

Fabrication of Microchambers Defined by Photopolymerized Hydrogels and Weirs within Microfluidic Systems: Application to DNA Hybridization

Gi Hun Seong, Wei Zhan, and Richard M. Crooks*

Department of Chemistry, Texas A&M University, P.O. Box 30012, College Station, Texas 77842-3012

This paper describes fabrication of serial microchamber arrays within the channels of a microfluidic device. The chambers are defined using a combination of weirs and UV-cross-linked hydrogel plugs (poly(ethylene glycol) diacrylates). This approach permits the microchambers to be addressed by pump-driven pressure in one dimension and by electrophoresis in the other. The function of the device is demonstrated by detecting DNA targets. Single-strand DNA (ssDNA) probes labeled with biotin were immobilized onto microbeads coated with streptavidin. The DNA-functionalized microbeads were packed into each of three microchambers by injection through inlet wells. Three oligonucleotides were designed as probes and four as targets. Hybridization reactions were performed by moving the targets across the array of probe-containing microchambers by electrophoresis. The hybridization of fluorescein-labeled ssDNA targets to complementary probes was observed by fluorescence microscopy. These studies resulted in four key observations: (1) there was no detectable binding of targets to noncomplementary probes; (2) hybridization was 90% complete within 1 min; (3) once captured, the targets could be independently released and recovered from the microbeads by treatment with 0.1 N NaOH; (4) multiple analyses could be performed using a single bead set, but there was degradation in performance after each capture/release cycle.

There is currently interest in combining the functional components necessary for performing complex chemical and biochemical analyses into small, integrated units. These integrated units have been described as microscale total analysis systems (μ TAS) or laboratories-on-a-chip.^{1–3} Much of the current research activity in this field is focused on DNA analysis devices that integrate multiple reaction, purification, and detection functions.^{4–6}

Here, we report the fabrication of on-chip microchamber array elements that can be independently addressed by pump-driven pressure in one dimension and by electrophoretic transport in another. The key components of these devices are photopolymerized hydrogels, which act as passive switches that are activated by modulating the mode of mass transport: no special solution conditions (such as pH change) are required. The versatility of this design strategy is demonstrated by selective bead-based capture and release of DNA oligonucleotides. Specifically, a solution containing one or more synthetic oligonucleotides can be flowed through a linear array of bead-containing microchambers. If the complement DNA (cDNA) for one of the targets is present on a bead, it is extracted from the mixture. The cDNA can subsequently be recovered from the microchamber.

Microbeads offer the advantages of solid-phase chemistry coupled with the convenience of fluidic handling.⁷ For example, microbeads provide a high degree of synthetic flexibility and very high surface-to-volume ratio compared to simple open-channel microfluidic devices. High surface area is important for applications such as certain types of bio/chemical reactions and separations, which rely on multiple interactions between analytes and surface-confined molecular species.⁸ These advantages have been recognized previously by others. For example, dynamic hybridization was reported using DNA probes pumped through target-bearing paramagnetic beads,⁹ and octadecyl-functionalized silica beads were packed from a side channel into a specifically designed chamber of the microchip and used for solid-phase extraction.¹⁰ Additionally, a microchip-based clinical diagnosis system that includes a bead-based sandwich immunoassay system has been reported.¹¹

* To whom correspondence should be addressed. Phone: 979-845-5629. Fax: 979-845-1399. E-mail: crooks@tamu.edu.

- (1) Bard, A. J. *Integrated Chemical Systems*; Wiley: New York, 1994.
- (2) Harrison, D. J.; Fluri, K.; Seiler, K.; Fan, Z.; Effenhauser, C. S.; Manz, A. *Science* **1993**, *261*, 895–897.
- (3) Figeys, D.; Pinto, D. *Anal. Chem.* **2000**, *72*, 330A–335A.
- (4) Anderson, R. C.; Su, X.; Bogdan, G. J.; Fenton, J. *Nucleic Acids Res.* **2000**, *28*, e60.

- (5) Burns, M. A.; Johnson, B. N.; Brahmasandra, S. N.; Handique, K.; Webster, J. R.; Krishnan, M.; Sammarco, T. S.; Man, P. M.; Jones, D.; Heldsinger, D.; Mastrangelo, C. H.; Burke, D. T. *Science* **1998**, *282*, 484–487.
- (6) Waters, L. C.; Jacobson, S. C.; Kroutchinina, N.; Khandurina, J.; Foote, R. S.; Ramsey, J. M. *Anal. Chem.* **1998**, *70*, 158–162.
- (7) Ruzicka, J.; Scampavia, L. *Anal. Chem.* **1999**, *71*, 257A–263A.
- (8) Yu, C.; Davey, M. H.; Svec, F.; Frechet, J. M. *Anal. Chem.* **2001**, *73*, 5088–5096.
- (9) Fan, Z. H.; Mangru, S.; Granzow, R.; Heaney, P.; Ho, W.; Dong, Q.; Kumar, R. *Anal. Chem.* **1999**, *71*, 4851–4859.
- (10) Oleschuk, R. D.; Shultz-Lockyear, L. L.; Ning, Y.; Harrison, D. J. *Anal. Chem.* **2000**, *72*, 585–590.
- (11) Sato, K.; Tokeshi, M.; Kimura, H.; Kitamori, T. *Anal. Chem.* **2001**, *73*, 1213–1218.

