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Sacrificial Layer Microfluidic Device Fabrication Methods

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Abstract

Over the past fifteen years, research in the field of microfluidics has experienced rapid growth due to significant potential advantages such as low cost, short analysis times and elimination of sources of contamination. Whereas etched and thermally bonded glass substrates have seen widespread use and offer solid performance, device fabrication remains cumbersome. Recent advances in sacrificial layer microfabrication methods for microfluidics should overcome many disadvantages of conventional fabrication approaches. Phase-changing sacrificial layers have been implemented in making inexpensive and high-performance polymer microchips for electrophoretic analysis, protein focusing and sample preconcentration. In addition, novel channel fabrication methods based on standard thin-film processes, which are readily integratable with microfabrication techniques used for electrical components, are being applied increasingly for the creation of microfluidic devices. These new sacrificial layer fabrication approaches will be instrumental in making low-cost and high-quality polymer microchips, and in interfacing electrical and fluidic systems on glass or semiconductor substrates.

Keywords

Capillary electrophoresis; Lab-on-a-chip; Microchip; Microfabrication; μ -TAS

Abbreviations

EFGE, electric field gradient focusing; **LED**, light-emitting diode; **PCSL**, phase-changing sacrificial layer; **PECVD**, plasma-enhanced chemical vapor deposition; **PMMA**, poly(methyl methacrylate); **PSG**, phosphosilicate glass; **μ -TAS**, micro-total analysis system

1 Introduction

Since the development of chromatography at the beginning of the twentieth century, separation science has emerged as one of the largest fields in analytical chemistry. Indeed, separations are used routinely in pharmaceutical development, medical diagnosis, forensics, environmental monitoring, fundamental scientific research and many other applications [1]. The wide popularity of separations has inspired considerable efforts to improve, automate and expand the capabilities of these tools. One emerging area of research is microfluidics, which focuses on miniaturizing and integrating separation systems [2].

Miniaturization is achieved effectively through the use of micromachining technologies applied in the semiconductor industry; these methods allow remarkable control over micrometer-sized features in sophisticated yet mass-producible miniaturized platforms. Thus,

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microfluidic systems should offer many of the advantages already realized in the semiconductor industry; namely, greater capabilities in increasingly cheaper, smaller and faster devices that can be made portable. Moreover, the reduced dimensions of microfluidic networks naturally require smaller sample volumes than traditional techniques, providing an advantage in analyses where specimen quantity is limited, such as in forensics or cerebrospinal fluid analysis [3]. Finally, by integrating and automating multiple sample handling steps on a single platform, microfluidic methods hold potential to decrease erroneous results due to human errors or contamination.

The earliest microfluidic devices were made from either glass or silicon, materials familiar to the semiconductor industry. For example, in the fabrication of CE microchips, channels were etched into a glass or silicon substrate, and a cover plate with access holes was bonded over the top [4]. Despite the prevalence of silicon in the microelectronics industry, glass substrates dominated microfluidic device output due to better electrical and optical properties.

More recently, many polymers have been added to the list of substrates used for the creation of microfluidic devices, due to their ease of fabrication and low cost [5]. Moreover, thermal imprinting and bonding of polymer microchannels avoid the disadvantages of high-temperature annealing and stringent cleaning, although microdevices made in polymer substrates generally suffer from comparatively weak bonding and channel deformation during enclosure [6]. The chemical resistivity and melting temperatures of some polymers also impose constraints on the microfabrication techniques that can be used in making microchips. The development of enhanced fabrication methodologies that overcome the disadvantages of polymer microfluidic device construction would facilitate the broader use of these easily made, low-cost systems.

