Fabricating Large Arrays of Microwells with Arbitrary Dimensions and Filling Them Using Discontinuous Dewetting

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This paper describes the fabrication of large (up to 45 cm²) arrays of microwells, with volumes as small as ~3 fL/well and densities as high as 10^7 wells/cm². These arrays of microwells are formed by casting an elastomer, poly(dimethylsiloxane) (PDMS), against "masters" prepared by photolithography; arrays of microwells in other polymers can be formed by using a master consisting of posts in PDMS. A straightforward technique, discontinuous dewetting, allows wells to be filled rapidly (typically on the order of 10^4 wells/s) and uniformly with a wide range of liquids. Several rudimentary strategies for addressing microwells are investigated, including electroosmotic pumping and gaseous diffusion.

Techniques for handling and analyzing small quantities of material are important for the development of new analytical methods in both chemistry and biology. The ability to isolate and manipulate small quantities of liquids is needed for studying single molecules in solution: a 1-fL (1 fL = 1 μm³) vessel filled with a 1-nM solution will contain, on average, one molecule. The use of small amounts of materials in chemical reactions limits their hazard and environmental impact. The combination of small volumes with microanalytical methods is also a component of microscale total analysis systems (μTAS).[1−3] Arrays of microscopic vessels are useful for the examination of statistical events, where large numbers of small, indistinguishable reactions are needed: nucleation of crystals[4] and the formation of drops of condensed liquids[5] are examples of such processes. In this paper, we apply a fabrication technique developed in our group to the preparation of arrays of microscopic vessels, and we present a straightforward, general method for filling these arrays with liquids. We also investigate several simple methods for delivering reagents to these wells and for analyzing the products of reactions in them.

To perform reactions on a small scale, methods are required to confine small volumes of liquids spatially in what we will refer to as micro*reactors* (to emphasize the focus on their use as containers for chemical reactions). There are a number of approaches to the formation of spatially localized arrays of small quantities of liquids. A nebulizer can be used to form disordered arrays of drops.[6−8] Precise control over drop size and placement by this method is not practical, however: the drops formed range in volume from picoliters to femtoliters and are randomly placed on the surface. Formation of arrays of microdrops with good control and more uniform volumes is possible, for example, by the assembly of microscopic drops of liquids on patterned self-assembled monolayers (SAMs).[5] Another approach for confining liquids is to generate microwells (or depressions in a surface) into which liquid can be delivered. Microwells[9−13] can be fabricated in silicon and glass using standard micromachining techniques.[14−16] These materials and processes are, however, relatively expensive, and the facilities required to use them are not routinely accessible to most chemists and biologists. Small arrays (~2 cm²) of picoliter microvials (0.4−300 pL; 10−120 μm each side) in polystyrene that overcome some of these problems have been formed by embossing using structures formed by photolithographic patterning techniques.[17] This embossing technique, however, is not convenient for the formation of large arrays of microwells with uniform, small volumes because the array cannot be released easily from the master. Recently, Walt et al.[18]

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demonstrated that densely packed ($\sim 4 \times 10^8$ wells/cm^2), ordered arrays of submicrometer wells (~ 0.25 μm diameter) can be formed by etching a bundle of optical fibers (1 mm diameter) and can be replicated by molding. These arrays, however, are small in area, and the size of wells and their density cannot be varied easily.

Methods for addressing microreactor vessels of the types described here typically involve the use of a micromanipulator to position a micropipet[6-8,17] or the use of an ink-jet printer.[19,20] Ink-jet printing of drops of liquid onto contoured or patterned surfaces is a promising technique that is being developed actively for the synthesis of arrays of oligonucleotides[19] and combinatorial libraries;[20] at this stage, however, the drops it forms are relatively large in size (>100 pL volume, >100 μm diameter).[19] Rapid delivery of reagents to microreactors is not simple and is currently the subject of research—new methods for delivery are needed.

The aim of the experiments described here was to produce a system for studying small volumes of liquid in vessels that were (i) manufactured by a flexible, rapid process that permits the volume of wells to be controlled easily; (ii) optically transparent at wavelengths required for spectroscopic analysis; (iii) low in background fluorescence, to enable analysis by fluorescence microscopy; (iv) chemically and physically inert; (v) easily filled with solution; (vi) addressable; (vii) inexpensive; and (viii) electrically insulating. Using low-cost, soft lithographic techniques that have been described previously,[21,22] we have fabricated large, regular, and planar arrays (<45 cm^2) of microwells in the surface of polymers. The microwells can be tailored to have dimensions and densities ranging between ~ 3 fL, with 10^7 wells/cm^2, and ~ 160 nL, with ~ 100 wells/cm^2. We present a method that we refer to as discontinuous dewetting that addresses the major problem associated with working with large arrays of wells: filling them uniformly and rapidly with a solution. Discontinuous dewetting fills wells by exploiting the differences in the interfacial free energies of the substrate and the liquid. It is a phenomenon that occurs when a drop of liquid is allowed to drain from a surface bearing discrete depressions and having surface free energy lower than that of the liquid: the holes remain filled as the liquid dewets the surface of the material.

We believe that the fabrication and filling methods presented here should find immediate use in applications that require the rapid distribution of small, uniform volumes of solutions or suspensions into spatially well-defined microreactors arranged in large ordered arrays. We have explored strategies for delivering reagents to and removing products from individual microwells in PDMS substrates, including electroosmotic pumping and gaseous diffusion, to demonstrate that chemical reactions can be performed in these microwells.

