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Phase Changing Sacrificial Layers in Microfluidic Devices: Adding another dimension to separations

Daniel J. Eves and Adam T. Woolley*

Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602-5700, USA

Abstract

The use of polymers in microchip fabrication affords new opportunities for the development of powerful, miniaturized separation techniques. One method in particular, the use of phase changing sacrificial layers, allows for simplified designs as well as many additional features to the now standard fabrication of microchips. With the possibility of adding a third dimension to the design of separation devices, various means of enhancing analysis now become possible. The application of phase changing sacrificial layers in microchip analysis systems is discussed, both in terms of current uses and future possibilities.

*Corresponding author; email: atw@byu.edu.

Keywords

bioanalytical methods; capillary electrophoresis; microfluidics/microfabrication; preconcentration; sacrificial materials; separations/instrumentation

Introduction

The “gold standard” microchip separations substrate is glass. Indeed, many great advances have been made in glass microchip analysis systems, including DNA sequencing [1,2], two dimensional capillary [3] and gel electrophoresis [4], and DNA fragment sizing [5,6]. With these significant advances in microchip fabrication also comes considerable cost in using microfabrication techniques to etch channel designs into chips. To reduce fabrication expenses, alternative materials have been explored, especially polymers [7]. Common polymer substrates for microchips are poly(dimethylsiloxane) (PDMS) [8], poly(methyl methacrylate) (PMMA) [9], cyclic olefin copolymer (COC) [10,11], and polycarbonate (PC) [12]. Polymers have key benefits in terms of cost, ease of fabrication and design flexibility, but separation performance has lagged compared to that of glass microdevices [13].

The growth of the use of polymer microdevices has spurred the development of new methods for microchip construction. In one such example, Kelly et al. [14] implemented phase changing sacrificial layers in polymer microchip fabrication. In the past, phase-changing materials had been used for creating micropumps [15], valves [16,17], and membrane actuators [18], but not in microdevice construction. The phase changing sacrificial layer method involves protecting imprinted channels with a sacrificial layer that can be removed easily without caustic chemicals or risk of damaging the patterned features. This approach can be used to streamline the construction of microfluidic separation devices and enable new functionality for micro-total analysis systems.

Applications of Phase Changing Sacrificial Layers

The principal idea behind phase changing sacrificial layers is that a series of channels are patterned onto a substrate and then protected from damage during subsequent processing steps. Once device fabrication is complete, the sacrificial material can be removed to leave the channels open and available for use. Phase changing sacrificial layers have been used for solvent-bonded capillary electrophoresis microchips [14,19], protein preconcentration systems [20], electric field gradient focusing [20], and multilayer devices with crossover channels [21].

A paraffin wax phase changing sacrificial layer was used in the initial demonstration to make solvent-bonded electrophoresis microdevices [14]. Very recently, water (ice) has also been shown to work as a sacrificial material in solvent-bonding capillary electrophoresis microchips [19]. A schematic overview of the device fabrication process using a paraffin wax sacrificial layer is shown in Figure 1. First, channels are imprinted in a PMMA substrate using a lithographically patterned master and hot embossing, for example. The patterned polymer substrate is covered with a PDMS film (Figure 1A) and heated to 75°C, after which melted wax is introduced into the channels (Figure 1B). Once the wax is throughout the channel network, the substrate is allowed to cool to room temperature and excess wax is removed from the surface of the substrate. Acetonitrile is then added to the sacrificial layer protected surface (Figure 1C), and the top polymer piece is placed atop the substrate (Figure 1D). Once the bonding is finished the completed device is placed on a hot plate at 75°C and the wax is vacuumed out of the channels, leaving open microcapillaries embedded within the PMMA

[14]. A key advantage of this approach over conventional thermal annealing is that a stronger bond between layers can be achieved, which results in a more robust device.