Control over the micrometer-sized dimensions in fluidic microdevices is generally obtained through photolithographic techniques. The logical combination of these with thin-film methods will be critical for the integration of electrical and fluidic control to form micro-total analysis systems (μ -TAS). Indeed, thin-film methods have enabled the integration of many components, such as electrodes [7,8], heaters [9,10], optical sources [11,12], waveguides [13], filters [14] and detectors [14–16] in fluidic systems. For example, gold thin-film electrodes have been used in microchips for both in-channel and end-channel amperometric detection [7]. Thin-film boron-doped diamond electrodes have been implemented for amperometric detection of purines, showing favorable signal-to-noise characteristics even at high detection voltages [8]. Microfluidic devices with thin-film heaters have been developed for use in PCR amplification [9] and the determination of DNA hybridization kinetics [10]. Thin-film polymer light-emitting diodes (LEDs) with peak emission at 488 nm have been integrated on microchips as excitation sources for CE analysis; the required potential for LED operation was as low as 3.7 V [11]. Subsequently, a thin-film organic LED with peak emission at 540 nm was integrated on-chip to excite fluorescence of Albumin Blue 580 reacted human serum albumin [12]. Hollow-core anti-resonant reflecting optical waveguides have been constructed on planar substrates; these systems provided single-molecule detection sensitivity in microchannels [13]. Thin-film optical filters based on Fabry-Perot optical resonators have been used in a microdevice capable of detecting absorbance at specific wavelengths from a broadband visible source and then outputting the information in bit streams to provide computer integration [14]. Finally, Du et al. have developed a composite thin-film electrochemiluminescence detector for microchip CE analysis [15].

Sacrificial etching has been developed in the field of microelectromechanical systems for various applications, including mechanical micromotors [17], acceleration sensors [18], switches [19] and micromirrors for optical projection [20]. Sacrificial and thin-film technologies also have potential to be applied in the fabrication of microchannels, with the

important advantage of straightforward integration with thin-film electrical components for device operation. Moreover, sacrificial and thin-film microfabrication processes are compatible with mass fabrication, a critical criterion for low-cost systems. Finally, sacrificially formed microchannels avoid the use of a high-temperature thermal bonding step. It has been noted that difficulties in thermal bonding need to be overcome before highly integrated, commercially viable microfluidic devices (e.g., μ -TAS) can be realized [6]. One challenge associated with the sacrificial layer approach is the slow, diffusion-limited nature of chemical etching from the channel ends to remove the sacrificial material. If this issue can be addressed, then sacrificial layer technologies offer promising potential to eliminate thermal bonding and thus facilitate the development of microfluidic analysis systems.

In this review paper, we discuss several fabrication schemes that have been developed using sacrificial layers to achieve enclosed microfluidic systems. We cover the use of phase-changing sacrificial layers (PCSLs) in polymeric substrates for the development of microchips for CE, electric field gradient focusing (EFGF) and preconcentration. Also described is the implementation of thin-film inorganic or photolithographically defined polymer sacrificial layers for the creation of tubular microstructures for CE or DNA sequencing. We next discuss an approach that utilizes a combination of metal and photoresist sacrificial layers for the fabrication of thin-film microsystems and show initial CE results. Finally, we present much improved separations recently obtained in these thin-film microdevices.

2 Phase-Changing Sacrificial Layers

Kelly et al. [21] have obtained promising results using paraffin wax PCSLs to create solvent-bonded poly(methyl methacrylate) (PMMA) microfluidic devices. In this approach, an imprinted polymer substrate has its channels filled with liquid paraffin wax, which forms a solid PCSL upon cooling. A second polymer piece is then solvent bonded to the imprinted substrate, where the PCSL prevents dissolved PMMA or solvent from entering and clogging the microchannels during enclosure. Once the substrates are sealed together, the device is heated to allow the straightforward removal of the liquefied paraffin wax, thus overcoming the issue of diffusion-limited chemical etching.

The PCSL fabrication process yielded microchips that could withstand at least an order of magnitude higher internal pressure than thermally bonded polymer devices. In addition, channel integrity was maintained better than in thermal bonding, and the fabrication time was reduced. PCSL-formed, solvent-bonded microchips were used for CE of FITC-labeled amino acids and peptides (Figure 1). Separations were rapid (~ 10 s) and efficient ($>40,000$ theoretical plates), and the use of electric fields two-fold higher than in other polymer microchips was possible.