RESULTS AND DISCUSSION

Fabrication of Microwells. Figure 1 illustrates the method that we used to produce large arrays of microwells.[23,24] This

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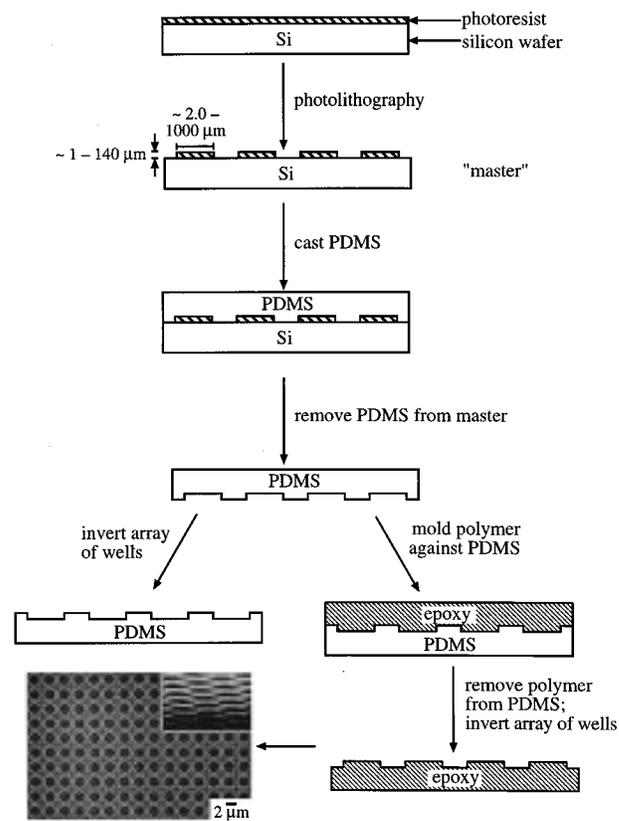**Figure 1.** Procedure for the fabrication of microreactor wells. Photolithography using either a chrome mask (for feature sizes < 50 μm) or a high-resolution transparency mask (for feature sizes > 50 μm) produced a master. Poly(dimethylsiloxane) (PDMS) prepolymer was cast against the master. After curing the PDMS at 65 °C for 2 h, we removed the polymeric mold with microwells defined on its surface from the master. To make arrays of microwells from other materials, an array of posts was made in PDMS by this procedure, and then the other material was molded against the PDMS master. A scanning electron micrograph of a section of an array of microreactor wells formed in epoxy (Epo-tek UVO114) is shown (bottom left); inset shows side view of wells.

method has been used previously for the fabrication of stamps and molds for soft lithographic techniques;[22] our approach has been to adapt this fabrication technique to the formation of arrays of microwells. An array of posts formed in photoresist on a silicon wafer was used as a "master" to form the arrays of microwells. The master was generated either by "rapid prototyping" (for feature sizes > 50 μm)[25]—a method that uses a high-resolution transparency as the photomask for photolithography—or by performing standard photolithography with a chrome mask (for feature sizes < 50 μm). The arrays of wells were fabricated by molding an elastomeric polymer, poly(dimethylsiloxane) (PDMS), against the master.[23] An elastomeric polymer was used because it could be separated easily from the master without damaging either the molded polymer or the master. The masters could be reused many (>20) times.[23] Figure 1 shows a scanning electron micrograph (SEM) of a section of an array of the smallest microwells (diameters ~ 2 μm) that we have obtained using this technique.

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We have used this molding technique to produce wells with a variety of geometries, with aspect ratios (depth:width) between 1:5 and 1:1. The lateral dimensions and shapes of the wells were controlled by using different masks for photolithography; the depth of the wells was controlled by using different photoresists and different speeds of spin coating. Diameters (and depths) of the wells ranged between 2 μm ($\sim$1 μm deep), 10 μm (1–2 μm deep), 50 μm (1–50 μm deep), and 1000 μm (40–200 μm deep); volumes ranged between $\sim$3 fL and $\sim$160 nL. The density of wells in the array was determined by the period of the features on the mask; the highest densities that could be achieved easily with the procedures we used were approximately 10^7 wells/cm^2 (for wells 2 μm in diameter, separated by 1 μm). It should be possible to fabricate smaller wells with higher densities using appropriate masters, since neither the fabrication nor the phenomenon of discontinuous dewetting used to fill the wells is intrinsically limited to the dimensions, densities, and circular shapes we have examined. The area over which these arrays can be produced is limited only by the mask aligner used for photolithography and the size of the mask itself: we have formed arrays as large as 45 cm^2, but typically worked with arrays of $\sim$10 cm^2.

Using the rapid prototyping methodology, the complete cycle from design of the array to its fabrication is less than 24 h; using a chrome mask, once the mask has been produced, the fabrication requires less than 6 h to make masters and to mold arrays. The ability to use the master many times (>20) for the fabrication of arrays is also a significant advantage over methods that require micromachining of glass or silicon each time an array is prepared.

