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Microchip-Based Purification of DNA from Biological Samples

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A microchip solid-phase extraction method for purification of DNA from biological samples, such as blood, is demonstrated. Silica beads were packed into glass microchips and the beads immobilized with sol−gel to provide a stable and reproducible solid phase onto which DNA could be adsorbed. Optimization of the DNA loading conditions established a higher DNA recovery at pH 6.1 than 7.6. This lower pH also allowed for the flow rate to be increased, resulting in a decrease in extraction time from 25 min to less than 15 min. Using this procedure, template genomic DNA from human whole blood was purified on the microchip platform with the only sample preparation being mixing of the blood with load buffer prior to loading on the microchip device. Comparison between the microchip SPE (*μ*chipSPE) procedure and a commercial microcentrifuge method showed comparable amounts of PCR-amplifiable DNA could be isolated from cultures of *Salmonella typhimurium*. The greatest potential of the *μ*chipSPE device was illustrated by purifying DNA from spores from the vaccine strain of *Bacillus anthracis*, where eventual integration of SPE, PCR, and separation on a single microdevice could potentially enable complete detection of the infectious agent in less than 30 min.

An underlying trend over the past several decades in analytical processes has been the development of miniaturized methods. Capillary electrophoresis (CE) was developed as a rapid and low-cost alternative to applications traditionally performed by slab-gel electrophoresis and HPLC. CE is currently being challenged by the development of microchip systems, which provide extremely rapid separations, with subminute[1−3] and, in some cases, subsecond separations already demonstrated.[4] While the CE field will continue to see growth and analytical utility, there are many advantages to the use of microchip systems, including the potential for sample volumes that are reduced (100-pL range) beyond that of CE. Also, the portability of the instrumentation for microchip analysis is increased due to the small physical dimensions of the separation device (microchip). Perhaps the greatest potential for microchips, however, lies in the ability to integrate multiple analytical processes onto a single device, the product of which forms the elusive "lab-on-a-chip" or "micro total analytical system" (*μ*TAS).[5] While this concept has received much attention, the generation of a functional device has yet to be demonstrated.

One of the greatest areas of potential impact for miniaturized devices is in molecular diagnostics, where detection of abnormal DNA sequences has diagnostic and prognostic value. Traditional DNA assays involve purification of DNA from a complex sample, such as blood or purified white blood cells, amplification of the target DNA sequence by the polymerase chain reaction (PCR), and electrophoretic size separation of the DNA fragments. The immense potential for implementation of these processes for clinical diagnostics in microchips was initially demonstrated with electrophoretic separations[6−12] and more recently with chip-based PCR.[10,13−16] Several recent reports focused on integration of both processes into a single microchip device, performing PCR and

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(1) Effenhauser, C. S.; Manz, A.; Fan, Z. H.; Ludi, H.; Widmer, H. M. *Anal. Chem.* **1993**, *65*, 2637−2642.

(2) Harrison, D. J.; Fluiri, K.; Seiler, K.; Fan, Z. H.; Effenhauser, C. S.; Manz, A. *Science* **1993**, *261*, 895−897.

(3) Harrison, D. J.; Fan, Z. H.; Seiler, K.; Manz, A.; Widmer, H. M. *Anal. Chem. Acta* **1993**, *283*, 361−366.

(4) Jacobson, S. C.; Culbertson, C. T.; Daler, J. E.; Ramsey, J. M. *Anal. Chem.* **1998**, *70*, 3476−3480.

(5) Manz, A.; Graber, N.; Widmer, H. M. *J. Chromatogr.* **1990**, 244−252.

(6) Munro, N. J.; Snow, K.; Kant, J.; Landers, J. P. *Clin. Chem.* **1999**, *45*, 1906−1917.

(7) Tian, H.; Jaquins-Gerstl, A.; Munro, N. J.; Trucco, M.; Brody, L. C.; Landers, J. P. *Genomics* **2000**, *63*, 25−34.

(8) Tian, H.; Brody, L. C.; Landers, J. P. *Genome Res.* **2000**, *10*, 1403−1413.

(9) Hofgaertner, W.; Hühmer, A. F. R.; Landers, J. P.; Kant, J. *Clin. Chem.* **1999**, *45*, 2120−2128.

(10) Cheng, J.; Waters, L. C.; Fortina, P.; Hvichia, G.; Jacobson, S. C.; Ramsey, J. M.; Kricka, L. J.; Wilding, P. *Anal. Biochem.* **1998**, *257*, 101−106.

(11) Shi, Y.; Simpson, P. C.; Scherer, J.; Wexler, D.; Skibola, C.; Smith, M.; Mathies, R. A. *Anal. Chem.* **1999**, *71*, 5354−5361.

(12) Hadd, A.; Jacobson, S. C.; Ramsey, J. M. *Anal. Chem.* **1999**, *71*, 6206−5212.

(13) Cheng, J.; Shoffner, M. A.; Hvichia, G. E.; Kricka, L. J.; Wilding, P. *Nucleic Acids Res.* **1996**, *24*, 380−385.

(14) Woolley, A. T.; Hadley, D.; Landre, P.; deMello, A. J.; Mathies, R. A.; Northrup, M. A. *Anal. Chem.* **1996**, *68*, 4081−4086.