Similarly, PCSLs have been employed for the creation of microfluidics interfaced with polymer membranes [22]. To make devices, microchannels were imprinted into a base polymer substrate and protected with a PCSL. A coverplate having PCSL-protected buffer and sample reservoirs, as well as an open membrane reservoir, was placed atop the lower piece, and the membrane reservoir was filled with a prepolymer solution. The membrane was polymerized using UV radiation, and once the PCSL was removed, the membrane was interfaced with an open microfluidic channel. Devices created in this manner were used for both EFGF and protein preconcentration. EFGF microchips delivered a threefold resolution improvement in protein focusing compared to capillary-based systems. Moreover, these EFGF microdevices were used in separating peptides (Figure 2); the resolution was similar to that in microchip CE, but the analytes were enriched more than 150 fold in microchip EFGF relative to CE. PCSL-formed membranes were also used to enrich R-phycoerythrin over 10,000 fold in a microdevice. These

examples illustrate the power of the PCSL approach in making improved microfluidic systems with increased functionality.

3 Thin-Film Sacrificial Layer Microfluidics

Sharma et al. employed thin-film techniques for the creation of microdevices for detecting surface potential changes [23]. A layer of silicon dioxide was sandwiched between two silicon films, and a thin (205 nm) channel was etched into the top silicon layer, except for at a few small bridge points. This thin channel provided access to the sacrificial SiO₂ film, which was then etched through to the second silicon layer, making a deeper and wider channel. Gold/titanium contacts were deposited to provide a source and drain at the silicon bridges, and a piece of PMMA was clamped over the top of the substrate to enclose the channels. These devices were used to manipulate the flow of rhodamine B; current vs. voltage curves on the silicon bridges were obtained at different reference potentials, and surface potential changes as low as 8 mV could be detected.

Guijt et al. [24] employed thin films and sacrificial etching to make silicon nitride/glass microchannels. Features etched into a silicon substrate had a thin film of silicon nitride deposited on top. Next, the silicon nitride coated substrate was bonded to a glass piece with thin-film platinum electrodes. Finally, the silicon was removed from the thin-film silicon nitride layer by back etching. The resultant channel with 360-nm-thick walls (Figure 3A) was used in CE separation of Li⁺, K⁺ and Na⁺, which were detected based on conductivity at the platinum electrodes (Figure 3B).

Although these previous examples of sacrificially formed thin-film microfluidic systems showed the integration of electrical and fluidic components, the fabrication was subject to difficulties associated with sealing a coverplate to enclose channels. To overcome this problematic bonding step while maintaining the device integration advantages of thin-film systems, several groups have devised fabrication methods that employ sacrificial layers that define microchannels, over which enclosing walls may be formed. Removal of the sacrificial material then provides open columns for microfluidic work.

A variety of materials and techniques have been employed in the creation and removal of sacrificial layers, as well as for the formation of microchannel walls. For example, Lee and Lin [25] layered thin films of polysilicon, silicon nitride and phosphosilicate glass (PSG) to create microfluidic channels. Two fabrication schemes were demonstrated, one employing a polysilicon sacrificial layer enclosed by PSG channel walls, and the other using a PSG sacrificial layer covered with polysilicon. Small openings in the channel walls provided multiple points of access for the etchants (KOH or HF) to reach the respective polysilicon or PSG sacrificial layers. These etch holes substantially decreased the time needed for sacrificial layer removal (relative to etching only from the channel ends), and the openings were sufficiently small that surface tension was expected to prevent fluid leakage during CE analysis; however, no separation work was reported.

An approach developed by Kohl's group incorporated spin-on photosensitive polycarbonate or polynorbornene sacrificial layers for the creation of silicon dioxide, silicon nitride or polymer-enclosed air gaps [26–29]. The patterned sacrificial layers were enclosed beneath plasma-enhanced chemical vapor deposition (PECVD) silicon dioxide or silicon nitride films, or under spun-on polymers. Removal of the sacrificial layer was achieved through decomposition at elevated temperature (110–425 °C) for up to 10 h as an alternative to chemical etching. Though the use of the resulting structures as microfluidic channels was suggested, it was not reported.