PDMS has several advantages over materials that are typically used for the fabrication of microwells and lends itself to several strategies of individually addressing microwells that will be described in the next section. In addition to its ability to be molded and its elasticity, it is optically transparent in the UV/visible region (down to $\sim$350 nm),[26] electrically insulating, and permeable to many gases.[27] The optical transparency of PDMS facilitates the study of events in microwells using optical microscopy and also makes spectroscopic assays possible. To pump liquids into microreactors using electroosmosis requires an electrically insulating substrate: PDMS is a suitable material for this application. The permeability of PDMS to gases allows air bubbles that form on immersion of the array in a solution to dissipate and also allows diffusion of reactive gases into the wells.

Chemical stability is an important consideration when selecting an appropriate material for the arrays of microwells. Although PDMS is swollen by many organic solvents,[28] it is unaffected by water, perfluorinated compounds, and other polar solvents (e.g., alcohols, ethylene glycol, tri(ethylene glycol)); the microwells are compatible with many chemical and biological systems. If an organic solvent that swells PDMS is required for a reaction, then arrays of wells can be formed from other polymers.

The fabrication of microwells in many polymers is hindered by their rigidity; it is difficult to remove them from a master formed by photolithography on a silicon wafer without damage to both the master and the molded polymer. To fabricate arrays from these rigid materials, we used elastomeric masters made
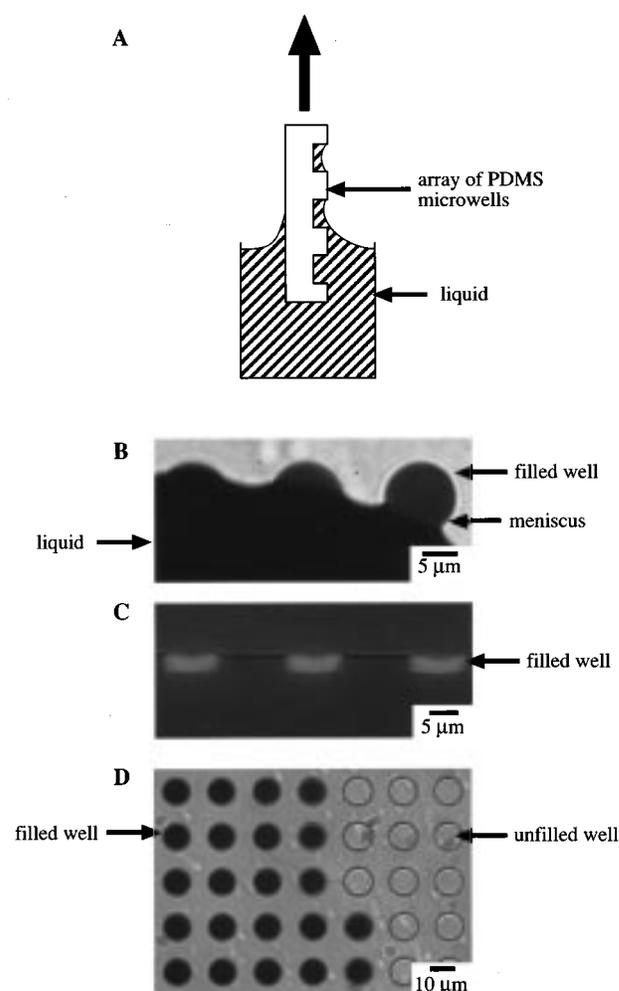

Figure 2. Discontinuous dewetting can fill large arrays of microwells. (A) Schematic illustration of an array of microreactors filling with liquid as they are pulled from a bulk solution. (B) Optical micrograph of wells (10 μm diameter; 2.2 μm deep) in a PDMS surface filling with tri(ethylene glycol) saturated with brilliant green. (C) Optical fluorescence micrograph of a microtomed section through wells filled with epoxy (Epo-tek UVO114) containing Rhodamine B. (D) Empty wells (right) and wells filled with a solution of brilliant green in tri(ethylene glycol) (left) by discontinuous dewetting.

from PDMS (Figure 1)[23] and molded polymers (epoxies, poly-urethanes, and poly(acrylic acid)) against them: this procedure makes release of the replica easier.[29] The polyurethanes that we used (Norland Optical Adhesives) are not attacked by most organic solvents but are degraded by strongly basic solutions.[30] Epoxy substrates are the most stable and inert materials we have examined.[31] Poly(acrylic acid), while soluble in methanol and water, is inert to most other organic solvents.

Discontinuous Dewetting: A Method for Filling Wells. Discontinuous dewetting is a method we have developed for filling large arrays of microwells with similar quantities of reagent rapidly: this method takes advantage of the difference in the interfacial free energies of the substrate and the liquid of interest and the controlled topology of the surface. Other methods (ink-

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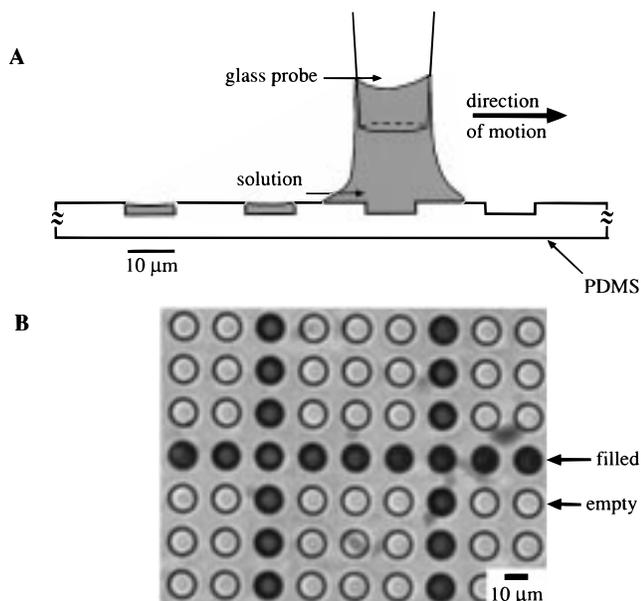(31) Technical Data Sheet, Epo-tek UVO114, Epoxy Technology, Billerica, MA.