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separation in a rapid manner with little user intervention.[17,18] However, this approach is somewhat limited, as PCR requires relatively pure DNA free of contaminants. Thus, integration of a chip-based DNA purification process before the PCR step would be of immense benefit.

Most modern DNA purification processes rely on adsorption of DNA onto a solid surface, either via hydrogen bonding to silica or via electrostatic interactions. This approach is more amenable to miniaturization than the traditional phenol extraction process, as demonstrated by Tian et al.,[19] who established a capillary-based micro-SPE (μSPE) DNA purification system in a 500-nL chamber packed with silica particles. The real potential of this approach was demonstrated by purifying DNA from whole blood into a PCR-ready form for mutation analysis of the BRCA1 gene, a known breast cancer susceptibility gene. Only a single mixing step was required with the blood sample before the purification was carried out.

Microchip-based DNA purification was first demonstrated by Christel et al.[20] using pillars created in a microchannel to increase the silica surface area available for DNA adsorption. While this is an elegant approach, the potential is restricted by the complex fabrication procedure used (reactive ion etching) and, consequently, the cost for fabrication of each microchip. As part of our continued effort to develop a μ-TAS device for clinical diagnostics, we recently examined several low-cost alternatives for increasing the silica surface area inside a microchannel. Silica particles and silica particles immobilized with sol−gel were examined, with the best results obtained using a combined silica particle/sol−gel hybrid approach.[21] The aim of the work described in this report was to thoroughly examine the performance of sol−gel-immobilized silica particles in a microchannel for purification of DNA on a microchip SPE (μchipSPE) device. The stability and reproducibility of μchipSPE devices were examined, the loading conditions of the DNA were optimized, and the system was used to purify human genomic DNA from whole blood and bacterial DNA from colony samples and spores.

MATERIALS AND METHODS

Reagents. Silica beads, 15 μm, were a gift from Mallinckrodt-Baker. Tetraethoxysilane (TEOS), guanidine hydrochloride, HNO_3, KOH, NaOH, Tris, EDTA, 2-propanol, Triton-X 100, and *Hin*DIII-digested λ-phage DNA were purchased from Sigma-Aldrich (St. Louis, MO). PicoGreen dsDNA intercalating dye was purchased from Molecular Probes. HCl, MES, and acetic acid were purchased from Fisher (Fairlawn, NJ). Taq polymerase, primers, and other PCR reagents for amplification of the 500-bp λ-DNA fragment were purchased from Perkin-Elmer (Santa Clara, CA). Primers for the 380-bp β-globin gene were purchased from MWG biosciences. Primers for detection of *Salmonella* using PCR were prepared in-house and target a 275-bp fragment of the invasive A (invA) gene.[22] Detection of anthrax by PCR used primers designed in our laboratory targeting the pOX1 plasmid gene tlf (toxin lethal factor). Borofloat glass for cover plates was purchased from S.I. Howard Glass (Worchester, MA), and borofloat glass coated with chrome and photoresist, for production of microchip devices, was purchased from Nanofilm (Westlake Village, CA).

All solutions were prepared in Nanopure water (Barnstead/Thermolyne, Dubuque, IA). TE buffer (10 mM Tris, 1 mM EDTA, titrated to pH 7.6 with HCl), 6 M guanidine hydrochloride (GuHCl) in TE buffer, and 80% 2-propanol were used for the μchipSPE procedure. Stock solutions of *Hin*DIII-digested λ-phage DNA and prepurified genomic DNA were prepared in 6 M guanidine hydrochloride solution and TE buffer at a concentration of 500 pg/μL. These were diluted as needed in the appropriate buffer for DNA extraction experiments and for use as standards in the DNA quantitation assay.

Salmonella typhimurium and *Bacillus anthracis*, the Sterne vaccine strain (nonencapsulated) (Colorado Sterum Co., Bolder, CO), were grown to appropriate levels in culture. The *Salmonella* had a final concentration of 7.08×10^8 colony forming units per milliliter (cfu/mL). The *B. anthracis* had a final concentration of 6.60×10^6 cfu/mL.

Device Preparation. Bottom plates for the microchips were fabricated using standard photolithographic techniques and consisted of an etched channel 2.2 cm long, 60 μm deep, with a width of 400 μm at the center. A cover plate was prepared by forming access holes at each end of the channel using a 1.1-mm-diameter diamond-tipped drill bit (Crystalite Corp., Lewis Center, OH). A complete device was formed by thermal bonding of the etched base to the cover plate at 690 °C.

Silica bead/sol−gel hybrid microdevices were packed using the following procedure: Sols were prepared by hydrolyzing a 27% (v/v) solution of TEOS in water by addition of 0.1% (v/v) HNO_3 and heating to 60 °C for 10 min and then 80 °C for 60 min with stirring at 200 rpm. A temporary frit was constructed by placing a hydrolyzed sol−gel silica bead slurry (~200 mg/mL) in the outlet access hole and heating at 70 °C for 60 min to accelerate aging and drying of the gel. The channel was then filled with silica beads by drawing a bead/water slurry through the channel toward the frit by application of a vacuum. Once packing was complete, the column bed was rinsed with 1 M HCl to hydrolyze the surface of the silica and the channel walls, and then the channel was filled with hydrolyzed TEOS. The filled chip was placed in an oven at 50 °C, heated to 300 °C at 8 °C/min, and held at this temperature for 3 h before being allowed to cool.