Analysis of multiple DNA targets using microfluidics will inevitably involve the use of intracolumn DNA arrays. As mentioned previously, we rely on photosensitive hydrogels as the main structural component to prepare the arrays described here. Hydrogels have attracted attention recently because of their hydrophilicity and good tissue biocompatibility and because they are synthetically flexible.^{12,13} Poly(ethylene glycol) (PEG) can be cross-linked into hydrogels by introducing terminal acrylate functional groups (PEG-DA), which can participate in photopolymerization reactions.^{13–15} Highly cross-linked PEG networks are capable of protein entrapment and have been used for numerous chemical and biological sensing applications.^{16,17} The use of hydrogels for microfluidic applications was recently extended by Beebe, Moore, and co-workers, who showed that hydrogel-based valves could be photolithographically fabricated within channels.¹⁸ Tarlov and co-workers recently showed that DNA oligonucleotides could be immobilized within a polyacrylamide matrix confined to a microfluidic channel.¹⁹ They also demonstrated that the polymer was porous under electrophoretic conditions and that the encapsulated DNA could undergo hybridization.

In this study, a UV-polymerizable PEG-based hydrogel was used to pattern microchambers within fluidic devices. Microbeads hosting different single-stranded DNA (ssDNA) probes on their surfaces were subsequently loaded into these microchambers and then used for the detection and the screening of specific DNA oligonucleotides in DNA mixtures. DNA was 90% captured within 1 min and could subsequently be released and recovered. The device could be used for multiple analyses, although there was degradation in performance after each capture/release cycle. This general approach should be useful for applications involving multiple sequential reactions, multiple sequential analyses, and high-throughput screening of biological materials.

EXPERIMENTAL SECTION

Materials. Positive photoresist (AZP4620) and developer solution (AZ421K) were obtained from the Clariant Co. (Somerville, NJ). Chrome-coated soda lime glass for fabrication of photomasks was purchased from Nanofilm (Westlake Village, CA). PDMS microfluidic devices were fabricated by curing the prepolymer components of Sylgard 184 (Dow Corning, Midland, MI). 3-(Trichlorosilyl)propyl methacrylate (TPM) for surface modification was purchased from the Fluka Chemical Co. (Milwaukee, WI). 2-Hydroxy-2-methylpropiophenone (the photoinitiator) and poly(ethylene glycol) diacrylates (PEG-DA, MW 575) were used as received from the Aldrich Chemical Co. (Milwaukee, WI). Microbeads were Proactive streptavidin-coated microspheres (Bangs Laboratories, Fishers, IN) that were 15.5 μm in diameter, as

determined by optical microscopy. DNA oligonucleotides, modified with biotin and fluorescein and purified by reversed-phase HPLC, were purchased from Integrated DNA Technologies (Coralville, IA). Milli-Q water (18 M Ω -cm) was used to prepare aqueous solutions.

Instrumentation. Photopolymerization of the hydrogel was performed using an uncollimated, 365-nm, 300 mW/cm² light source (EFOS Ultracure 100ss Plus, UV spot lamp, Mississauga, ON, Canada). Optical and fluorescence images of the hydrogel microstructures and DNA hybridization on the microbeads within the microchambers were acquired with a fluorescence microscope (Nikon Eclipse TE 300, Nikon Co., Tokyo, Japan) equipped with band-pass filters, a 100-W mercury lamp, and a charge-coupled device camera (Photometrics Ltd., Tucson, AZ). Fluorescein isothiocyanate (FITC) microscope fluorescence filters with excitation and emission wavelengths of 480 \pm 30 and 535 \pm 40 nm, respectively, were used for observing DNA hybridization in microchambers.

Fabrication of Microfluidic Channels. High-resolution transparencies containing the design of the channels and the weirs were created in a computer drawing package and patterned onto chrome-coated soda lime glass to yield photomasks. Positive photoresist was spin-coated onto a glass substrate at 800 rpm for 2 min, followed by baking at 92 $^{\circ}\text{C}$ for 15 min. The same procedure was repeated twice to increase the thickness of the resist layer. The photoresist-coated glass substrate was exposed to UV light through the mask for 5 min and developed in AZ421K solution to create the master. To fabricate the weirs, a second UV exposure was performed through a slit-type, dark-field photomask having a width of 100 μm . This mask was aligned perpendicular to the photoresist lines corresponding to the outlet channels. This additional UV exposure (5 min) followed by a second development step (60% AZ421K solution for 30 s) resulted in removal of a fraction of the photoresist left behind after the first development step. When PDMS is finally cast over this photoresist master, the weir will form in the depression present at the location of the second exposure. The depth of weir could be controlled by varying the UV irradiation time, the concentration of developing solution, and the development time. The depth and width of individual microchannels were measured using a Veeco Dektak 3 profilometer (Veeco Instruments, Plainview, NY).