Kelly et al. [14] constructed capillary electrophoresis microchips using this method. They separated fluorescently tagged amino acid and peptide samples in these devices, with excellent speed and efficiency. Hundreds of replicate separations were also carried out in the same device. More recently, Koesdjojo et al. [19] used water (ice) as a sacrificial layer in constructing a solvent-bonded separation microdevice. As a demonstration of microchip electrophoresis, two fluorescent dyes each at a 1 mM concentration, were separated.

Phase changing sacrificial layers can also enable the integration of polymerized, ion-permeable membranes with micromachined channels [20]. In this process a sacrificial layer protected channel is combined with an open well in the top layer (Figure 2A). The well is next filled with monomer solution as shown in Figure 2B and photopolymerized (Figure 2C). Finally the device is heated and the sacrificial material removed, leaving an open channel interfaced with a ion permeable membrane (Figure 2D). Microfluidic devices constructed in this fashion were used in protein analysis. As much as 1000 fold sample enrichment was achieved by electrokinetically loading a protein at the membrane. Furthermore, electric field gradient focusing in microchips fabricated by this approach demonstrated improved performance relative to capillary-based systems [20].

Fuentes et al. [21] constructed a three-dimensional multichannel separation device in PMMA with crossover channels using phase changing sacrificial layers. Three-dimensional devices have been previously assembled from PDMS [22], but sacrificial layers increase the range of materials that can be used to make multilayer microfluidic devices. The implementation of crossing channels requires that one channel have a discontinuity on the base layer of the PMMA substrate (Figure 3A). Vias in an intermediate layer connect the discontinuous channels to a bridging channel in the top layer, enabling microchannels to cross paths, without fluidic connection. Once the channel network is filled with the sacrificial material (Figure 3B) the device can be assembled through solvent bonding (Figure 3C). Filling channels with different dye solutions confirmed that there was no fluidic cross talk in the device and that the channels were independent of each other (Figure 3D). Capillary electrophoresis was conducted in these devices, and the separation performance was unaffected by either an applied potential or a fluid flow in a crossing channel. That processes in the crossover channel did not affect separations is proof that independent function was possible with intersecting channels in a three-dimensional structure.

Outlook

The ability to form multilayer systems with phase changing sacrificial materials enables additional structural and design advantages in microdevice construction. First, because the overall device format incorporates three dimensions, the designs can be more sophisticated. Second, selected channels can be functionalized before bonding, providing additional flexibility for creating modules for preconcentration or targeted analyte retention. Finally, the three-dimensional opportunities of fabrication facilitate interfacing devices with detection methods, such as mass spectrometry. Whereas other methods may be used individually to construct one of these device layouts, phase changing sacrificial layers allow the fabrication of all these designs and structures using a single platform. We now explore the outlook for these possibilities, which are enabled by phase changing sacrificial layer construction.

The nature of the phase changing sacrificial layer, coupled with solvent bonding and multiple-level designs, reduces the need to create a layout wherein channels avoid intersection, such as the one in Figure 4A. Microdevices with crossover channels can have a smaller overall device footprint, potentially enabling greater sample throughput. Furthermore, the number of access

holes can be decreased, which simplifies the interfacing of the device with sample and can also reduce device footprint. Therefore, more straightforward and space economical designs can be obtained using phase changing sacrificial layers. Indeed, Figure 4B shows a multilayer layout with the same separation channel lengths as Figure 4A. Importantly, the non-crossover design (Figure 4A) has 60% more reservoirs and occupies more than twice the surface area as the comparable, but much more compact crossover layout in Figure 4B.