An early example of a microfluidic device made using thin-film and sacrificial layer technologies was developed in Craighead's group [30]. Thin films of PMMA, aluminum, silicon dioxide, polysilicon and silicon nitride were used to define and create a microfluidic structure with 100-nm-wide pillars. The polysilicon sacrificial layer was wet etched using tetramethylammonium hydroxide, leaving silicon nitride pillars supporting PECVD silicon dioxide. The electrophoretic velocities of two different types of DNA were determined in these microfluidic structures.

Subsequently, Craighead's group created DNA fragment sizing devices using thin-film and sacrificial layer technologies [31]. Polysilicon sacrificial layers were enclosed by silicon dioxide, and etch holes were patterned into the silicon dioxide layer to provide rapid (4 h) removal of the sacrificial layer by wet chemical etching. Finally, a second layer of silicon dioxide was deposited to seal the etch holes. The small feature dimensions (270 nm height $\times$ 1–10 μ m width, Figure 4A) obtained through this micromachining approach made the channels ideal for single-molecule DNA fragment sizing (Figure 4B). Although the devices had appropriate dimensions for the DNA work performed, scalability to taller or wider channels desirable for many other types of analyses was not reported.

We have also explored the use of sacrificial and thin-film technologies to create microfluidic devices on planar substrates [13,32]. Like some of the work described previously, our construction technique obviates the need to thermally or otherwise seal a cover plate to a micromachined substrate for channel enclosure, thus eliminating one of the most difficult steps in the fabrication process [6]. Importantly, our method is compatible with a broad range of sacrificial layers, channel walls and base materials, providing unique fabrication flexibility. We have explored various sacrificial layers (SU-8, aluminum and AZ3330 photoresist), wall materials (silicon dioxide, silicon nitride and amorphous silicon) and base substrates (silicon, quartz and Borofloat glass). Furthermore, we anticipate facile expansion to other materials by making minor adjustments to the fabrication steps, such as altering the method of overcoat material deposition. We also take advantage of the high precision obtainable through photolithographic patterning of the sacrificial layer.

Our fabrication process involves the deposition of a thin layer of aluminum on quartz or Borofloat glass wafers, followed by the spinning on of AZ3330 photoresist (Figure 5A). Next, the photoresist is patterned and developed, and the unprotected aluminum is etched away, leaving an aluminum/photoresist sacrificial layer (Figure 5B). Then, PECVD silicon dioxide is deposited over the patterned features, forming enclosure walls for the microchannels (Figure 5C). Lastly, the channel ends are exposed, and the aluminum, followed by the photoresist, is etched away (Figure 5D) to form microfluidic structures; the two-stage etching approach provides more rapid sacrificial material removal than can be obtained on a solid photoresist core.

Figures 6A–B show SEM images of channels fabricated using our method. In initial studies, these channels were used to separate FITC-labeled amino acids (Figure 6C) in under 30 s [32]. More recently, we have used UltraTrol Dynamic Precoat reagents (Target Discovery, Palo Alto, CA) and higher applied potentials to obtain significantly improved CE separations. Figure 7 shows the same three amino acids as Figure 6, separated under these new conditions. Reduced electroosmotic flow reversed the migration order, peak widths well under 1 s were observed and the separation efficiency was improved by as much as a factor of ten. Moreover, rapid separations of FITC-tagged peptides were also feasible in these devices (Figure 8), illustrating potential for proteomics applications. Finally, a single 100-mm-diameter wafer processed using our fabrication scheme can contain over 30 microdevices of about 1 cm $\times$ 1 cm dimensions. Using larger wafers and batch processing could generate significantly greater numbers of these microchips, similar to what is done in the microelectronics industry. Thus,

our approach shows important progress toward realizing the mass fabrication potential of microfluidic structures.

4 Conclusions

Sacrificial microfabrication technologies provide important advantages, such as the straightforward integration of electrical components, the avoidance of problematic thermal bonding steps, and the use of an established format for mass fabrication, all of which are critical for the development of broadly applicable μ -TAS platforms. Furthermore, this approach should enable the development of microstructures in materials that were previously unused or underused due to fabrication constraints.