Apparatus. The μchipSPE apparatus consisted of a Harvard Apparatus model 22 syringe pump (Harvard Apparatus, Holliston, MA), with a 250-μL Hamilton gastight syringe (Hamilton, Las Vegas, NV). The syringe was connected to the microchip using 0.75-mm-i.d. PEEK tubing and minitight fittings (Upchurch Scientific, Oak Harbor, WA) with a noncommercial Teflon/plexiglass microchip interface. A second piece of tubing was secured over the outlet hole and facilitated collection of the column

(15) Simpson, P. C.; Roach, D.; Woolley, A. T.; Thorsen, T.; Johnston, R.; Sensabaugh, G. F.; Mathies, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2256−2261.

(16) Giordano, B. C.; Ferrance, J.; Swedberg, S.; Hühmer, A. F. R.; Landers, J. P. *Anal. Biochem.* **2001**, *291*, 124−132.

(17) Khandurina, J.; McKnight, T.; Jacobson, S. C.; Waters, L. C.; Foote, R. S.; Ramsey, J. M. *Anal. Chem.* **2000**, *72*, 2995−3000.

(18) Lagally, E. T.; Simpson, P. C.; Mathies, R. A. *Sens. Actuators, B* **2000**, *63*, 138−146.

(19) Tian, H.; Hühmer, A. F. R.; Landers, J. P. *Anal. Biochem.* **2000**, *283*, 175−191.

(20) Christel, L. A.; Petersen, K.; McWilliam, W.; Northrup, M. A. *Trans. ASME* **1999**, *121*, 272−279.

(21) Wolfe, K. A.; Breadmore, M. C.; Ferrance, J. P.; Power, M. E.; Conroy, J. F.; Norris, P. M.; Landers, J. P. *Electrophoresis* **2002**, *23*, 727−733.

(22) Wang, R. F.; Cao, W. W.; Cerniglia, C. E. *J. Appl. Microbiol.* **1997**, *83*, 727−736.

effluent in microcentrifuge tubes. The dead volume of the connecting tubing and the microchip chamber was less than 1 μL.

***μ*chipSPE Extraction Procedure.** New chips were conditioned with MeOH for 30 min at a flow rate of 250 μL/h. Prior to each extraction, chips were washed with elution buffer (TE) for 30 min, followed by GuHCl load solution without DNA for a further 30 min. The extraction procedure itself consisted of load, wash, and elution steps. In the load step, 20 μL of load buffer (6 M GuHCl, 1% Triton X-100 in 1× TE buffer) containing the DNA to be extracted was passed through the device. Proteins and possible PCR inhibitors that were adsorbed onto the silica during the load step were removed by passing 20 μL of wash buffer (2-propanol/water 80/20 (v/v)) through the solid phase. Finally, the DNA was eluted in a low ionic strength TE buffer. After elution of DNA from a sample, the microchip was conditioned with load solution (without DNA) for 5 min to prepare the surface for a subsequent extraction.

The load and wash solutions, along with aliquots of the eluent, were collected in microcentrifuge tubes and analyzed using PicoGreen intercalating dye in a TD-700 fluorometer (Turner Designs, Sunnyvale, CA) using separate calibration curves for each solution. Extracted DNA samples were PCR amplified using a Perkin-Elmer Thermocycler (Santa Clara, CA) and a standard PCR protocol.[22] This involved, for example, 95 °C for 5 min (hot start), up to 40 cycles with 94 °C for 1 min/60 °C for 1 min/72 °C for 1 min followed by extension at 72 °C for 10 min. All amplified samples were analyzed on the Bio-Analyzer 2100 (Agilent Technologies, Palo Alto, CA) using the DNA 500 kits according to the manufacturer's instruction.

RESULTS AND DISCUSSION

The development of a solid-phase extraction procedure would be of immense benefit and a considerable step toward the development of a DNA diagnostic device. However, the difficulty in creating such a device remains the creation of the solid phase. Using a weir-type approach similar to one described by Oleschuk et al.,[23] we adapted the procedure of Tian et al.,[19] placing silica particles inside a microchannel but achieved only limited success due to compression of the packed bed during extraction. This was overcome by immobilizing the particles by using a sol−gel to act as an "interparticle glue", a procedure that has been successfully employed by several groups with capillary electrochromatography.[24−28] Figure 1 shows a microchip channel filled with silica beads and immobilized with sol−gel. As can be seen from Figure 1B, the channel is evenly packed, with no large voids in the column. Figure 1C is a SEM image of the cross section of a filled microchip where the distribution of the packing material in the cross section of the channel can be seen. It is interesting to note that visualization of the interconnecting sol−gel matrix is difficult, but this is consistent with images shown by other groups using capillaries.[24−26]