The advent of a general approach for three-dimensional construction afforded by phase changing sacrificial layers allows for the addition of functional portions of an analysis channel to aid in preconcentration or sample cleanup. Indeed, increasing interest in single cell analysis makes it important for scientists to be able to purify and concentrate small volume samples on-line, reducing loss and contamination. To this end, parts of a channel that can be addressed individually during fabrication and then combined with a full microfluidic network during a bonding step would be useful in customizing an analysis system. Thus, a crossover channel could be derivatized with an affinity element such as an antibody and protected by a sacrificial layer (Figure 4C). The affinity channel could then be coupled to unmodified fluidic channels to enhance analyte specificity. Also, this approach opens the door to greater customization of an analysis channel. It should be feasible to have multiple crossover channels (each customized for a different analyte) in a single microfluidic network. Such a format is especially exciting for improving detection of proteins with a low copy number. For example, a single cell could be introduced into a sample reservoir and lysed; then, the proteins of interest could be captured and concentrated in different affinity crossover channels, allowing multiple target analysis without the need for additional characterization by mass spectrometry, etc. A similar approach could be applied to integrating ion exchange materials such as nafion, which could remove contaminants and improve limits of detection. The nafion could be localized in a crossover channel and then coupled to a sacrificial layer protected channel network via solvent bonding. Hence, the use of sacrificial layer methods should increase the ability to characterize diverse compounds in microdevices.

Three-dimensional fabrication with phase changing sacrificial layers would also allow for the design of off-chip detection coupling systems to be placed at the end of a channel. For example, the ability to fabricate an electrospray tip in one layer integrated with a channel in another layer should facilitate coupling to a mass spectrometer (Figure 4E). Importantly, the multilayer approach allows the tip geometry to be optimized independently from the fluidic layers.

Moreover, phase changing sacrificial layers could be used to implement magnetic bead based sample preconcentration (Figure 4E). For example, a thin polymer film could be added as an intermediate layer separating an electromagnet from a separation channel. Phase changing sacrificial materials would enable optimal placement of the thin polymer layer and spacing between the magnet and channel. Flowing analytes targeted by the magnetic nanoparticles would localize near the magnet in its "on" state; once a sufficient time had elapsed, the magnetic field could be released and the concentrated analytes detected. This approach would offer another means by which phase changing sacrificial layers could aid in assembling sophisticated microchip analysis systems.

The use of sacrificial layers and polymers in microfluidic analysis systems is providing a greater degree of customization to increase specificity, sensitivity, and utility. Building on previous results, it should be possible to adapt these sacrificial materials for new devices to increase our ability to analyze and detect molecules of interest. Phase changing sacrificial layers already enable the integration of membranes with channels, and the construction of three-dimensional fluidic systems to simplify device layouts. Further development of sacrificial layers in microchip fabrication should open many new possibilities in microscale analysis, such as integrating affinity columns and facilitating coupling to a mass spectrometer. These established

and emerging capabilities will further the field of microfluidics to provide bioanalysis systems that advance the disciplines of chemistry, biology, and medicine.