For polymer molding on the patterned glass master, a 10:1 mixture of PDMS prepolymer and the curing agent were stirred thoroughly and then degassed under vacuum. The polymer mixture was poured onto the master and cured for 1 h at 65 $^{\circ}\text{C}$. After curing, the PDMS replica was peeled from the master and wells were punched to define reservoirs.

To create the fluidic system, a PDMS replica was sonicated in ethanol for 10 min and rinsed with deionized water. A cover glass was cleaned overnight in 2% glass cleaning agent and rinsed with Milli-Q water and ethanol. The PDMS and cover glass were placed in a low-energy plasma cleaner (PDC-32G, Harrick Scientific Ossining, NY) and oxidized at medium power for 1 min. Immediately after removal from the plasma cleaner, the substrates were brought into conformal contact and an irreversible seal formed spontaneously.²⁰

(12) Andreopoulos, F. M.; Roberts, M. J.; Bentley, M. D.; Harris, J. M.; Beckman, E. J.; Russell, A. J. *Biotechnol. Bioeng.* **1999**, *65*, 579–588.

(13) Andreopoulos, F. M.; Deible, C. R.; Stauffer, M. T.; Weber, S. G.; Wagner, W. R.; Beckman, E. J.; Russell, A. J. *J. Am. Chem. Soc.* **1996**, *118*, 6235–6240.

(14) Mellott, M. B.; Searcy, K.; Pishko, M. V. *Biomaterials* **2001**, *22*, 929–941.

(15) Sawhney, A. S.; Pathak, C. P.; Hubbell, J. A. *Biomaterials* **1993**, *14*, 1008–1016.

(16) Scott, R. A.; Peppas, N. A. *Biomaterials* **1999**, *20*, 1371–1380.

(17) Quinn, C. P.; Pathak, C. P.; Heller, A.; Hubbell, J. A. *Biomaterials* **1995**, *16*, 389–396.

(18) Beebe, D. J.; Moore, J. S.; Bauer, J. M.; Yu, Q.; Liu, R. H.; Devadoss, C.; Jo, B. H. *Nature* **2000**, *404*, 588–590.

(19) Olsen, K. G.; Ross, D. J.; Tarlov, M. J. *Anal. Chem.* **2002**, *74*, 1436–1441.

(20) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. *Anal. Chem.* **1998**, *70*, 4974–4984.

Fabrication of Hydrogel Patterns within Microchannels.

Before fabricating the hydrogel pattern, the cover glass and PDMS surfaces within the microchannel were functionalized with TPM. Briefly, a 1% (v/v) TPM solution was prepared by dilution with paraffin oil, and then the solution was evacuated to remove bubbles.²¹ The silane solution was injected into the microchannel immediately after sealing the PDMS to glass. The silane solution was incubated for 10 min, and the channel was rinsed with ethanol, dried with N₂ gas, and then baked at 95 °C for 30 min.

Following surface modification, the microfluidic channel was filled with a solution consisting of 1% (v/v) photoinitiator and 50% PEG-DA diluted with tris-acetate/EDTA (TAE) buffer (pH 8.0, 40 mM tris-acetate and 1 mM EDTA). The slit-type dark-field photomask was aligned atop the glass substrate, and then the solution below the clear areas of the mask were cross-linked by exposure to UV light (365 nm, 300 mW/cm²) for 1.5 s. Specifically, formation of hydrogel microstructures from PEG-DA is based on free-radical polymerization of acrylate end groups appended to the PEG derivatives. The photoinitiator dissociates upon exposure to UV radiation, creating highly reactive methyl radicals that attack unsaturated carbon-carbon double bonds of the acrylate functionality, thus initiating free-radical polymerization. Because two reactive centers per monomer are created, propagation results in the formation of a highly cross-linked PEG-DA network.²² Following polymerization, the channel was flushed with TAE buffer to remove the unpolymerized liquid.

Preparation of Microbead-DNA Complexes. DNA probe oligonucleotides modified with biotin at the 5' terminal consisted of biotin and a 15-carbon mixed polarity spacer arm based on a triethylene glycol (biotin-TEG). Oligonucleotides modified with fluorescein were used as DNA targets. The conjugation of streptavidin-coated microbeads with the biotinylated, ssDNA probes was carried out using the following procedure. 30 μ L of stock beads (4.8×10^6 bead/mL) were rinsed in 200 μ L of phosphate-buffered saline (PBS) solution (pH 7.4, 150 mM NaCl, 4 mM KCl, 8.1 mM Na₂HPO₄, and 1.47 mM KH₂PO₄) and centrifuged at 3000 rpm for 5 min. The microbead pellet was resuspended in 20 μ L of PBS buffer, and then 10 μ L of the biotinylated ssDNA probe (100 ng/ μ L) was added to the microbeads to yield the microbead-DNA complex. The mixture of microbeads and biotinylated DNA was incubated for 30 min at room temperature (18–25 °C) with gentle mixing. After conjugation, the mixture was centrifuged to remove unreacted biotinylated ssDNA probes, and the beads were resuspended in 100 μ L of TAE buffer. These microbead-DNA conjugates were stored at 4 °C prior to use.

