Glucose	8 s	77
	BMA-EDMA	AchE	Covalent	Acetylthiocholine iodide	10 min	74
<i>Unconventional</i>	Agarose gel	Trypsin, pepsin	Covalent	BSA, lysozyme	4 h	76
	TEOS, TMOS, alkoxysilanes, EGMS	G6PD	Sol-gel based encapsulation and covalent	Glucose	5 min	78
	Poly(vinyl alcohol)	Protease	Entrapment via cryogelation	BSA	20–90 s	79
	Poly(vinyl alcohol)	α -amylase	Entrapment via cryogelation	Starch	25–225 s	80

1. Proteolytic enzyme reactors

Protein digestion, because of its significance in proteomics, is one of the most extensively explored applications of enzymatic microreactors. A recent review by Safdar *et al.*¹⁰ outlined the latest developments in enzyme microreactors with special focus on proteolysis by immobilized trypsin and its application in proteomics. Several examples of such reactors are discussed below.

Liang *et al.*⁶⁷ formed a hydrophilic monolith on a microchip by photopolymerization of N-acryloxysuccinimide and PEGDA for immobilization of trypsin. This reactor was coupled with MS through a C18 particle-packed electrospray emitter tip (Figure 5(a)); BSA, myoglobin, and cytochrome C were digested, separated, and identified using this setup with 50%–60% sequence coverage. In another study, a BMA-based monolith was prepared with a polyE-323 coating on the channel walls to reduce nonspecific adsorption. Digestion of cytochrome C and BSA was performed by retaining them on the monolith, electrokinetically injecting trypsin through the column, and incubating at a desired pH. The digested peptides were then eluted and detected by MS. Sequence coverages of 88% and 56% were reported for cytochrome C and BSA, respectively.⁶⁸ Zhang *et al.*⁶⁹ reported a hybrid monolith made from TEOS, 3-amino-propyltriethoxysilane, and SBA-15-NH₂ nanoparticles that increased the surface area. Glutaraldehyde was used as a bridging agent to immobilize trypsin; BSA, myoglobin and cytochrome C were then digested and analyzed by MS with a sequence coverage of 50%, 93%, and 71%, respectively. Protein digestion in microreactors is faster and enzyme contamination free, which is advantageous over traditional in-solution proteolytic methods, and offers promising possibilities for advancement of bottom-up proteomics.

2. Non-proteolytic enzyme microreactors

Apart from proteolysis, enzymatic reactors have also been developed for catalyzing other biochemical reactions. A silica monolith-based microreactor was fabricated using TEOS and methyltrimethoxysilane, and functionalized with polyethylenimine to facilitate immobilization of glucose oxidase (GOD) or choline oxidase through electrostatic attraction. Increased stability (~95%) and similar activity was observed for immobilized GOD compared to in-solution GOD (Figure 5(b)).⁷⁰ Using a similar monolith, He *et al.*⁷¹ also developed a bienzyme reactor by co-immobilizing acetylcholinesterase and choline oxidase for evaluation of enzyme inhibition. Yesil-Celiktas *et al.*⁷² described another silica monolith-based reactor using TEOS and an ethylene glycol modified silane for immobilization of glucose 6-phosphate dehydrogenase and cellulase. Reactors were evaluated for aging with respect to enzyme activity, and conversion rates of 20%–30% were reported. A GOD-based microreactor was developed by forming a porous surface on a PMMA microchannel by modifying it with polyethylenimine and glutaraldehyde. Using this reactor, glucose concentration was determined by electrochemical detection of hydrogen peroxide liberated from the oxidation reaction.⁷³ Acetylcholinesterase microreactors were

FIG. 5. Monolithic microreactors with immobilized enzyme. (a) Schematic diagram of an integrated microchip-based system. Adapted with permission from Liang *et al.* J. Chromatogr. A **1218**, 2898 (2011). Copyright 2011 Elsevier. (b) Comparison of stability of GOD immobilized on a monolith and in-solution. Adapted with permission from He *et al.*, Microfluid. Nanofluid. **8**, 565 (2010). Copyright 2010 Springer.

also developed recently on methacrylate-based monoliths that used enzyme inhibition for applications in determination of copper(II) in natural water⁷⁴ and organophosphorus pesticides.⁷⁵