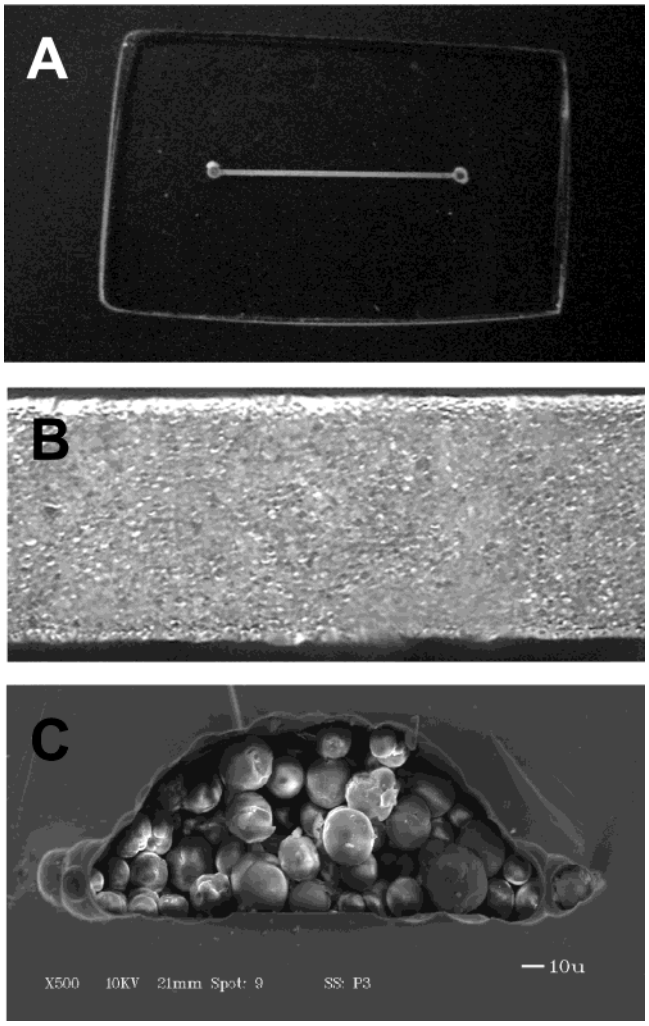

Figure 1. Microchip packed with silica particles and immobilized with sol−gel: (a) 1× magnification; (b) 10× magnification; (c) cross section of packed channel at 500× magnification.

It is difficult to visualize the presence of the sol−gel matrix on SEM images; however, a telling test is the stability of these devices for repetitive DNA extractions. Using a single microchip containing sol−gel-immobilized silica particles, it was possible to perform over 10 successive extractions (Figure 2A), with the recovery of λ-phage DNA averaging 67% (10% RSD), a significant improvement over the one or two extractions possible without sol−gel immobilization. Furthermore, the chip-to-chip reproducibility (Figure 2b) was exceptional, with an average of 68% (6% RSD) of the λ phage DNA recovered, evaluated with data from at least three extractions on 15 different immobilized silica bead microchips. When compared to channels packed with silica particles alone, the performance is considerably better, suggesting that inconsistent chip-to-chip results observed in the absence of immobilization stem from the dynamic nature of the packing.

Optimization of Load pH and Flow Rate. The above results illustrate the suitability of the immobilized silica bead *μ*chipSPE devices for successful integration into a *μ*-TAS device; however, a few other criteria must be met. First, the DNA must be of sufficient quantity and quality for PCR amplification, second, the DNA must be contained in a volume suitable for microchip-based PCR, and third, the procedure must be rapid and efficient. The

(23) Newton, C. R.; Graham, A. *PCR*; BIOS Scientific Publishers Ltd.: New York, 1997.
(24) Oleschuk, R. D.; Shultz-Lockyear, L. L.; Ning, Y.; Harrison, D. J. *Anal. Chem.* **2000**, *72*, 585−590.
(25) Tang, Q.; Lee, M. L. *J. Chromatogr., A* **2000**, *887*, 265−275.
(26) Tang, Q.; Xin, B.; Lee, M. L. *J. Chromatogr., A* **1999**, *837*, 35−50.
(27) Chirica, G.; Remcho, V. T. *Electrophoresis* **1999**, *20*, 50−56.
(28) Dulay, M. T.; Kulkarni, R. P.; Zare, R. N. *Anal. Chem.* **1998**, *70*, 5103−5107.