RESULTS AND DISCUSSION

Microfluidic devices were fabricated in PDMS using standard photolithographic techniques.²⁰ Figure 1 shows the layout of the microfluidic device. It consists of serial microchambers A, B, and C, weirs at outlets of each microchamber, and microbead-loading inlets (I1–I3) and outlets (O1–O3). The weirs were prepared lithographically using a photomask containing slits having widths of 100 μ m (see Experimental Section). Figure 2 shows an optical image of the microchambers and an illustration of the weir cross

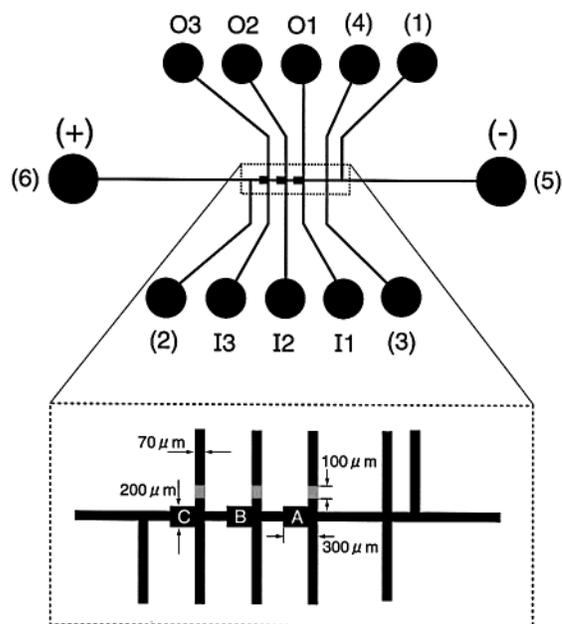


Figure 1. Layout and dimensions of the microchannel network. After photopolymerization of the PEG-DA hydrogel the unpolymerized liquid is flushed out through 1–3 and I1–I3. Microbeads conjugated with biotinylated ssDNA probes are introduced into the microchambers through I1–I3. DNA solution containing the ssDNA targets is loaded into well 3. The driving potential is applied via electrodes in wells 5 and 6.

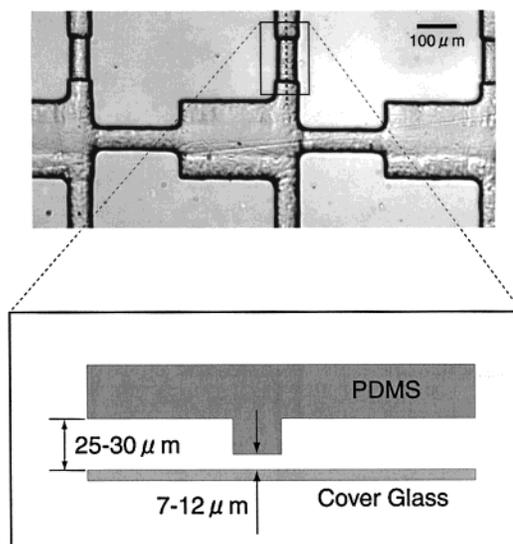


Figure 2. Optical image of the three microchambers illustrated schematically in Figure 1. The structure and dimensions of the weir are shown schematically at the bottom of the figure.

section. The width and depth of weirs ranged from 40 to 60 μ m and 7–12 μ m, respectively.

The PEG-based hydrogel microstructures were prepared by UV exposure of PEG-DA through a photomask (Figure 3A). However, we found that adhesion between the cross-linked hydrogel (α PEG-DA) and PDMS was insufficient to prevent movement of the hydrogel plug within the channel when pressure was applied. Accordingly, prior to PEG-DA UV exposure, both the glass and PDMS interior channel surfaces were functionalized with the coupling agent (3-(trichlorosilyl)propyl methacrylate

(21) Chaudhury, M. K.; Whitesides, G. M. *Science* **1992**, *255*, 1230–1232.

(22) Revzin, A.; Russell, R. J.; Yadavalli, V. K.; Koh, W. G.; Deister, C.; Hile, D. D.; Mellott, M. B.; Pishko, M. V. *Langmuir* **2001**, *17*, 5440–5447.