3. Unconventional enzyme microreactors

Hydrogels are another type of porous material that has been employed for protein immobilization and enzymatic reactions. An excellent review was published by Chung *et al.*¹¹ on fabrication techniques for hydrogels and their applications in protein engineering and cell biology. Luk *et al.*⁷⁶ developed a hydrogel-based DMF reactor where agarose gel discs were functionalized with glycidol and periodate to generate glyoxyl-agarose, followed by trypsin or pepsin immobilization (Figure 6(a)). These reactor hydrogels were then used for proteolytic digestion of BSA and lysozyme in a droplet DMF setup (Figure 6(b)). Proteolysis was evaluated for sequence coverage by in-droplet vs. on-gel tryptic digestion. A high sequence coverage for both BSA and lysozyme in on-gel tryptic digestion was reported. Additionally, successful parallel protein digestion using two hydrogel discs containing trypsin and pepsin was demonstrated. A novel microreactor reported by Xiong *et al.*⁷⁷ exhibited reversible hydrophobicity-hydrophilicity based on shrinking or swelling of a poly(N-isopropylacrylamide) monolith. Reversible adsorption and release of GOD was demonstrated, and a linear electrochemical response for a range of glucose concentrations (0.05–5 mM) was reported. Cumana *et al.*⁷⁸ compared various silica-based gels for encapsulation of glucose 6-phosphate dehydrogenase. The enzyme activity and pore size distribution with aging in different materials were also compared. Nakagawa *et al.* developed microreactors based on freeze-dried poly(vinyl alcohol) foam for immobilization of protease⁷⁹ and α -amylase.⁸⁰ Enzymes were entrapped within the monoliths during cryogelation, and the enzyme activity was evaluated for the proteolysis of BSA or hydrolysis of starch at different residence times.

B. Non-enzymatic microreactors

Monolithic microreactors for catalysis of non-enzymatic processes have been less explored than enzymatic reactors. A silica-based reduction reactor was recently developed via a sol gel technique using tetramethoxysilane and 3-aminopropyltriethoxysilane, with the reducing reagent, tris(2-carboxyethyl)phosphine hydrochloride. The reactor reduced protein disulfide bonds to facilitate proteolytic digestion.³⁰ An efficient microreactor was reported containing Pd nanoparticles on a titania monolith for hydrogenation of unsaturated hydrocarbon chains. Pd nanoparticles were immobilized by flowing aqueous $\text{Pd}(\text{NO}_3)_2$ solution through the monolith, and Pd nanoparticles were well distributed throughout the monolith (0.24% Pd w/w), as shown in Figure 7. The hydrogenation efficiency was evaluated for a series of reactions, and a conversion rate up to 100% was reported with an increase in H_2 flow.⁸¹ Pd nanoparticles have also been immobilized on a silica monolith by impregnation with an aqueous solution of $\text{Pd}(\text{NH}_3)_4(\text{NO}_3)_2$. The structure was used for selective hydrogenation of 1,5-cyclooctadiene and 3-hexyn-1-ol, with conversion rates of 95% and 85%, respectively.⁸² In a recent report, the Heck–Mizoroki reaction of aryl iodides and methacrylate was catalyzed using Pd immobilized on a silica-based microfluidic flow reactor.⁸³ Iodobenzene coupling with 4-tolyl boronic acid was also performed on GMA-EDMA monoliths functionalized with a phenanthroline ligand and Pd, formed in COC and borosilicate chips.⁸⁴ Overall, such structures show promise for efficient and durable metal nanoparticle catalytic monolithic continuous flow microreactors.

V. ANALYSIS OF SUBCELLULAR AND LARGER STRUCTURES

Porous materials have attracted attention not only for analysis and catalysis, but also for biological studies that leverage advantages provided by microfluidics. As the requirements of the support material depend mostly on the application, rigidity and very high surface area of porous structures are not necessarily key factors for fabricated polymers, whereas biocompatibility and degradability of materials are often essential for studying living objects or use as scaffolds in tissue engineering. Soft, porous materials like hydrogels and cryogels with various surface

FIG. 6. A hydrogel based DMF proteolytic microreactor. (a) Immobilization of enzymes onto hydrogel discs. (b) Series of images from a movie (left) and a schematic (right) showing protein digestion. Adapted with permission from Luk *et al.*, *Proteomics* **12**, 1310 (2012). Copyright 2012 Wiley.