A

glass probe

direction of motion

solution

10 μm

PDMS

B

filled

empty

10 μm

Figure 3. Filling of small, adjacent groups of wells by discontinuous dewetting. (A) Schematic illustration of filling small groups of wells by discontinuous dewetting. (B) Optical micrograph of small groups of wells filled by dragging a sharp glass probe (~15 μm diameter) holding a drop of tri(ethylene glycol) containing brilliant green dye across the surface of the microreactors using a small *xyz* stage. The probe was made by dipping an optical fiber (125 μm diameter) into 48% HF for ~10 min. Wells that appear gray are filled with solution (brilliant green in tri(ethylene glycol)), and wells that appear colorless are empty.
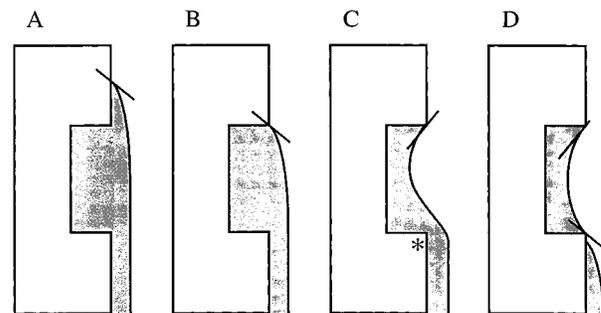

A   B   C   D

*

Figure 4. Qualitative description of the method of discontinuous dewetting used for filling wells. (A) Bulk liquid dewets the surface at an equilibrium receding contact angle. (B) On reaching the top edge of the well, the abrupt change in contact angle pins the drop at this edge.[32-35] (C) The drop hinges on the edge of the well as the bulk liquid recedes. As the liquid drains, it approaches its equilibrium contact angle at the top edge of the well, and the film at the lower edge of the well starts to thin. (D) Liquid is left behind in the well when the thin film at the bottom lip of the well (marked *) ruptures.

jet printing, micropipetting, or using a picospritzer) for fluid delivery exist, but these methods normally require complex engineering and precise measurement to deliver small volumes of liquid in a serial fashion and are unnecessarily complex for filling an array uniformly with liquid.

In discontinuous dewetting, liquid is allowed to drain off an array of microwells either by gravity or by pulling the array from a bulk solution. For most liquids, the microwells remain filled with approximately equal volumes of solution as the liquid dewets the surface of the material. Figure 2 shows a schematic illustration of discontinuous dewetting and optical micrographs of microreactors filled and in the process of filling. This technique, in combination with a simple delivery system, can also be used to fill small groups of wells: a small (~1 pL) drop of liquid is dragged across the surface of small areas of the array using a small glass probe mounted on a micropositioning stage (Figure 3). A single 25-nL drop of liquid (~1 mm diameter), in principle, could be portioned into thousands of picoliter wells by dragging it along a surface with a hydrophobic glass fiber.

Figure 4 summarizes a qualitative explanation for discontinuous dewetting. As an array of microwells is pulled from a solution, the bulk liquid retracts from the surface at a constant receding contact angle. When the liquid front meets a microwell, the abrupt change in angle at the edge of the well causes the drop to be pinned: it is well known that liquids have a tendency to pin both on heterogeneities on planar surfaces and on geometrical heterogenities.[32-35] As the bulk liquid continues to recede, the pinned drop hinges on the edge of the well, and the drop tends toward its equilibrium contact angle at the top edge of the well. Drainage of the liquid at the lower edge of the well causes the film to thin until, finally, it ruptures and pins at the bottom lip (marked *) of the well. This process leaves each of the microwells filled with an equal volume of liquid (Figure 4). We have quantified the filling of the microwells by measuring the fluorescence from a large number (~500) of microwells (~10 μm diameter) and have determined that the deviation in the volume of solution contained in each well is smaller than ±5% at the 1σ level.

Discontinuous dewetting has been used to fill wells in PDMS with diameters between 2 and 1000 μm and with aspect ratios (depth:width) typically between 1:1 and 1:5. The size of the wells that we have filled by discontinuous dewetting has been limited only by the dimensions of masters that we can fabricate easily: it should be possible to fill wells with submicrometer dimensions in the same manner. Liquid in wells with a low aspect ratio (i.e., <1:20), however, tends to bead up to minimize free energy. While this retraction from the boundaries of the well should not hinder performing chemistry in the wells, it could be solved, in principle, by the use of deeper wells, or by selectively increasing the surface free energy of the interior of the well. The frequency of filling wells depends on the rate at which the array is pulled from the liquid, the density of wells in the array, and the lateral dimension of the array: for an array (~5 cm wide) of wells (10 μm diameter, spaced by 5 μm on a square grid), the rate of filling was ~10^4 wells/s. When wells were filled by the discontinuous dewetting procedure, improper filling occurred only on areas of the array where there were defects in the wells (due to photolithography) or where there was debris on the surface of the array: for an array ~6 cm² pulled from a solution of tri(ethylene glycol), ~99% of wells could be filled.