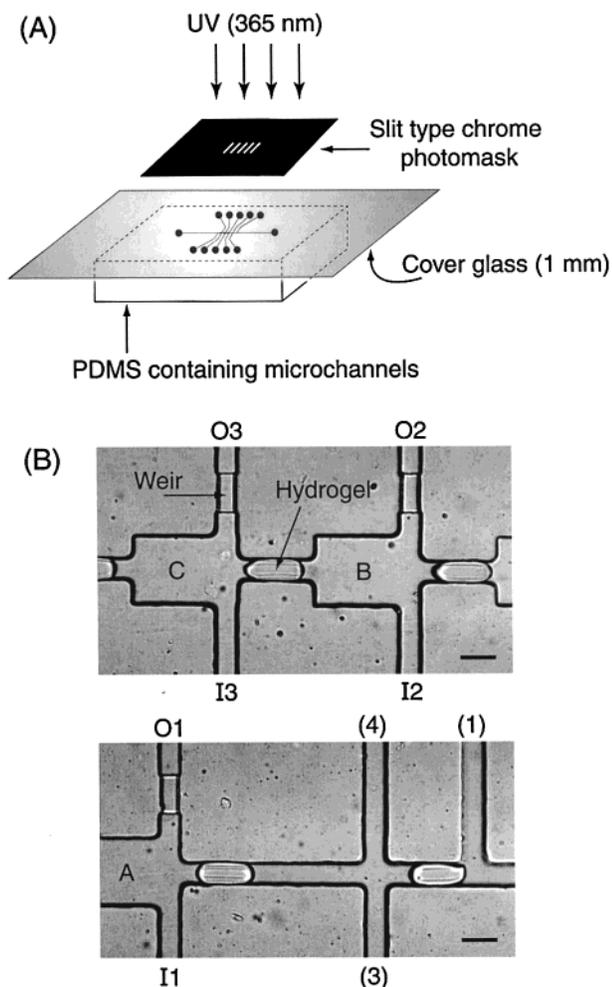


Figure 3. (A) Schematic illustration of the method used to photopolymerize the hydrogel plugs. (B) Optical image of the xPEG-DA hydrogel microstructures photopolymerized within the microchannels. The three microchambers (A–C) are shown in Figure 1 and referred to in the text. Scale bar, 100 μm .

(TPM)).^{18,22} In the presence of the coupling agent, the PEG hydrogel microstructures (Figure 3B) did not detach from the channel, even at high pressures.

As shown in Figure 3, each DNA detection microchamber was defined by a weir and a hydrogel plug. ssDNA probes were immobilized onto the surface of the microbeads through streptavidin–biotin conjugation, and then these DNA–microbead complexes were introduced into each microchamber via the corresponding inlet and outlet channels (Figure 1) using pump-driven pressure. The volume of the microchambers was 1.5 nL, and 350–450 microbeads could be packed into each microchamber. A key aspect of this device design is that the inlet and outlet channels have much lower flow resistance than the main channel, because the PEG-DA hydrogel resists hydrostatic pressure.¹⁸ Therefore, the microchambers continue to pack until they are entirely filled with microbeads. Difficulties in retaining packing materials in conventional capillaries have been described previously,^{23,24} but the hydrogel blocks and weirs used in the present work circum-

vent these problems. Additionally, microbead packing by hand pumping is very simple and results in an even distribution of microbeads throughout the microchamber. The time required to pack the microbeads was less than 30 s/microchamber.

For genetic applications, DNA targets sometimes need to be interrogated by several probes, each bearing a different DNA sequence. There are four general approaches for doing this: (1) independent analysis in microtiter plates,^{25–27} (2) parallel analysis using DNA chips,^{28–31} (3) parallel analysis using liquid arrays, such as fluorescence-encoded beads,^{32,33} semiconductor nanocrystals (quantum dots),^{34,35} or barcoded nanoparticles,^{36,37} and (4) continuous flow serial analysis.^{4,5,9,38} Our approach falls into the latter category, and of the methods that have been described in this general family, the work of Fan et al. is the most relevant.⁹ They described DNA hybridization onto paramagnetic beads contained within microfluidic devices. In their experiments, pneumatic pumping was used to deliver DNA probes and washing solutions. In contrast, our approach uses electrokinetic pumping to deliver target DNA to the probes and pump-driven pressure to recover the captured target.

Experiments were performed by flowing ssDNA targets through the linear array of bead-filled microchambers. The targets were labeled with fluorescein so that hybridization of the target with the bead-bound probes could be detected by fluorescence microscopy. Hybridization reactions took place in tris-acetate buffer, which enhances discrimination against mismatched probes.³⁹ However, before introduction of the targets, a 100-V potential was applied across the main channel (wells 5 and 6, Figure 1) for 10 min to eliminate unstable biotin–streptavidin couplings and nonspecifically bound ssDNA probes.

The first experiments were designed to investigate nonspecific binding or absorption of ssDNA targets onto the microbeads. This was accomplished by examining the extent of probe adsorption onto unfunctionalized microbeads and comparing this to beads modified with ssDNA probes. To carry out this experiment, bare microbeads and ssDNA probe (BT-NFB)-labeled microbeads were

(23) Kutter, J. P.; Jacobson, S. C.; Matsubara, N.; Ramsey, J. M. *Anal. Chem.* **1998**, *70*, 3291–3297.

(24) He, B.; Tait, N.; Regnier, F. *Anal. Chem.* **1998**, *70*, 3790–3797.

(25) Kolakowski, B.; Battaglini, F.; Lee, Y. S.; Klironomos, G.; Mikkelsen, S. R. *Anal. Chem.* **1996**, *68*, 1197–1200.