FIG. 7. A titania monolith based hydrogenation microreactor with immobilized Pd nanoparticles. (a) and (b) Optical images, (c) X-ray tomography, (d) SEM image, and (e) overall mechanism. Adapted with permission from Linares *et al.*, *ACS Catal.* **2**, 2194 (2012). Copyright 2012 American Chemical Society.

modifications have been utilized and recently reviewed.^{11,85–87} Here, we discuss their applications in microfluidic systems.

A. Analysis of vesicles and bacteria

Extracellular membrane vesicles with dimensions in the 50–1000 nm range are promising biomarkers in the diagnosis and prognosis of cancer. Current methods based on centrifugation and filtration are laborious, so microfluidics offers an interesting approach for rapid and efficient purification of these vesicles from whole blood. Davies *et al.*⁸⁸ employed a microfiltration system involving a porous polymer monolith membrane for passing vesicles downstream for further analysis and quantification. Different pore sizes were investigated for filtration performance. Fast filtration of vesicles was achieved in a pressure-driven mode, whereas electric field-driven filtration allowed selective elimination of proteins, resulting in a higher purity extract.

Porous polymer monoliths have found use as efficient tools for bacteria analysis. A polyacrylamide-based monolith was formed in glass/urethane diacrylate/glass microfluidic channels,⁸⁹ and streptavidin acrylamide was used to follow functionalization by biotinylated antibodies. These monoliths allowed specific capture of *Rickettsia typhi*, and subsequent immunofluorescent staining and imaging of the bacteria inside the chip indicated detection limits of about 100 bacteria per ml of blood sample. Aly *et al.*^{90,91} developed a method for lysis of both gram-positive and gram-negative bacteria using a monolith based on in-house-synthesized N-(tert-butyloxycarbonyl) aminoethyl methacrylate, which has antibacterial properties. The influence of the flow rate and of the hydrophilic-lipophilic balance in the monolith on the cell lysis efficiency was investigated, and they found that the contribution of contact killing to cell lysis was more significant than that of mechanical shearing. The monolith also served as a filter that isolated cell debris, permitting PCR-amplifiable DNA to be transmitted. The chip could be reused and showed better lysis efficiency than off-chip chemical, mechanical, and thermal lysis techniques.

B. Cells, tissues, and organs

Microfluidics have been widely explored for recreating cellular and tissue microenvironments, screening drug responses, and mimicking cellular physiology. Structures supporting growth and viability have complex mass transport requirements that need to be satisfied to provide nutrients and remove metabolites. This enables the development of models that mimic *in vivo* conditions, and observation of responses to different stimuli. Various approaches for formation of perfusable channels and their applications are discussed below.

Biocompatible porous materials like hydrogels offer great potential for providing robust yet elastic 3D extracellular matrix mimics for encapsulation of cells. A review by Wong *et al.*⁹² discussed the use of hydrogels in creating vascular-like physiology in microfluidic models. Zhang *et al.*⁹³ demonstrated a hydrogel-based programmable cell capture and release platform. The hydrogel was conjugated with an oligonucleotide to which a complementary oligonucleotide also carrying a cell receptor specific aptamer was hybridized. This allowed capture of live cells by cell receptor-aptamer interaction. Importantly, captured cells were released by introducing another complementary oligonucleotide sequence that detached the aptamer from the hydrogel. A key advantage of this approach was its nondestructive nature that facilitated the use of live cells that could be further extracted to create specific cell lines.

Jeon *et al.*⁹⁴ created bone and muscle tissue mimicking 3D vascular microenvironments using endothelial and mural-like cells suspended in a fibrin gel surrounded by culture media channels made of PDMS. These vascular microenvironments were used to study the effect of adenosine on the metastasis of breast cancer cells in the microvasculature mimic. In another study on engineering a 3D vascularized construct, a sacrificial template of sucrose-glucose fibers was 3D printed and encapsulated in a variety of gel extracellular matrices containing living cells. The carbohydrate fibers were then dissolved and flushed with liquid to provide a vascularized, rigid extracellular matrix for living cells.⁹⁵ These studies demonstrate the potential of such artificial microenvironments for conducting drug screening studies.

FIG. 8. A bilayer membrane microfluidic device. (a) Schematic, (b) photograph, and (c) cross-sectional schematic illustrating the spatial arrangement of cells. Adapted with permission from Chen *et al.*, Lab Chip **13**, 2591 (2013). Copyright 2013 Royal Society of Chemistry.