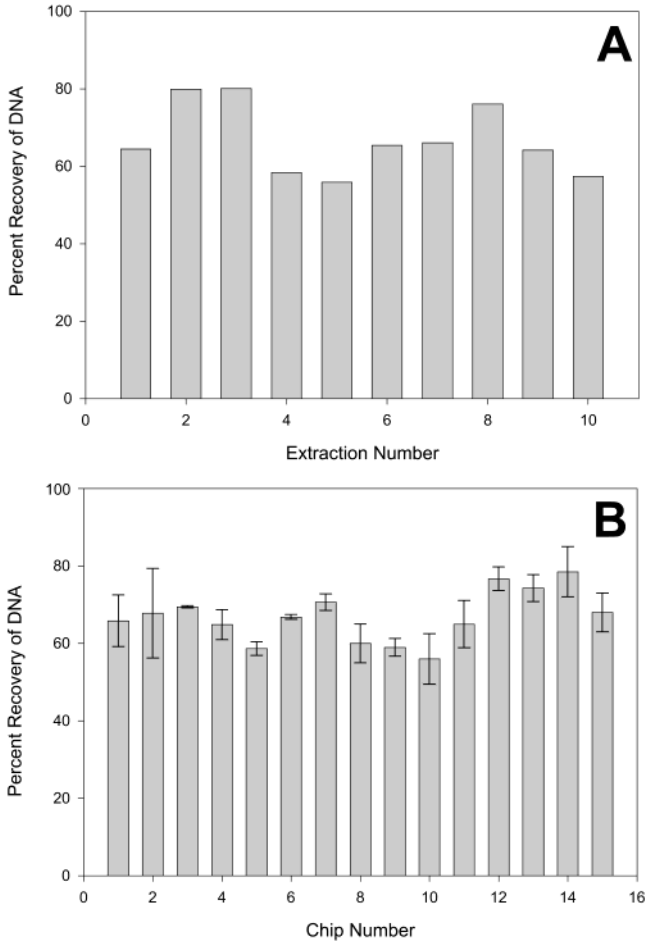

Figure 2. Intrachip (A) and interchip (B) reproducibility of λ-DNA extractions on microchips filled with sol−gel-immobilized silica particles. Extraction conditions: load solution contained 500 pg/μL DNA in 6 M GuHCl in 1× TE, pH 7.6. 20 μL of load, wash, and elution buffer passed through device at 150 μL/h. For interchip reproducibility studies, three extractions were performed on each microchip.

initial demonstration of the potential of these immobilized devices, previously reported by Wolfe et al.,[21] produced PCR-amplifiable DNA in an extraction volume of ~5 μL. The total extraction time of 25 min was somewhat lengthy, however, given that the PCR and separation processes can be performed in less than 5 min each. It would, therefore, be desirable to decrease the extraction time to a length similar to that possible for PCR and separation.

The easiest method to decrease the extraction time is to increase the flow rate; this must be done in a manner that does not reduce DNA extraction efficiency and, therefore, requires careful examination of the DNA extraction process. Since the most critical component of the extraction process is initial adsorption of the DNA onto the silica surface, attention was focused on the load step and on ways to improve the flow rate without sacrificing DNA adsorption efficiency. The DNA purification process employed here utilizes the adsorption of DNA onto bare silica under high ionic strength chaotropic conditions. The high ionic strength serves to shield the negative surface, reducing the electrostatic repulsion between the negative DNA and the surface of the silica, while the chaotropic salt dehydrates the silica surface and DNA, thus promoting hydrogen bonding between the DNA molecules and the protonated silanol groups. These two factors combine to allow DNA to adsorb onto silica surfaces.

In one of the few works addressing DNA adsorption onto silica surfaces, Melzak et al.[29] examined the buffer properties to elucidate the DNA binding mechanism. They found that the type of salt, concentration, and pH of the solution significantly affected the adsorption of DNA onto silica surfaces. Of interest here is the significant effect of pH on DNA adsorption, with lowering the pH of the solution from 8 to 5 having two pronounced effects. First, the saturation level (DNA binding capacity) of the surface increased on decreasing the pH to 6 by at least a factor of 2, with further reduction of the pH producing no further changes in capacity. Second, the initial slope of the adsorption isotherm was much higher at pH 5 than at pH 8, indicating more rapid adsorption of the DNA onto the surface. These two effects were explained by a reduction in the extent of protonation of the silanol groups, thus reducing electrostatic repulsion between the DNA and the silica surface while also providing more protonated silanol groups capable of hydrogen bonding to the DNA. It seemed likely that reducing the pH of the load buffer while increasing the flow rate would potentially maintain DNA adsorption, allowing a reduction in extraction time without sacrificing performance.

To examine the potential of this approach, the extraction efficiency of DNA was examined using a flow rate of 250 μL/h for the load, wash, and elution steps (previous studies were performed at 150 μL/h) and three different buffers: (1) pH 7.6 (6 M GuHCl in 10 mM Tris, 1 mM EDTA, pH adjusted with HCl), (2) pH 6.1 (6 M GuHCl in 10 mM Tris, 1 mM EDTA, pH adjusted with MES), and (3) pH 4.8 (6 M GuHCl in 10 mM Tris, 1 mM EDTA, pH adjusted with acetic acid). Using the same microchip, three extractions were performed at each different pH with 53% ± 4%, 81% ± 3%, and 79% ± 2% of the DNA recovered for pH 7.6, 6.1, and 4.8, respectively. As anticipated, the DNA extraction performance improved as the pH decreased due to the combined increases in binding capacity and binding isotherm as suggested by Melzak et al.[30] Interestingly, there was no further benefit when the pH was lowered to 4.8, a result also observed by Melzak et al.[29] While it is feasible that this is due to complete protonation of the silanol groups, given the complexity of our system, a more thorough investigation in flowing streams would be required to determine the exact cause.