Discontinuous dewetting can be used to fill microwells with many solutions and suspensions of interest to chemists and biologists. Table 1 presents a set of liquids that will fill wells in

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Table 1. Interfacial Free Energies[36] (γ_{lv}) and Advancing (θ_a) and Receding (θ_r) Contact Angles of Liquids on PDMS ($\gamma_{sv} \approx 21$ dyn/cm)[28] That Will Fill Wells (Diameter $\approx 10~\mu$m; 2.2 μm deep) in PDMS

	γ_{lv} (dyn/cm)	θ_a (deg $\pm$ 5 deg)	θ_r (deg $\pm$ 5 deg)
water	73.0	108	81
glycerol	63.4	104	70
ethylene glycol	48.0	92	54
tri(ethylene glycol)	45.6	76	59
dimethyl sulfoxide	43.5	88	40
dimethylformamide	37.0	63	35
butanol	24.6	36[a]	16[a]
ethanol	22.4	31[a]	20[a]
perfluorodecalin	18.3	36[a]	27[a]

[a] Errors on these measurements are closer to ± 10 deg because of the low contact angles and high volatility of the liquids.

PDMS, together with their interfacial free energies and contact angles on PDMS ($\gamma_{sv} \approx 21$ dyn/cm).[28] For discontinuous dewetting to be successful, there are three criteria that the liquid must meet: (i) it must not swell PDMS (many nonpolar organic liquids, e.g., heptane, toluene, methylene chloride, and acetone, swell PDMS, so other substrates must be used); (ii) it must have a low viscosity (<500 cP) so that it begins to dewet the surface on a reasonable time scale; and (iii) it must have a receding contact angle on the substrate that falls in an appropriate range. All solvents we have tried (other than those that swell PDMS, and mercury) meet these criteria. Empirically, we observed that liquids that have receding contact angles (on PDMS) between approximately 16° and 81° will fill wells by discontinuous dewetting. For liquids with contact angles beneath this lower limit, discontinuous dewetting will begin to fail because the liquid will tend to coat the entire surface, both wells and background. We have not found any liquids that exhibit this behavior on PDMS. On surfaces with higher free energy (e.g., PDMS treated with a plasma oxidation ($\gamma_{sv} \approx 59$ dyn/cm)),[28] some liquids will, however, wet completely. Liquids with receding contact angles above 81° tend to bead on the substrate and do not spread to fill the wells at all—mercury is an example. Based on our observations, water ($\theta_r \approx 81° \pm 5°$) seems to represent the upper limit in receding contact angle for discontinuous dewetting on PDMS to work. Care is required when filling with pure water (and other liquids with high contact angles) to ensure that air bubbles do not remain trapped in the microwells.

We[37] and others[38] have overcome a problem associated with filling the microreactors with pure, deionized water (and other, lower boiling solvents): that is, the rapid (<1 s) evaporation of the picoliter volumes under ambient conditions because of the high surface-to-volume ratio of the liquid. Solutions to this problem have included the use of high ionic strength, buffered, aqueous solutions (which may, in any event, be necessary for biological experiments) and closed, humidity-controlled environments. Often, in the experiments presented here, we were able to avoid the problem of evaporation completely by using less

volatile solvents or cosolvents (e.g., tri(ethylene glycol)). We have also, for some applications, sealed the arrays of wells with a microscope coverslip or another piece of PDMS. Other strategies to overcome the problem of evaporation have involved covering drops or reactors with an immiscible layer of mineral oil[13] or heptane,[6-8] or adding glycerol to the water to lower vapor pressure.[10,17]

Addressing Individual Wells in Arrays of Microreactors. The ability to deliver well-defined quantities of reagents to individual microwells is critical for the use of arrays of these wells as vessels in which to perform microchemical reactions. There is, however, a scarcity of methods developed for addressing small microreactors. We have explored several simple ways to deliver fluid to the microreactors to illustrate that chemical reactions can be performed in them. Each method takes advantage of the specific properties of PDMS.

(a) With a Glass Probe. A microdelivery system, consisting of an etched glass fiber mounted on an *xyz* stage, allowed us to deliver material to single wells. We could pick up a drop of liquid on the tip of the probe (diameter $\sim$10 μm), position the needle over a selected microwell (50 μm diameter) to within $\sim$5 μm, and then lower it into the well to deliver the reagent. For example, Figure 5A shows a set of individual wells filled by this method with tri(ethylene glycol) saturated with brilliant green; Figure 5B shows the addition of sulfuric acid or potassium hydroxide using a glass probe (diameter $\sim$10 μm) to individual wells in an array filled by discontinuous dewetting with an acid−base indicator. These experiments also illustrated that the optical transparency of PDMS enables optical microscopy on these arrays. The ability to position a glass probe to within 5 μm suggests that it should also be possible to position an optical fiber over a microwell: this capability would enable the delivery of light to these wells to perform photochemistry.