The organ-on-chip concept is also intriguing and has been recently explored by a number of research groups endeavoring to show the ability of microfluidics to mimic tissue- or organ-level physiology.^{96,97} A similar cardiovascular microfluidic model was evaluated by Chen *et al.*⁹⁸ using gelatin-methacrylate hydrogels as a 3D matrix for encapsulation of valvular interstitial cells. An organ-on-chip platform was developed consisting of a 3D matrix containing valvular interstitial cells and an endothelial cell layer to mimic cell-cell interactions in a vascular micro-environment (Figure 8). A high cell viability ($\sim 84\%$) in this hydrogel was reported after 4 days. Additionally, the endothelial layer of cells protected the interstitial cells from shear stress, consistent with macroscale models.

Similarly, gelatin-methacrylate hydrogels photopolymerized in a microfluidic flow focusing PDMS device were used as an elastic scaffold by Cha *et al.*⁹⁹ Cells proliferating on the hydrogel surface were promising for transplantation into a host. To reduce the oxidative and mechanical stress, as well as decrease the immune response associated with transplantation, a protective biodegradable silica hydrogel shell was formed over the cardiac side population cell seeded microgels. Importantly, this approach resulted in $\sim 90\%$ cell viability even after oxidative stress.

Overall, these porous microfluidic systems may prove beneficial for a variety of tissue or organ physiological response studies in drug discovery and development.

VI. CONCLUSIONS

Since the introduction of monolithic stationary phases in separation science and more recently in microfluidics, many procedures have been developed for their preparation, functionalization and use in many applications. With a variety of substrates, different strategies need to be employed for successful attachment of the porous structure to the microchannel surface. Ease of preparation enables the wide use of monoliths with spatial control over their fabrication. However, additional preparation methods need to be invented in order to provide easier coupling of the monolithic material to the microfluidic channel wall with less dependence on multistep treatment processes for poorly reactive substrates. Recently introduced polymerization techniques for preparation of monoliths, such as living radical or atom transfer radical polymerization, could be exploited in microfluidic devices. As microfluidics enables precise manipulation of small liquid volumes, new techniques could be used for fabrication of novel materials with well-controlled and distinct porosities. 3D printing as an emerging technique in microfluidics^{100,101} may also bring new approaches for preparation of porous structures from various

materials, provided the printing resolution is sufficient to produce desired pore dimensions. Long-established materials such as methacrylates and silica are widely used; however, they are limited by lower surface area or hydrolytic stability, respectively. Combining both organic and inorganic monomers to produce hybrid monoliths¹⁰² and metal organic frameworks¹⁰³ can provide resistant and high-surface-area structures, leading to further improvements.

In general, there are some stand-alone parameters that largely control SPE-based device performance. Selection of the type of monolith material is important because hydrophobicity, affinity, and pore size can specifically affect liquid-solid phase interactions, and hence analyte retention, elution, and separation. Choosing the device material is crucial too, as different materials have advantages and disadvantages such as compatibility with eluent. Along with device design the sample flow rate, elution time, pH, and ionic strength are key parameters that need to be considered in designing experiments. For monolith stability, the device material compatibility and microfluidic channel pre-treatment processes are important too. With electrokinetic sample loading, the selection of functional groups that support electroosmotic flow is also essential to maintain flow rate uniformity throughout the device.

The majority of studies so far have been performed with model analytes and/or sample matrices containing few interferents; however, to more fully exploit the potential of this technology for health-care diagnostics, future directions should focus more on processing samples from blood or other complex matrices and containing real analytes. Miniaturization is a key aspect of microfluidics-based SPE; however, there are still components such as sample injection and detection systems that need to be miniaturized to enhance the applicability of this technology.

Reactors in microfluidics offer a wide range of applications including protein digestion and catalytic reduction. These microscale reactors require less reagent and demonstrate faster reaction times relative to their benchtop counterparts. Monoliths, having high surface area and facile modification capabilities, offer an excellent solid support for immobilization of catalysts in reactors. Employment of monolithic supports in microreactors also offers advantages like low catalyst contamination, and high reaction efficiency and catalyst stability. Further advancements in microreactors may offer solutions to current day complications in fields like proteomics, drug screening, enzymatic assays, catalytic synthesis, and point-of-care diagnostics.

A number of bioapplications have been demonstrated in tubing, capillaries, discs, or other formats, but they could be transitioned into microfluidic platforms and integrated with other processes to enhance analysis capabilities. Hydrogels and cryogels with biocompatible porous structures have been extensively researched as matrices for exploring cell or tissue level interactions.