Having verified that lowering the pH enabled increased flow rates through the system to be utilized, the rate was varied to determine the optimal flow rate for the different pH solutions. Given that there was essentially no difference in extraction efficiency of the DNA between buffers with a pH of 4.8 and 6.1, the lower pH buffer was not tested, as this load buffer could introduce detrimental effects relating to protein and lipid adsorption on the silica surface. The effect of varying the flow rate on DNA extraction efficiency can be seen in Figure 3 for buffers at pH 6.1 and 7.6. While a similar profile is observed at the two different pHs, the position of the maximums occurs at the much higher flow rate of 250 μL/h when a buffer at pH 6.1 is used; at pH 7.6 it is only 175 μL/h. This translates to a total extraction time of less than 15 min at pH 6.1 compared to 25 min at pH 7.6, representing a significant reduction in extraction time. Once

(29) Kato, M.; Dulay, M. T.; Bennett, B.; Chen, J.-R.; Zare, R. N. *Electrophoresis* **2000**, *21*, 3145−3151.
(30) Melzak, K. A.; Sherwood, C. S.; Turner, R. F. B.; Haynes, C. A. *J. Colloid Interface Sci.* **1996**, *181*, 635−644.
(31) Product information of Promega Wizard PCR Preps DNA purification system.

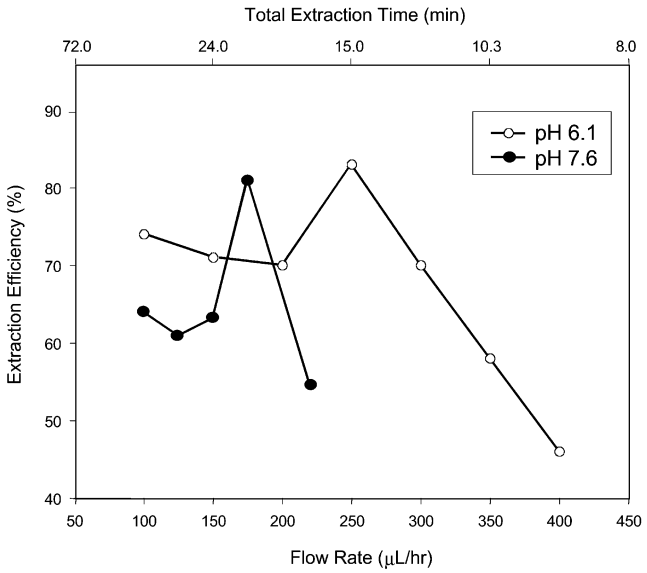

Figure 3. Optimization of the flow rate and pH of load buffer on the extraction performance of λ-DNA in a μchipSPE device. Load buffer contained 500 pg/mL λ-DNA in 6 M GuHCl buffer at pH 7.6 (1× TE buffer) or pH 6.1 (1× Tris/MES buffer).

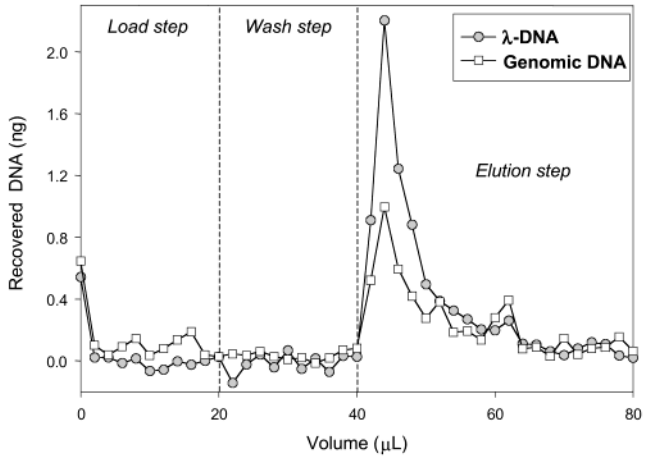

Figure 4. Extraction profiles of λ- and genomic-DNA on μchipSPE devices. Load solution contained 500 pg/μL of either λ- or genomic-DNA in 6 M GuHCl in 1× Tris/MES buffer, pH 6.1. The flow rate for all solutions was 250 μL/h.

integrated into a total analysis device, it should be possible to perform purification, PCR amplification, and separation in less than 30 min.

Extension to Human DNA. For the μchipSPE device to be used for biological samples, it is imperative that the procedure be capable of extracting human genomic DNA fragments (>50 kb) with reasonable efficiency. To examine this, 20 μL of load solution containing 500 pg/μL λ-DNA or prepurified human genomic DNA was prepared and passed through the extraction device using the optimum procedure developed above: a pH 6.1 load solution and a flow rate of 250 μL/h. Effluent from the microchip was collected every 2 μL and assayed using the PicoGreen fluorescence method to obtain the extraction traces shown in Figure 4. As can be seen, the extraction efficiency of DNA from the stationary phase is somewhat lower for human genomic DNA when compared to λ-phage DNA. The elution profile is almost identical for the two, however, as well as consistent with previous results reported by Tian et al.;[19] ~80% of the eluted DNA was collected within 10 μL. The lower extraction efficiency of human genomic DNA appears to result from inefficient elution of the adsorbed DNA from the silica phase. The lack of DNA detected in the effluent load solution (Figure 4) indicates it was not due to inefficient retention of the DNA onto the silica surface as previously reported.[30] Even given this lower extraction efficiency of DNA (50% for human genomic versus 70% for λ-DNA), there was sufficient DNA obtained for PCR amplification of the β-globin gene (discussed below).