(b) With Gaseous Reagents. We have taken advantage of the permeability of PDMS to gases to generate a gradient in a gaseous reagent in an array of microwells. The ability to generate gradients in concentration offers a method to vary the amount of reagent delivered to rows of wells in an array; it could be useful for performing combinatorial chemistry. We sealed an array of wells (10 μm diameter, spaced by 5 μm), filled by discontinuous dewetting with an acid−base indicator solution, against a microscope coverslip. A small drop (<1 μL) of ammonium hydroxide was placed on top of the PDMS array, and then the assembly was sealed in a vessel containing another small drop of solution. The production and diffusion of ammonia gas into the microreactors was observed as a change in color of the in the wells (Figure 5C).

(c) By Electroosmotic Pumping and Electrophoresis. Electroosmotic pumping provides the ability to deliver controlled volumes of reagents to the microwells, and capillary electrophoresis offers the ability to analyze the products of a reaction performed in the wells. PDMS is a suitable choice for the array of microwells for these experiments because it is electrically insulating: this property is necessary to avoid shorting of the applied high voltage. Discontinuous dewetting was used to fill an array of larger microwells ($\sim$1 mm diameter; 140 μm deep; $\sim$100 nL) with a solution of *p*-nitrophenol−galactopyranoside (a substrate for β-galactosidase, E.C. 3.2.1.23). We then delivered

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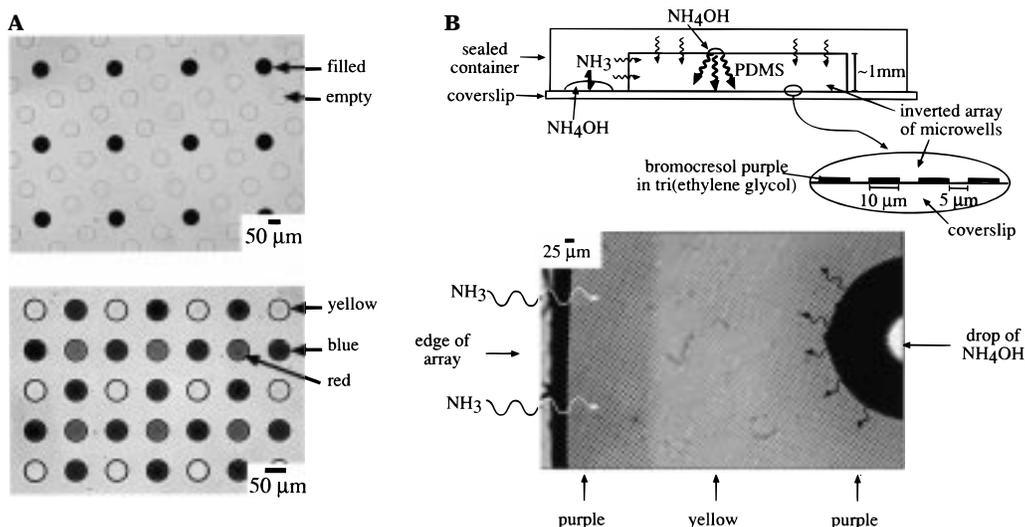
Figure 5. Delivery of chemical reagents to individual microwells molded in PDMS. (A) Using a glass probe: (Top) Empty wells (50 μm diameter) in an array can be filled using a glass probe. A small drop ($\sim$0.5 pL) of tri(ethylene glycol), saturated with brilliant green, was picked up on the tip of a glass probe by passing it through a large drop ($\sim$10 μL) of solution. An *xyz* stage allowed a probe holding the drop to be positioned over a selected microwell (to within $\sim$5 μm) and then to be lowered into the well to deliver the drop of liquid. (Bottom) Reagents can be delivered to wells already containing solution. A $\sim$10^4 $\times$ 10^4 array of microwells (50 μm diameter, 2.2 μm deep) was filled with a solution of tri(ethylene glycol) saturated with bromocresol purple and thymol blue by discontinuous dewetting. A small ($\sim$0.5 pL) drop of either concentrated sulfuric acid or potassium hydroxide was then delivered serially to selected wells in the array. Addition of acid or base caused the indicator (yellow at approximately neutral pH) mixture to become red (acidic) or blue (basic), respectively. Wells that were untouched (i.e., still at neutral pH) appear colorless in this black-and-white micrograph; wells that were made acidic appear gray; wells that received base appear black. (B) By gaseous diffusion: Discontinuous dewetting was used to fill a 10^4 $\times$ 10^4 array of microwells (10 μm diameter, 2.2 μm deep) with a solution of tri(ethylene glycol) saturated with bromocresol purple (which appeared yellow at approximately neutral pH). The array of microwells was sealed by placing a microscope coverslip over it. A small drop of NH$_4$OH was placed on top of the PDMS array, and then the inverted array was sealed in an atmosphere containing ammonium hydroxide. The presence of ammonia gas was observed as a change in color (yellow to purple) of the indicator as the ammonia diffused through the PDMS into the wells. A gradient in color appeared first in wells along the edges of the array: the shortest path length for NH$_3$ gas to the filled wells was laterally in from the edge of the array. A radial gradient in color appeared, later, under the drop of NH$_4$OH. In this black-and-white micrograph, yellow wells appear colorless, and purple wells appear gray.