The quest for preparation and application of various supports with extended surface area in many research areas has already provided promising tools helpful in solving numerous scientific challenges, and many more advances will certainly follow.

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¹I. Nischang, O. Brueggemann, and F. Svec, *Anal. Bioanal. Chem.* **397**, 953 (2010).

²R. Wu, L. G. Hu, F. J. Wang, M. L. Ye, and H. Zou, *J. Chromatogr. A* **1184**, 369 (2008).

³O. G. Potter and E. F. Hilder, *J. Sep. Sci.* **31**, 1881 (2008).

⁴J. Wen, L. A. Legendre, J. M. Bienvenue, and J. P. Landers, *Anal. Chem.* **80**, 6472 (2008).

⁵A. Podgornik and N. L. Krajnc, *J. Sep. Sci.* **35**, 3059 (2012).

⁶D. Kim and A. E. Herr, *Biomicrofluidics* **7**, 041501 (2013).

⁷M. Vázquez and B. Paull, *Anal. Chim. Acta* **668**, 100 (2010).

⁸K. Faure, *Electrophoresis* **31**, 2499 (2010).

⁹D. A. Mair, T. R. Schwei, T. S. Dinio, F. Svec, and J. M. J. Frechet, *Lab Chip* **9**, 877 (2009).

¹⁰M. Safdar, J. Spross, and J. Jänis, *J. Chromatogr. A* **1324**, 1 (2014).

¹¹B. G. Chung, K.-H. Lee, A. Khademhosseini, and S.-H. Lee, *Lab Chip* **12**, 45 (2012).

¹²Y. Temiz, R. D. Lovchik, G. V. Kaigala, and E. Delamarche, *Microelectron. Eng.* **132**, 156 (2015).

¹³F. Svec, *J. Chromatogr. A* **1217**, 902 (2010).

¹⁴R. Knob, C. Kulsing, R. I. Boysen, M. Macka, and M. T. W. Hearn, *Tr. Anal. Chem.* **67**, 16 (2015).

¹⁵R. D. Arrua, M. Talebi, T. J. Causon, and E. F. Hilder, *Anal. Chim. Acta* **738**, 1 (2012).