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References

1. Woolley AT, Mathies RA. *Anal Chem* 1995;67:3676–3680. [PubMed: 8644919]
2. Paegel BM, Emrich CA, Wedemayer GJ, Scherer JR, Mathies RA. *Proc Natl Acad Sci USA* 2002;99:574–579. [PubMed: 11792836]
3. Tsai S-W, Loughran M, Karube I. *J Micromech Microengin* 2004;14:1693–1699.
4. Demianov Z, Shimmo M, Poysa E, Franssila S, Baumann M. *Electrophoresis* 2007;28:422–428. [PubMed: 17177246]
5. Woolley AT, Mathies RA. *Proc Natl Acad Sci USA* 1994;91:11348–11352. [PubMed: 7972062]
6. Foquet M, Korlach J, Zipfel W, Webb WW, Craighead HG. *Anal Chem* 2002;74:1415–1422. [PubMed: 11922312]
7. Soper SA, Ford SM, Qi S, McCarley RL, Kelly K, Murphy MC. *Anal Chem* 2000;72:642A–651A.
8. McDonald JC, Duffy DC, Anderson JR, Chiu DT, Wu HK, Schueller OJA, Whitesides GM. *Electrophoresis* 2000;21:27–40. [PubMed: 10634468]
9. Qi S, Liu X, Ford SM, Barrows J, Thomas G, Kelly K, McCandless A, Lian K, Goetert J, Soper SA. *Lab Chip* 2002;2:88–95. [PubMed: 15100840]
10. Kameoka J, Craighead HG, Zhang HW, Henion J. *Anal Chem* 2001;73:1935–1941. [PubMed: 11354473]
11. Stachowiak TB, Mair DA, Holden TG, Lee LJ, Svec F, Frechet JMJ. *J Sep Sci* 2007;30:1088–1093. [PubMed: 17566345]
12. Liu YJ, Ganser D, Schneider A, Liu R, Grodzinski P, Kroutchinina N. *Anal Chem* 2001;73:4196–4201. [PubMed: 11569809]
13. Becker H, Locascio LE. *Talanta* 2002;56:267–287. [PubMed: 18968500]
14. Kelly RT, Pan T, Woolley AT. *Anal Chem* 2005;77:3536–3541. [PubMed: 15924386]
15. Sethu P, Mastrangelo CH. *Sens Actuators A* 2003;104:283–289.
16. Liu RH, Yang J, Lenigk R, Bonanno J, Grodzinski P. *Anal Chem* 2004;76:1824–1831. [PubMed: 15053639]
17. Pal R, Yang M, Johnson BN, Burke DT, Burns MA. *Anal Chem* 2004;76:3740–3748. [PubMed: 15228349]
18. Klintberg L, Svedberg M, Nikolajeff F, Thornell G. *Sens Actuators A* 2003;103:307–316.
19. Koesdjojo MT, Tennico YH, Remcho VT. *Anal Chem* 2008;80:2311–2318. [PubMed: 18303914]
20. Kelly RT, Li Y, Woolley AT. *Anal Chem* 2006;78:2565–2570. [PubMed: 16615765]
21. Fuentes HV, Woolley AT. *Anal Chem* 2008;80:333–339. [PubMed: 18031061]
22. Unger MA, Chou HP, Thorsen T, Scherer A, Quake SR. *Science* 2000;288:113–116. [PubMed: 10753110]

Figure 1. Schematic overview of using phase changing sacrificial layers for solvent bonding microfluidic devices

(A) A PDMS layer (crosshatched) is placed on top of an imprinted PMMA chip. (B) The polymer chip is heated and the channels are filled with liquid sacrificial material (gray with white vertical lines). (C) After the sacrificial layer is cooled and solidified (gray with horizontal white lines) the PDMS covering is removed and solvent (black) is added to the top of the PMMA chip. (D) A covering plate is placed on top of the imprinted chip and bonded. (E) The bonded chip is heated and the wax is removed from the channels in the completed device. Reprinted with permission from ref. 14; copyright 2005, American Chemical Society (ACS).

Figure 2. The integration of a semipermeable membrane with an open microfluidic channel using phase changing sacrificial layers

(A) A PMMA piece with an opening is placed on top of a protected channel. (B) A monomer solution is placed in the PMMA well. (C) The mixture is photopolymerized. (D) The device is heated and the wax is removed. Reprinted with permission from ref. 20; copyright 2006, ACS.

Figure 3. Key features of a multichannel separation device with a crossover channel
(A) Optical image of channels embossed in PMMA at the crossover interface. (B) Optical micrograph of channels filled with a sacrificial material. (C) Photograph of a completed multilayer device. (D) Channels filled with colored solution to show fluidic independence. Reprinted with permission from ref. 21; copyright 2008, ACS.

Figure 4. Potential multilayer microdevice designs enabled by sacrificial fabrication methods

(A) A layout with four standard microchip capillary electrophoresis systems. (B) A crossover channel format that reduces reservoir numbers and makes better use of space. (C) Three pieces of a device that illustrates how crossover channels can be individually addressable and available for functionalization before assembly into a more complex microfluidic network. (D) A three-dimensional illustration of the formation of an electrospray nozzle on a chip to facilitate coupling to a mass spectrometer. (E) Integration of an electromagnet in a microfluidic device near a separation channel, using phase changing sacrificial materials and a thin polymer film.