Phase-changing sacrificial layers for polymeric microdevices have shown promise for performing rapid and efficient CE separations. PCSL-formed microchips should also enable the fabrication of more complex systems with increased numbers of lanes, due to reduced channel deformation and stronger substrate bonding. Moreover, the PCSL approach has proven valuable for interfacing ion-permeable membranes with microfluidic systems to enable new separation techniques and provide on-chip sample preconcentration. Finally, polymeric microdevices have low materials costs and simplified fabrication procedures, which may be advantageous in many applications.

Thin-film sacrificial layer fabrication shows promise for the development of sophisticated microdevices capable of performing comprehensive analyses, although further research needs to be done in this regard. For example, issues such as etch holes, residues from thermally decomposed sacrificial layers, relatively long etch times for sacrificial layer removal, and interfacing between the macro and micro worlds, must be addressed for this approach to have maximum impact. Importantly, the thin-film sacrificial layer fabrication strategy offers a straightforward route to making three-dimensional microfluidic arrays containing multiple layers of thin-film channels that can cross over one another without interference. Lastly, combining successful thin-film sacrificial layer methods with established techniques for the fabrication of electrical components should lead to intricate separation systems having integrated sample preparation and detection capabilities that draw near to the goal of μ -TAS.

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Figure 1. Electropherograms of FITC-labeled amino acids and peptides separated in PCSL-formed, solvent-bonded PMMA microdevices. (A) Separation of FITC-tagged amino acids; peaks are: (1) Gly, (2) Asn, (3) Phe and (4) Arg. (B) CE of FITC-labeled peptides; peaks are: (a) FLEEI; (b) FA; (c) FGGF; (d) Leu enkephalin; (e) angiotensin II, fragment 3–8; (f) angiotensin II; and (g) GGYR. Adapted with permission from [21]; copyright 2005, American Chemical Society.

Figure 2. Focusing of peptides in a sacrificially formed EFGF microchip. Peaks are: (a) FLEEI; (b) FGGF; (c) angiotensin II, fragment 3–8; and (d) GGYR. Reprinted with permission from [22]; copyright 2006, American Chemical Society.

Figure 3. Thin-film silicon nitride/glass CE microchannels. (A) SEM image of a microchannel and (B) electropherogram of three ions separated at 80 V/cm. Reprinted with permission from [24].

Figure 4. Thin-film sacrificially formed microchannels for DNA analysis. (A) SEM image of a channel and (B) photon burst histogram of a mixture of DNA fragments assayed. Reprinted with permission from [31]; copyright 2002, American Chemical Society.

Figure 5. Fabrication schematic for thin-film sacrificial layer microfluidic devices. (A) Deposition of aluminum (black) and photoresist (crosshatched gray). (B) Patterning of photoresist and aluminum sacrificial layers. (C) Deposition of PECVD silicon dioxide (light gray) to form channel cover. (D) Removal of sacrificial layers through wet chemical etching.

Figure 6. Thin-film sacrificially formed microdevices for CE analysis. (A–B) SEM images of microchannels; scale bar is 20 μm in (A) and 5 μm in (B). (C) CE separation of three FITC-labeled amino acids in a thin-film microdevice. Reproduced from [32] by permission of The Royal Society of Chemistry.

Figure 7.

Electropherograms of same amino acids as in Figure 6. Coatings were UltraTrol Dynamic Precoat HR in (A) and (C) and UltraTrol Dynamic Precoat LN in (B) and (D). The electric fields for the separations were 660 V/cm in (A–B) and 830 V/cm in (C–D). Peaks are: (1) glycine, (2) phenylalanine, (3) FITC, and (4) arginine.

Figure 8. Electropherogram of three FITC-labeled peptides separated at 830 V/cm in planar thin-film microfluidic channels treated with UltraTrol Dynamic Precoat HR. Peaks are: (1) FLEEI, (2) Leu enkephalin and (3) GGYR.