- ¹⁶Z. Walsh, B. Paull, and M. Macka, *Anal. Chim. Acta* **750**, 28 (2012).
- ¹⁷C. L. Gao, X. H. Sun, and A. T. Woolley, *J. Chromatogr. A* **1291**, 92 (2013).
- ¹⁸J. M. Burke and E. Smela, *Biomicrofluidics* **6**, 016506 (2012).
- ¹⁹K. Faure, M. Albert, V. Dugas, G. Crétier, R. Ferrigno, P. Morin, and J.-L. Rocca, *Electrophoresis* **29**, 4948 (2008).
- ²⁰K. K. R. Tetala and M. A. Vijayalakshmi, *Anal. Chim. Acta* **906**, 7 (2016).
- ²¹S.-L. Lin, T.-Y. Lin, and M.-R. Fuh, *Electrophoresis* **35**, 1275 (2014).
- ²²J. Hereijgers, G. Desmet, T. Breugelmans, and W. De Malsche, *Microelectron. Eng.* **132**, 1 (2015).
- ²³M. He, J.-B. Bao, Y. Zeng, and D. J. Harrison, *Electrophoresis* **31**, 2422 (2010).
- ²⁴P. A. Levkin, S. Eeltink, T. R. Stratton, R. Brennen, K. Robotti, H. Yin, K. Killeen, F. Svec, and J. M. J. Frechet, *J. Chromatogr. A* **1200**, 55 (2008).
- ²⁵B. Gorey, J. Galineau, B. White, M. R. Smyth, and A. Morrin, *Electroanalysis* **24**, 1318 (2012).
- ²⁶A. S. Chan, M. K. Danquah, D. Agyei, P. G. Hartley, and Y. G. Zhu, *Sep. Sci. Tech.* **49**, 854 (2014).
- ²⁷M. Araya-Farias, M. Taverna, M. Woytasik, F. Bayle, M. Guerrouache, I. Ayed, H. H. Cao, B. Carbonnier, and N. T. Tran, *Polymer* **66**, 249 (2015).
- ²⁸R. Jindal and S. M. Cramer, *J. Chromatogr. A* **1044**, 277 (2004).
- ²⁹K. J. Shaw, D. A. Joyce, P. T. Docker, C. E. Dyer, G. M. Greenway, J. Greenman, and S. J. Haswell, *Lab Chip* **11**, 443 (2011).
- ³⁰E. Alzahrani and K. Welham, *Anal. Methods* **6**, 558 (2014).
- ³¹M. H. Levy, S. Goswami, J. Plawsky, and S. M. Cramer, *Chromatographia* **76**, 993 (2013).
- ³²E. L. Kendall, E. Wienhold, O. D. Rahmanian, and D. L. DeVoe, *Sens. Actuators, B* **202**, 866 (2014).
- ³³N. Nordman, B. Barrios-Lopez, S. Laurén, P. Suvanto, T. Kotiaho, S. Franssila, R. Kostianen, and T. Sikanen, *Electrophoresis* **36**, 428 (2015).
- ³⁴Z. Walsh, P. A. Levkin, S. Abele, S. Scarmagnani, D. Heger, P. Klán, D. Diamond, B. Paull, F. Svec, and M. Macka, *J. Chromatogr. A* **1218**, 2954 (2011).
- ³⁵H. Yang, J. M. Mudrik, M. J. Jebrail, and A. R. Wheeler, *Anal. Chem.* **83**, 3824 (2011).
- ³⁶K. Q. Jiang, A. Sposito, J. K. Liu, S. R. Raghavan, and D. L. DeVoe, *Polymer* **53**, 5469 (2012).
- ³⁷M. T. Gokmen, B. Dereli, B. G. De Geest, and F. E. Du Prez, *Part. Part. Syst. Charact.* **30**, 438 (2013).
- ³⁸C.-C. Lin, J.-L. Hsu, and G.-B. Lee, *Microfluid. Nanofluid.* **10**, 481 (2011).
- ³⁹A. Ríos and M. Zougagh, *Tr. Anal. Chem.* **43**, 174 (2013).
- ⁴⁰F. Cui, M. Rhee, A. Singh, and A. Tripathi, *Ann. Rev. Biomed. Eng.* **17**, 267 (2015).
- ⁴¹J. Wu, R. Kodzius, W. Cao, and W. Wen, *Microchim. Acta* **181**, 1611 (2014).
- ⁴²Z. Wang, A. B. Jemere, and D. J. Harrison, *Electrophoresis* **33**, 3151 (2012).
- ⁴³Y. J. Hua, A. B. Jemere, J. Dragoljic, and D. J. Harrison, *Lab Chip* **13**, 2651 (2013).
- ⁴⁴Y. Ladner, G. Crétier, and K. Faure, *J. Chromatogr. A* **1217**, 8001 (2010).
- ⁴⁵Z. P. Xu and R. D. Oleschuk, *Electrophoresis* **35**, 441 (2014).
- ⁴⁶P. N. Nge, J. V. Pagaduan, M. Yu, and A. T. Woolley, *J. Chromatogr. A* **1261**, 129 (2012).
- ⁴⁷R. Yang, J. V. Pagaduan, M. Yu, and A. T. Woolley, *Anal. Bioanal. Chem.* **407**, 737 (2015).