Purification of Genomic DNA from Whole Blood. While the ability of the microchip device to provide PCR-amplifiable human genomic DNA is a significant step, the process is meaningless unless DNA can be purified from a real biological sample (as opposed to a standard). Given that the eventual μ-TAS may potentially be portable and useable in a point-of-care situation, the best sample with which to test the device is whole blood. Blood serves as a stringent test of the μchipSPE purification ability, as it is a complex mixture of cells, proteins, peptides, lipids, carbohydrates, and other low molecular weight compounds that are known to inhibit the amplification of nucleic acids by PCR.

To test the performance of the μchipSPE method, 10 μL of thawed frozen blood was mixed with 1000 μL of GuHCl (pH 6.1) with 1% Triton X-100. The Triton X-100 is added to help lyse the cells and solubilize organic matter and has no impact on the extraction process.[19] The optimum procedure developed above was used for purification: 20 μL of load (6 M GuHCl, 1% Triton-X 100 in 1× TE buffer at pH 6.1), wash, and elution buffer was passed through the device at a flow rate of 250 μL/h, with each step taking ~5 min. During the elution stage, fractions were collected every 4 μL and submitted for PCR. Amplification of the 380-bp fragment of the β-globin gene, as identified by microchip electrophoresis separations, was used to indicate successful extraction and purification of the DNA. Figure 5A shows the results from a positive control in which 2 ng of purified human genomic DNA was directly added to the PCR mixture and thermocycled. Figure 5B shows the presence of the same 380-bp fragment amplified from μchipSPE of prepurified human genomic DNA, and Figure 5C shows the absence of the target fragment when μchipSPE is carried out with no DNA loaded onto the microchip device. Microchip separation of the PCR amplification product using template DNA purified from whole blood in the μchipSPE device is shown in Figure 5D. The presence of the 380-bp peak shows the potential of this procedure to isolate DNA from a crude matrix, such as blood, which normally would severely inhibit the PCR reaction (Figure 5E). It is worthy of note that, when the whole blood sample was processed, there was slight discoloration of the silica indicating absorption of the yellow heme onto the surface.[21] This was subsequently removed as indicated by the silica turning from a pinkish hue to opaque during the wash step and by successful amplification of the β-globin gene, where even trace amounts of heme are known to be destructive.

Purification of Bacterial and Viral DNA. Having demonstrated the suitability of the μchipSPE device to purify DNA from whole blood, the full potential of the procedure was examined by comparing the extraction performance of the μchipSPE procedure with a commercial purification procedure for bacterial DNA from

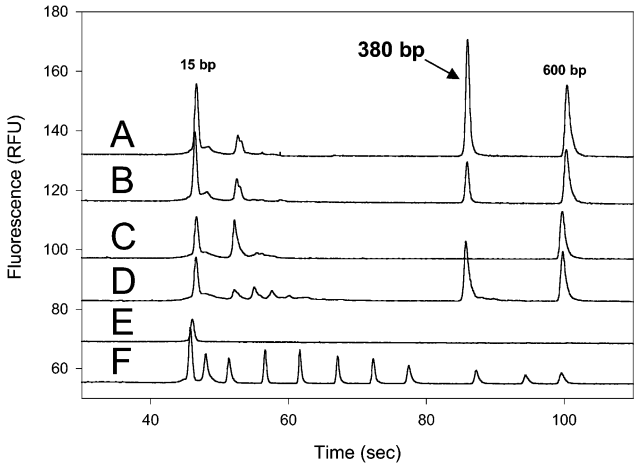

Figure 5. Electropherograms of PCR product microchip separations after amplification of the β-globin gene in human genomic DNA: (A) 2 ng of prepurified genomic DNA added directly to PCR mix. (B) μchipSPE of prepurified human genomic DNA. Approximately 10 ng of DNA loaded onto the device and PCR performed with the second 4-μL elution fraction. (C) Negative control, no DNA included in the load buffer during μchipSPE. PCR amplification of second 4-μL elution fraction. (D) Whole blood; 10 μL of whole blood added to 1 mL of 6 M GuHCl and 1% Triton X 100; 20 μL of this solution loaded onto μchipSPE device; (e) negative control. 2 μL; whole blood added directly to amplification reaction; (F) DNA ladder.

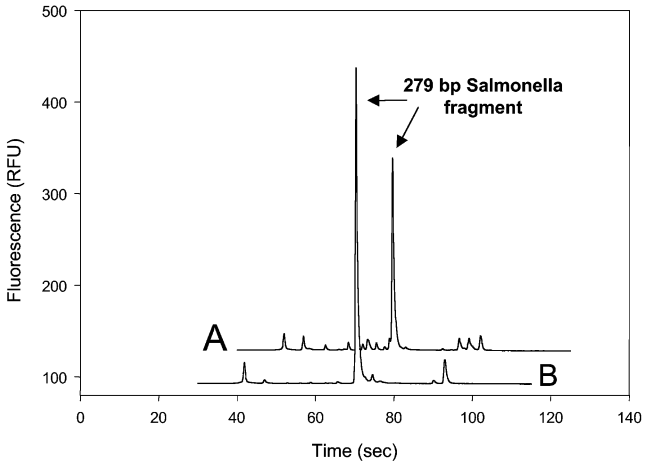

Figure 6. PCR amplification of bacterial DNA purified from *S. typhimuirium* by μchipSPE (A) and a commercial purification procedure (B). Commercial method used 200 μL of bacterial colony and was finally reconstituted in 100 μL of elution buffer. The μchipSPE procedure used 20 μL of sample load solution (20 μL of bacterial colony in 1 mL of 6 M GuHCl, pH 6.1), loaded onto the microchip device at 250 μL/h. PCR was performed using 5 μL of reconstituted DNA solution from the commercial method or 5 μL of the second elution fraction from the microchip (~12 μL total volume).