a plug of solution containing β-galactosidase ($\sim$2−4 nL) to an individual well by placing an electrode and the end of a microcapillary into it; by applying a voltage between a vial containing buffer solution and microwell, electroosmotic flow toward the microwell caused the plug of enzyme to be delivered into the solution of substrate. The procedure is illustrated schematically in Figure 6. The capillary remained in place during the course of the reaction. A sample of the reaction mixture was removed from the microwell[39] and analyzed by CE (Figures 6B,C) in the same capillary as used for delivery.[40]

CONCLUSIONS

We have applied simple soft lithographic techniques to the fabrication of large arrays of microwells with densities of wells as high as 10^7 wells/cm^2. All dimensions of the wells can be varied to produce wells with volumes ranging from 3 fL to 160 nL. These dimensions do not represent intrinsic limitations: smaller wells will be possible using different masters for the molding steps. The use of rapid prototyping to produce masks with features in any geometrical pattern greater than $\sim$50 μm significantly reduces both the cost and the turnaround time for the fabrication of masters. We believe that these advantages make this technique

preferable to other fabrication methods, such as embossing and micromachining.

Discontinuous dewetting is a straightforward technique that exploits the interfacial properties of the system and addresses the problem of filling large arrays of microwells uniformly with solution at high rates of filling. It can fill an array ($\sim$5 cm wide) of microwells with equal volumes within 5% (10 μm diameter, 5 μm spacing) at $\sim$10^4 wells/s; this frequency of filling makes discontinuous dewetting a technique that is complementary to more complex serial techniques, such as ink-jet printing. For applications that require the rapid distribution of small, uniform volumes of solutions or suspensions into spatially well-defined microreactors arranged in large ordered arrays, our fabrication technique combined with discontinuous dewetting may be preferable: for example, we are currently investigating applications that include single enzyme molecule reactions, cell-based sensors,[37] cell-based assays of combinatorial libraries,[41] and pixelated optical displays.[42]

Methods for addressing microwells for performing chemical reactions are still primitive and slow. We have investigated several simple methods for addressing individual wells in PDMS, including gas diffusion and capillary electrophoresis. The microreactor

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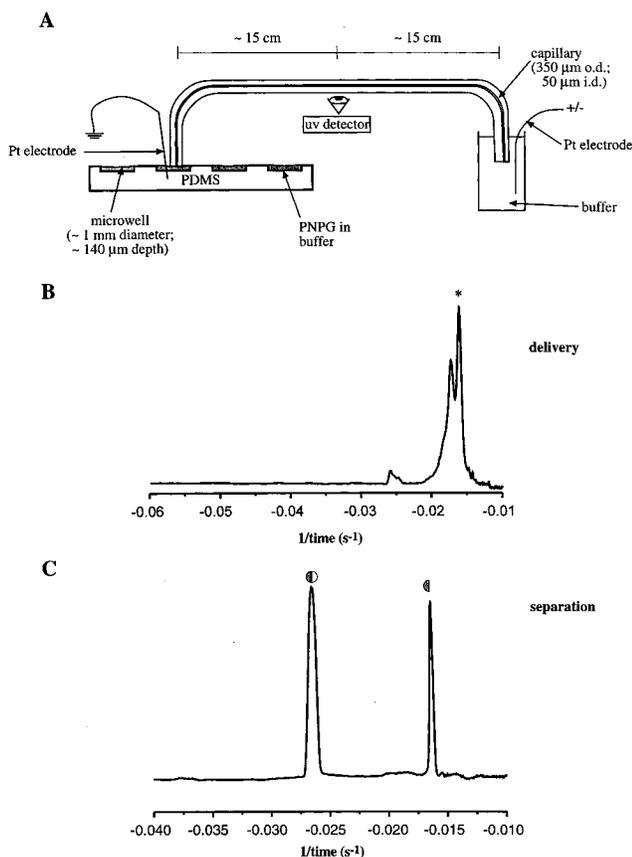
Figure 6. Delivery of an enzyme to a microwell filled with a solution of substrate using electroosmotic pumping, and analysis of the components of the enzyme−substrate reaction using capillary electrophoresis (CE). (A) Experimental arrangement: A plug (∼2−4 nL) of β-galactosidase (0.2 mg/mL in 25 mM Tris and 192 mM glycine buffer; ∼5 × 10^9 molecules of the homotetramer (∼2 × 10^{10} active sites)) was injected onto one end of a 30-cm section of fused silica capillary (o.d. 365 μm; i.d. 50 μm) that was filled with buffer. This end of the capillary was placed in a vial containing buffer; the other end was placed into a microwell containing a solution of p-nitrophenol−galactopyranoside (PNPG; 10 mM in buffer). Voltage (−15 kV) was then applied between the vial and microwell; electroosmotic flow toward the microwell caused the plug of enzyme to be delivered into the solution of substrate. After the reaction was allowed to proceed, the end of the capillary in the microwell was removed and placed in a vial containing buffer. The removal of the capillary from the microwell loaded a plug of the reaction mixture onto the capillary;[39] the plug was separated using CE by applying a voltage (+15 kV) between the two vials. (B) Electropherograms: The upper electropherogram was recorded during the delivery process; the peak (∗) arises from the injected enzyme. The lower electropherogram shows the separation of the mixture in the microwell: the first peak to elute (full circle) is assigned to the substrate; the appearance of a peak (half circle) from the product (p-nitrophenolate) indicates that enzyme was delivered to the microwell. The 1/t axis is proportional to electrophoretic mobility.[40]

system described here could be used in applications requiring rapid throughput (frequency ∼10^4 wells/s)[43] by introducing parallel addressing and analysis and by adding a higher level of automation to the system.