- ⁴⁸J. Kim, S. Abdulwahab, K. Choi, N. M. Lafrenière, J. M. Mudrik, H. Gomma, H. Ahmado, L.-A. Behan, R. F. Casper, and A. R. Wheeler, *Anal. Chem.* **87**, 4688 (2015).
- ⁴⁹L. Kashkary, C. Kemp, K. J. Shaw, G. M. Greenway, and S. J. Haswell, *Anal. Chim. Acta* **750**, 127 (2012).
- ⁵⁰P. Pruijm, M. Ohman, P. J. Schoenmakers, and W. T. Kok, *J. Chromatogr. A* **1218**, 5292 (2011).
- ⁵¹Z. W. Wang, W. J. Wang, G. N. Chen, W. Wang, and F. F. Fu, *J. Sep. Sci.* **33**, 2568 (2010).
- ⁵²Q.-S. Kang, Y. Li, J.-Q. Xu, L.-J. Su, Y.-T. Li, and W.-H. Huang, *Electrophoresis* **31**, 3028 (2010).
- ⁵³S. Kumar, V. Sahore, C. I. Rogers, and A. T. Woolley, *Analyst* **141**, 1660 (2016).
- ⁵⁴P. N. Nge, W. C. Yang, J. V. Pagaduan, and A. T. Woolley, *Electrophoresis* **32**, 1133 (2011).
- ⁵⁵H. G. Chun, T. D. Chung, and J. M. Ramsey, *Anal. Chem.* **82**, 6287 (2010).
- ⁵⁶J. M. Mudrik, M. D. M. Dryden, N. M. Lafrenière, and A. R. Wheeler, *Can. J. Chem.* **92**, 179 (2014).
- ⁵⁷L. Lin, H. Chen, H. B. Wei, F. Wang, and J.-M. Lin, *Analyst* **136**, 4260 (2011).
- ⁵⁸E. L. Kendall, E. Wienhold, and D. L. DeVoe, *Biomicrofluidics* **8**, 044109 (2014).
- ⁵⁹J. L. Zhang, G. Chen, M. M. Tian, R. G. Li, X. J. Quan, and Q. Jia, *Talanta* **115**, 801 (2013).
- ⁶⁰C. Cakal, J. P. Ferrance, J. P. Landers, and P. Caglar, *Anal. Chim. Acta* **690**, 94 (2011).
- ⁶¹M. H. Levy, J. Plawsky, and S. M. Cramer, *J. Sep. Sci.* **36**, 2358 (2013).
- ⁶²X. J. Liu, H. Wang, A. Y. Liang, Y. L. Li, H. W. Gai, and B. C. Lin, *J. Chromatogr. A* **1270**, 340 (2012).
- ⁶³Q.-S. Kang, X.-F. Shen, N.-N. Hu, M.-J. Hu, H. Liao, H.-Z. Wang, Z.-K. He, and W.-H. Huang, *Analyst* **138**, 2613 (2013).
- ⁶⁴V. Krivitsky, L.-C. Hsiung, A. Lichtenstein, B. Brudnik, R. Kantaev, R. Elnathan, A. Pevzner, A. Khatchourints, and F. Patolsky, *Nano Lett.* **12**, 4748 (2012).
- ⁶⁵X. J. Yao, Y. Zhang, L. Y. Du, J. H. Liu, and J. F. Yao, *Renewable Sustainable Energy Rev.* **47**, 519 (2015).
- ⁶⁶Y. Asanomi, H. Yamaguchi, M. Miyazaki, and H. Maeda, *Molecules* **16**, 6041 (2011).
- ⁶⁷Y. Liang, D. Y. Tao, J. F. Ma, L. L. Sun, Z. Liang, L. H. Zhang, and Y. K. Zhang, *J. Chromatogr. A* **1218**, 2898 (2011).
- ⁶⁸Y. J. Hua, A. B. Jemere, and D. J. Harrison, *J. Chromatogr. A* **1218**, 4039 (2011).
- ⁶⁹Z. D. Zhang, L. Y. Zhang, C. G. Zhang, and W. B. Zhang, *Talanta* **119**, 485 (2014).
- ⁷⁰P. He, G. Greenway, and S. J. Haswell, *Microfluid. Nanofluid.* **8**, 565 (2010).
- ⁷¹P. He, J. Davies, G. Greenway, and S. J. Haswell, *Anal. Chim. Acta* **659**, 9–14 (2010).
- ⁷²O. Yesil-Celiktas, S. Cumana, and I. Smirnova, *Chem. Eng. J.* **234**, 166 (2013).
- ⁷³L. M. C. Ferreira, E. T. da Costa, C. L. do Lago, and L. Angnes, *Biosens. Bioelectron.* **47**, 539 (2013).
- ⁷⁴P. Rattanakit and S. Liawruangrath, *J. Chem.* **2014**, 757069 (2014).
- ⁷⁵P. Rattanakit, G. M. Greenway, and S. Liawruangrath, *Int. J. Environ. Anal. Chem.* **93**, 739 (2013).
- ⁷⁶V. N. Luk, L. K. Fiddes, V. M. Luk, E. Kumacheva, and A. R. Wheeler, *Proteomics* **12**, 1310 (2012).
- ⁷⁷M. Xiong, B. Gu, J.-D. Zhang, J.-J. Xu, H.-Y. Chen, and H. Zhong, *Biosens. Bioelectron.* **50**, 229 (2013).
- ⁷⁸S. Cumana, J. Simons, A. Liese, L. Hilterhaus, and I. Smirnova, *J. Mol. Catal. B* **85–86**, 220 (2013).
- ⁷⁹K. Nakagawa, A. Tamura, and C. Chaiya, *Chem. Eng. Sci.* **119**, 22 (2014).