(26) Piuino, P. A. E.; Krull, U. J.; Hudson, R. H. E.; Damha, M. J.; Cohen, H. *Anal. Chim. Acta* **1994**, *288*, 205–214.

(27) Wang, J. *Nucleic Acids Res.* **2000**, *28*, 3011–3016.

(28) Southern, E. M. *Trends Genet.* **1996**, *12*, 110–115.

(29) Pease, A. C.; Solas, D.; Sullivan, E. J.; Cronin, M. T.; Holmes, C. P.; Fodor, S. P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5022–5026.

(30) Eggers, M.; Hogan, M.; Reich, R. K.; Lamture, J. B.; Ehrlich, D.; Hollis, M.; Kosicki, B.; Powdrill, T.; Beattie, K.; Smith, S.; Varma, R.; Gangadharan, R.; Mallik, A.; Burke, B.; Wallace, D. *BioTechniques* **1994**, *17*, 516–524.

(31) Tillib, S. V.; Mirzabekov, A. D. *Curr. Opin. Biotechnol.* **2001**, *12*, 53–58.

(32) Fulton, R. J.; McDade, R. L.; Smith, P. L.; Kienker, L. J.; Kettman, J. R. *Clin. Chem.* **1997**, *43*, 1749–1756.

(33) Walt, D. R. *Science* **2000**, *287*, 451–454.

(34) Mitchell, G. P.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1999**, *121*, 8122–8123.

(35) Pathak, S.; Choi, S. K.; Arnheim, N.; Thompson, M. E. *J. Am. Chem. Soc.* **2001**, *123*, 4103–4104.

(36) Zhou, H.; Roy, S.; Schulman, H.; Natan, M. J. *Trends Biotechnol.* **2001**, *19*, S34–S39.

(37) Nicewarner-Pena, S. R.; Freeman, R. G.; Reiss, B. D.; He, L.; Pena, D. J.; Walton, I. D.; Cromer, R.; Keating, C. D.; Natan, M. J. *Science* **2001**, *294*, 137–141.

(38) Soper, S. A.; Williams, D. C.; Xu, Y.; Lassiter, S. J.; Zhang, Y.; Ford, S. M.; Bruch, R. C. *Anal. Chem.* **1998**, *70*, 4036–4043.

(39) Fan, Z. H.; Kumar, R.; Deffley, G.; Dong, Q.; Stabile, P.; Fare, T. Technical Digest of 1998 Solid-State Sensor and Actuator Workshop, Transducers Research Foundation, Inc., 1998; pp 97–100.

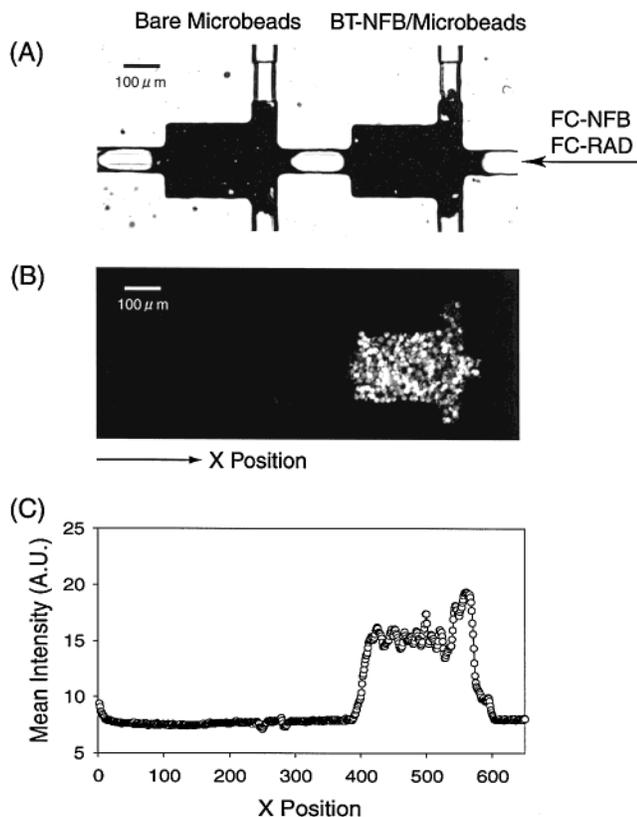


Figure 4. (A) Optical image of the microchambers after microbead packing. The left and right chambers are packed with naked microbeads and microbeads modified with a ssDNA probe (BT-NFB), respectively. (B) Fluorescence microscopy image following exposure of the beads to FC-NFB and FC-RAD. (C) Mean fluorescence intensity of the image shown in (B).

loaded into adjacent microchambers (Figure 4A). Next, the DNA target solution was injected into well 3 with a syringe. This action fills the main channel between the two hydrogel plugs, shown at the bottom of Figure 3B, as well as the channel connecting wells 3 and 4 (Figure 1). A potential of 70 V was then applied along the main channel of the device for 10 min. This results in continuous electrokinetic transport of the target DNA solution through the hydrogel plugs and over the beads within the microchambers. After turning off the driving potential, unhybridized target DNA was removed from each microchamber by rinsing with buffer solution injected into wells I1–I3 and 1–3 using a syringe. The fluorescence micrograph shown in Figure 4B shows that the FC-NFB target is only captured by the BT-NFB probe. Quantitation of the data in the micrograph indicates that the signal-to-background ratio measured for chambers B (left) and A (right) are 1.0 and 2.3 (Figure 4C), respectively, indicating no detectable nonspecific binding of the ssDNA targets on the unfunctionalized beads.