EXPERIMENTAL SECTION

Materials. The depth of microwells was determined by the photoresist used for photolithography (1−3 μm, Shipley 1800

Series; 10−40 μm, Shipley 1075 STR; 50−200 μm, SU-8 50; Microlithography Chemical Corp., Newton, MA). Photomasks for performing photolithography were either rigid chrome masks (Advanced Reproductions, North Andover, MA; features < 50 μm) or high-resolution transparencies (produced using Herkules PRO image setter, 3387 dpi, Linotype-Hell Co., Hauppauge, NY; features > 50 μm).[25]

We normally used poly(dimethylsiloxane) (PDMS; Sylgard 184, Dow Corning, Midland, MI) prepolymer to fabricate the arrays of microwells. Other materials from which we prepared arrays were epoxy (Epo-tek UVO 114, Epoxy Technology, Billerica, MA), polyurethanes (Norland Optical Adhesive, NOA 73 and 81, Edmund Scientific, Barrington, NJ), and poly(acrylic acid) (MW 450 000, Aldrich, Milwaukee, WI). Poly(acrylic acid) was cast from a methanol solution onto the PDMS master and then backed with a layer of epoxy. All other chemicals were obtained from Aldrich Chemical Co.

Instrumentation. (a) Controlled Discontinuous Dewetting. An array of microwells (mounted on a glass microscope slide) filled with liquid when pulled by a syringe pump (Harvard Apparatus, South Natick, MA) from an liquid (at 90° to the surface) at a rate of ∼10−15 cm/h, or when a drop of the liquid drained from a similar surface.

(b) Microscopy. For imaging arrays of wells, we used an upright microscope (Leica DMRX microscope, Heidelberg, Germany) equipped with both halogen (12 W) and mercury arc (50 W) lamps, and filters for fluorescence imaging. A TV monitor displayed images captured in real time by a CCD camera mounted on the microscope. A 35-mm SLR camera (Nikon) or a CCD camera connected to a Scion Corp. CG-7 frame grabbing card in a Power Macintosh 8500 captured still images.

(c) Contact Angles. Using a contact angle goniometer (Rame-Hart, Mountain Lakes, NJ) and a CCD camera connected to a TV monitor and VCR, we recorded the dynamic advancing and receding contact angles of various liquids on PDMS by delivering and withdrawing aliquots (25 μL) of solvent with a Matrix Technologies Microelectrapette at a constant rate, keeping the tip of the pipet in the drop. Images from the videotape were digitized using the frame grabbing card. Advancing and receding contact angles were measured from the grabbed frames. The dynamic contact angles were averaged over measurements on at least three drops.

(d) Measurement of Fluorescence from Microwells. An array of microwells (10 μm diameter, spaced on a square grid with a period of 15 μm) was filled by discontinuous dewetting with fluorescein (0.1 mg/mL) in tri(ethylene glycol). Fluorescence micrographs of areas (∼200 μm × 160 μm) of this array were captured using the CCD camera and frame grabber. Fluorescence intensities were analyzed in NIH Image 1.61. For each captured frame (∼100 microwells), the intensity of fluorescence from each well was measured, and the average and standard deviation in the intensity were calculated. Statistical data from frames of five different areas of microwells were averaged to obtain the typical percent standard deviation in volume.

(43) We choose an addressing frequency of 10^4 wells/s as a convenient rate of manipulating a large combinatorial array. This number corresponds to ∼3.6 million analyses in an hour or, for example, manipulation of a combinatorial library with 1 million compounds in ∼15 min.

(e) Microdelivery System. All experiments requiring delivery of small quantities of liquid to individual wells or small numbers of wells were performed on the stage of an inverted microscope (Galen III, Edmund Scientific, NJ). A TV monitor displayed images captured in real time by a CCD camera mounted on the microscope. We used a glass probe mounted on an *xyz* micropositioning stage (Line Tool Co., Allentown, PA) as a microdelivery system. We formed the sharp glass probe by first removing the polymeric coating from one end of an optical fiber (125 μm diameter; Thor Labs, Newton, NJ) with a razor blade and then etching the exposed glass in concentrated HF ($\sim$10 min) until its tip was $\sim$15 μm in diameter.

(f) Electroosmotic Pumping and Capillary Electrophoresis. Delivery of solutions containing enzyme into microwells and the subsequent separation of the product of the enzyme−substrate reaction were carried out using a 50-μm-i.d./365-μm-o.d. fused silica capillary (Polymicro Technology, Phoenix, AZ). A power supply (CZE1000R, Spellman, Hauppauge, NY) generated high voltages; detection of enzyme, substrate, and product was carried out using a UV detector (Crystal 100, Thermo CE, Franklin, MA) at 214 nm. A 16-bit I/O board (PCI-MIO-16XE-50, National Instruments, Austin, TX) and software (Labview, National Instruments, Austin, TX) on a personal computer recorded the signal from the UV detector; the computer also controlled the power supply. *Warning: extreme caution should be exercised when using high voltages!*