- ⁸⁰K. Nakagawa and Y. Goto, *Chem. Eng. Process* **91**, 35 (2015).
- ⁸¹N. Linares, S. Hartmann, A. Galarneau, and P. Barbaro, *ACS Catal.* **2**, 2194 (2012).
- ⁸²A. Sachse, N. Linares, P. Barbaro, F. Fajula, and A. Galarneau, *Dalton Trans.* **42**, 1378 (2013).
- ⁸³B. Urban, D. Srankó, G. Sáfrán, L. Úrge, F. Darvas, J. Bakos, and R. Skoda-Földes, *J. Mol. Catal. A* **395**, 364 (2014).
- ⁸⁴J. A. Deverell, T. Rodemann, J. A. Smith, A. J. Canty, and R. M. Guijt, *Sens. Actuator B* **155**, 388 (2011).
- ⁸⁵R. Schirhagl, *Anal. Chem.* **86**, 250 (2014).
- ⁸⁶S. Selimovic, J. Oh, H. Bae, M. Dokmeci, and A. Khademhosseini, *Polymers* **4**, 1554 (2012).
- ⁸⁷H. N. Chia and B. M. Wu, *J. Biol. Eng.* **9**, 4 (2015).
- ⁸⁸R. T. Davies, J. Kim, S. C. Jang, E.-J. Choi, Y. S. Ghoo, and J. Park, *Lab Chip* **12**, 5202 (2012).
- ⁸⁹J. Y. Mai, V. V. Abhyankar, M. E. Piccini, J. P. Olano, R. Willson, and A. V. Hatch, *Biosens. Bioelectron.* **54**, 435 (2014).
- ⁹⁰M. A. S. Aly, M. Gauthier, and J. Yeow, *Anal. Bioanal. Chem.* **406**, 5977 (2014).
- ⁹¹M. A. S. Aly, O. Nguon, M. Gauthier, and J. T. W. Yeow, *RSC Adv.* **3**, 24177 (2013).
- ⁹²K. H. Wong, J. M. Chan, R. D. Kamm, and J. Tien, *Ann. Rev. Biomed. Eng.* **14**, 205 (2012).
- ⁹³Z. Zhang, N. Chen, S. Li, M. R. Battig, and Y. Wang, *J. Am. Chem. Soc.* **134**, 15716 (2012).
- ⁹⁴J. S. Jeon, S. Bersini, M. Gilardi, G. Dubini, J. L. Charest, M. Moretti, and R. D. Kamm, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 214 (2015).
- ⁹⁵J. S. Miller, K. R. Stevens, M. T. Yang, B. M. Baker, D.-H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, S. N. Bhatia, and C. S. Chen, *Nat. Mater.* **11**, 768 (2012).
- ⁹⁶S. N. Bhatia and D. E. Ingber, *Nat. Biotechnol.* **32**, 760 (2014).
- ⁹⁷J. H. Sung, M. B. Esch, J. M. Prot, C. J. Long, A. Smith, J. J. Hickman, and M. L. Shuler, *Lab Chip* **13**, 1201 (2013).
- ⁹⁸M. B. Chen, S. Sriganapalan, A. R. Wheeler, and C. A. Simmons, *Lab Chip* **13**, 2591 (2013).
- ⁹⁹C. Cha, J. Oh, K. Kim, Y. Qiu, M. Joh, S. R. Shin, X. Wang, G. Camci-Unal, K.-t. Wan, R. Liao, and A. Khademhosseini, *Biomacromolecules* **15**, 283 (2014).
- ¹⁰⁰H. Gong, M. Beauchamp, S. Perry, A. T. Woolley, and G. P. Nordin, *RSC Adv.* **5**, 106621 (2015).
- ¹⁰¹C. I. Rogers, K. Qaderi, A. T. Woolley, and G. P. Nordin, *Biomicrofluidics* **9**, 016501 (2015).
- ¹⁰²J. Ou, Z. Liu, H. Wang, H. Lin, J. Dong, and H. Zou, *Electrophoresis* **36**, 62 (2015).
- ¹⁰³S. Qiu, M. Xue, and G. Zhu, *Chem. Soc. Rev.* **43**, 6116 (2014).