To investigate the feasibility of gene expression profiling and screening, microbeads functionalized with different ssDNA probes were packed into each of the three microchambers. We used a total of seven oligonucleotides as DNA targets and probes to demonstrate this screening function: three were designed as probes and four as targets (Table 1). As shown in Figure 5, the hybridization of ssDNA targets to complementary probes was observed by fluorescence microscopy and no binding was ob-

Table 1. Sequence of Oligonucleotides Used in This Work

name	sequence ^a
BT-NFB probe	5'-Bio-TEG-GTTGAGGGGACTTTCACAGG
FC-NFB target	5'-Fc-CCTGGGAAAGTCCCCCTCAAC
BT-TATA probe	5'-Bio-TEG-ACCTCACTTATATGCTCTG
FC-TATA target	5'-Fc-CAGAGCATATAAAGTGAGGT
BT-PBS probe	5'-Bio-TEG-CGGTATTATCCCGTATTGAC
FC-PBS target	5'-Fc-GTCAATACGGGATAATACCG
FC-RAD target	5'-Fc-TAACACCCGTATGATAGTCT

^a Bio, biotin; Fc, fluorescein; TEG, triethylene glycol.

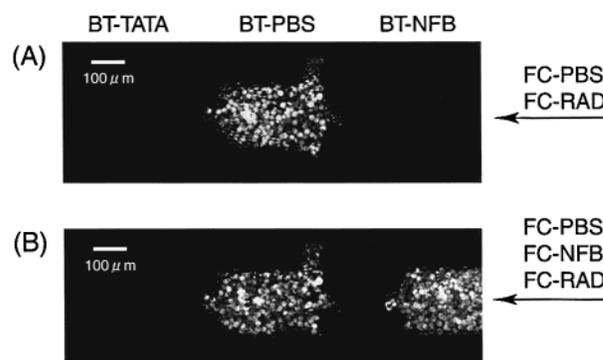


Figure 5. Fluorescence images of microbeads after hybridization with 100 ng/μL of a mixture of (A) FC-PBS and FC-RAD ssDNA targets and (B) FC-PBS, FC-NFB, and FC-RAD ssDNA targets. Microbeads conjugated with BT-TATA (left), BT-PBS (middle), and BT-NFB (right) are packed in each microchamber.

served to noncomplementary probes. The hybridization reactions were 90% complete 1 min after applying the 70-V potential. This is comparable to the time scale reported by Fan et al. for dynamic hybridization onto magnetic beads⁹ and substantially faster than most other DNA hybridization array strategies where hybridization times are typically on the hour time scale.^{40,41} The rapid hybridization time in our experiments is a consequence of fast mass transport by dynamic electrokinetic pumping and the high surface area of the beads.

It is sometimes desirable not only to capture particular DNA but also to release it for subsequent processing. Our approach is effective for this application. ssDNA targets were released from the beads by injecting 0.1 N NaOH into a particular microchamber and then immediately (~3 s later) washing out the denatured target with TAE buffer. The base is required to denature the hybridized DNA, but extended exposure of the cross-linked gel to a high-pH solution results in shrinkage¹⁸ and thus potential leakage of the target along the main channel rather than exclusively into one of the output wells (O1, O2, or O3, Figure 1). Figure 6 shows the results of this experiment. Prior to denaturation, the fluorescence signal-to-background ratios in the left, center, and right microchambers were 4.0, 3.1, and 3.9, respectively. Following denaturation and capture of DNA in only the center microchamber, these values changed to 3.7, 1.0, and 3.8, respectively, indicating that the specific DNA target hybridized in the middle chamber was fully released.

(40) Chee, M.; Yang, R.; Hubbell, E.; Berno, A.; Huang, X. C.; Stern, D.; Winkler, J.; Lockhart, D. J.; Morris, M. S.; Fodor, S. P. *Science* **1996**, *274*, 610–614.

(41) Schema, M.; Shalon, D.; Davis, R. W.; Brown, P. O. *Science* **1995**, *270*, 467–470.