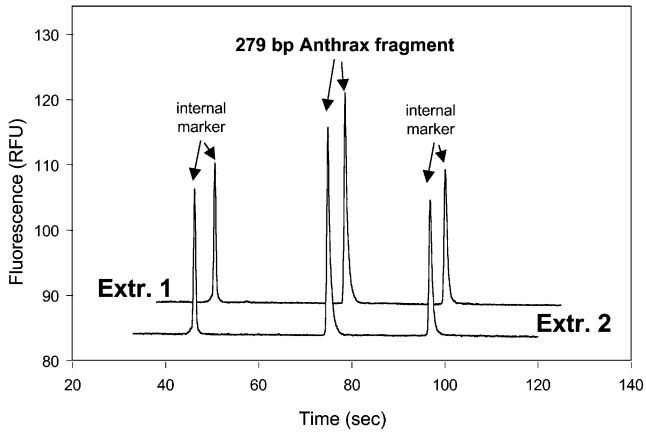

Figure 7. Successive extractions of bacterial DNA from the vaccine strain of *B. anthracis* (anthrax) using the μchipSPE procedure. A total of 100 μL of bacterial colony was added to 500 μL of load buffer (6 M GuHCl, pH 6.1); 20 μL of this solution was passed through the microchip device at 250 μL/h. PCR was performed with 5 μL of the first elution fraction (~12 μL).

cultured *S. typhimurium* samples. A commercial ion-exchange-based purification method (Qiagen) was carried out in a microcentrifuge tube according to the manufacturer's instructions. The procedure consists of mixing a bacterial sample with load solution, passing it through a small column by centrifugation, washing twice using ethanol with spinning after each step, and finally eluting the DNA in a suitable volume of buffer. The total time for the processes was ~30 min, using 200 μL of cultured sample (~13.8 μg of DNA), and reconstituting the DNA in 100 μL of buffer. In contrast, the μchipSPE method involves preparing a sample load solution (20 μL of the bacterial culture in 1 mL of load buffer), passing 20 μL of this solution (~28 ng of DNA) through the μchipSPE device, washing with 20 μL of wash buffer, and then eluting the DNA in TE buffer. Using a flow rate of 250 μL/h, the entire μchipSPE procedure was completed within 15 min. Successful DNA extractions were determined by the presence of the 275-bp amplified invA fragment using the extracted DNA as a template for PCR. This was shown by microchip electrophoresis, the results of which are presented in Figure 6, where the μchipSPE procedure and the commercial kit produce similar size peaks for the amplified fragment. Since PCR is a nonquantitative process, the slightly less DNA in the μchipSPE trace may be related to the number of starting copies of template available for PCR but could also be due to differences in PCR efficiency. Given that the μchipSPE process requires only 400 nL of bacterial culture sample per extraction, as opposed to the 200 μL required for the Qiagen kit, and the extraction time is half that of the commercial kit, the μchipSPE procedure is ideally suited for implementation of a portable DNA diagnosis device.

Potential Application in a Portable Device. One of the most important areas for portable devices, recently identified by terrorist attacks on the United States, will be the detection of biological warfare agents. This was recently illustrated by a problem in the United States where anthrax spores (*B. anthracis*) were sent via the postal system. As such, purification of DNA from the anthrax virus vaccine (*B. anthracis*) is a judicious choice to determine the feasibility of the μchipSPE to aid in identifying this type of infectious agent threat. The sample was prepared by adding 100 μL of bacterial culture to 500 μL of load buffer and passing 42 μL of this sample (~30.8 ng of DNA) through the μchip SPE device according to the developed optimum procedure. PCR was used to determine the successful purification of DNA by amplifying the 279-bp fragment of the tlf plasmid gene. Microchip electrophoresis results from two consecutive extractions are illustrated in Figure 7, where the peak corresponding to the 279-bp fragment can clearly be seen. This, again, illustrates the suitability of this μchipSPE procedure to provide rapid purification of DNA on a microscale and its suitability for integration with on-chip PCR and separation.

CONCLUSIONS

We have demonstrated the purification of DNA from a complex biological sample in the form of whole blood, using a μchipSPE procedure. Purification was performed on a silica particulate phase immobilized using sol−gel to "glue" the particles together forming a continuous network. The inter- and intrachip reproducibility of DNA extraction was excellent, enabling the load buffer composition and flow rate to be optimized. Using the optimum conditions (pH of the load buffer of 6.1 and a flow rate of 250 μL/h), we successfully amplified target DNA purified from blood in less than 15 min. The purification performance of the microchip device for bacterial DNA was shown to be comparable with a commercial microcentrifuge method but was faster and required significantly less sample. The importance of this procedure was further illustrated by purifying DNA from a bacterial culture of anthrax. Continued development will enable integration of μchipSPE with microchip PCR and separation, where it should be possible to perform all three steps within 30 min.

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