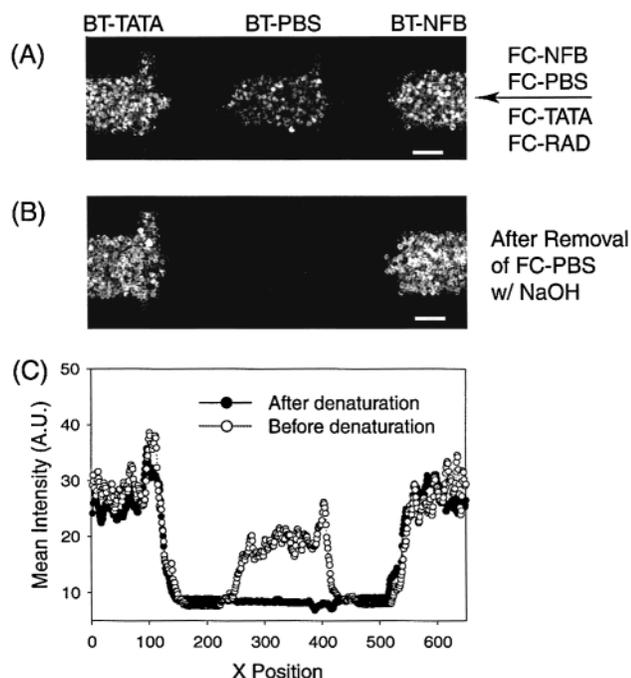


Figure 6. Fluorescence images (A) after hybridization with a mixture of FC-NFB, FC-PBS, FC-TATA, and FC-RAD ssDNA targets and (B) after isolation of the FC-PBS ssDNA target with 0.1 N NaOH solution. Microbeads conjugated with BT-TATA (left), BT-PBS (middle), and BT-NFB (right) are packed in each microchamber. (C) Mean fluorescence intensity after hybridization and isolation. Scale bar, 100 μm .

We also investigated the number of times that capture and release of DNA could be repeated using the same probe-modified microbeads. We found that the signal-to-background ratio of the hybridized beads dropped to only somewhat higher than the detection limit after three capture/release cycles (data not shown). The signal decrease is probably a result of deterioration of streptavidin at high pH. Although each streptavidin molecule contains tetravalent binding capacity, streptavidin predominantly behaves as a bivalent linker molecule. One binding site is attached to the bead and only one among the remaining three is primarily responsible for the binding of biotinylated DNA probes. The restricted formation of tetraadducts, previously reported for the binding of short single-stranded DNA oligomers,^{42,43} indicates that electrostatic repulsion of the negatively charged phosphate backbone hinders biotinylated DNA from approaching the biotin-

binding sites. Deterioration of the streptavidin/biotin linkage increases with each denaturation cycle, and therefore, the amount of DNA probe on each bead is reduced. Covalent linking of the probe DNA to the beads should eliminate this limitation.

SUMMARY AND CONCLUSIONS

Here, serial microchamber arrays were fabricated within microchannels using UV-cross-linked hydrogels and photolithographically defined weirs. Microbeads functionalized with different ssDNA probes were easily packed into the microchambers, and we demonstrated that it was possible to capture and release complementary DNA targets from a complex mixture using this approach.

A significant innovation associated with this methodology is that the hydrogel plugs act as passive switches that can distinguish between electrokinetic and syringe pumping. That is, the DNA target solution can be moved through the hydrogel plugs by applying a potential (hydrogel switch open), and subsequently, the hybridized DNA targets can be isolated via the weirs using pump-driven pressure (hydrogel switch closed). This switching action is completely passive: no external reagents or interconnects are required for it to be enabled. Likewise, the use of microbeads provides several desirable attributes for DNA manipulation, including simple fluidic handling of the microbeads, a high degree of synthetic flexibility, and a high surface-to-volume ratio.

This approach could be important as a detection principle for gene expression analysis. Specifically, cDNA can be synthesized from expressed mRNA extracts by reverse-transcription PCR and subsequent digestion of the cDNA with a restriction enzyme to yield the DNA fragments of interest. Subsequent PCR amplification of the DNA fragments results in sufficient DNA for this bead-based approach. It seems reasonable that all of these functions could be integrated onto a single chip.^{44–46} More generally, we expect that this type of microfluidic system will contribute to the fields of gene expression, clinical diagnostics, and drug discovery and screening.

ACKNOWLEDGMENT

We gratefully acknowledge support from the U.S. Army Medical Research & Materiel Command, the National Science Foundation (9818302), and the Texas Higher Education Coordinating Board through the Advanced Technology Program (0082-1999). Some of the equipment used in this work was provided by the Center for Integrated Microchemical Systems at Texas A&M University.

Received for review January 31, 2002. Accepted April 30, 2002.

AC020069K

(42) Niemeyer, C. M.; Adler, M.; Pignataro, B.; Lenhart, S.; Gao, S.; Chi, L.; Fuchs, H.; Blohm, D. *Nucleic Acids Res.* **1999**, *27*, 4553–4561.

(43) Niemeyer, C. M.; Ceyhan, B.; Blohm, D. *Bioconjugate Chem.* **1999**, *10*, 708–719.

(44) Krishnan, M.; Namasivayam, V.; Lin, R.; Pal, R.; Burns, M. A. *Curr. Opin. Biotechnol.* **2001**, *12*, 92–98.

(45) McGlennen, R. C. *Clin. Chem.* **2001**, *47*, 393–402.

(46) Bruin, G. J. M. *Electrophoresis* **2000**, *21*, 3931–3951.