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PUBLICATION DATE

01-01-2008

HANDLE

10536/DRO/DU:30017240

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Received 30th August 2007, Accepted 20th November 2007
First published as an Advance Article on the web 6th December 2007
DOI: 10.1039/b713332d

We present a fully automated DNA purification module comprised of a micro-fabricated chip and sequential injection analysis system that is designed for use within autonomous instruments that continuously monitor the environment for the presence of biological threat agents. The chip has an elliptical flow channel containing a bed (3.5 × 3.5 mm) of silica-coated pillars with height, width and center-to-center spacing of 200, 15, and 30 μm, respectively, which provides a relatively large surface area (*ca.* 3 cm^2) for DNA capture in the presence of chaotropic agents. We have characterized the effect of various fluidic parameters on extraction performance, including sample input volume, capture flow rate, and elution volume. The flow-through design made the pillar chip completely reusable; carryover was eliminated by flushing lines with sodium hypochlorite and deionized water between assays. A mass balance was conducted to determine the fate of input DNA not recovered in the eluent. The device was capable of purifying and recovering *Bacillus anthracis* genomic DNA (input masses from 0.32 to 320 pg) from spiked environmental aerosol samples, for subsequent analysis using polymerase chain reaction-based assays.

Introduction

In recent years, there has been a growing recognition that autonomous sample processing technologies for point-of-care or field-deployable detection systems have not received the same amount of attention as chip-based detector platforms. Many of these systems continue to rely on benchtop sample purification methods to provide purified DNA or RNA as an input sample, limiting their practical use and increasing the time, cost, and complexity of the entire analysis. Sample preparation is particularly critical for assays that rely on the polymerase chain reaction (PCR), due to its known susceptibility to inhibitory soluble and insoluble constituents in a given complex sample. Given the potential dilution between collected and analyzed samples, pre-concentration of the DNA or RNA is also desirable to ensure adequate sensitivity levels. One application where the development of new sample processing technologies for PCR is particularly important is in environmental surveillance of biological threat agents.[1]

We have previously described an autonomous pathogen detection system (APDS) that performs aerosol collection, sample processing and detection based on multiplexed immunoassays[2] and real-time PCR,[3] with continuous operation benchmarked at seven days.[4] For the APDS and other biological detectors, the need for sample purification prior to PCR may depend on several factors, including the instrument location (subway, airport), type of aerosol collector used (dry filter, wetted-wall cyclone), collection time, collection efficiency and robustness of the detection assays. Given these considerations, we sought to identify and evaluate a suitable DNA purification module for processing environmental aerosol samples that inhibit the PCR. Reusable components in autonomous instruments reduce maintenance frequency and consumable costs; we have already reported reusable flow-through reaction vessels used in the APDS for conducting multiplexed immunoassays[5] and real-time PCRs.[6]

Purification of DNA by solid-phase extraction using silica surfaces in the presence of a chaotropic salt (*e.g.* guanidine thiocyanate, pH 6.5) is well established; surface-bound DNA is washed with aqueous alcohol (*e.g.* ethanol or isopropanol, 70% v/v), then eluted in a low concentration buffer (*e.g.* 10 mM Tris/1 mM EDTA, pH 8.0), with heating to improve recovery. Numerous small-scale devices that utilize this chemistry have been reported, including capillary columns packed with silica beads[7,8] or monolithic materials,[9] micro-centrifuge tubes incorporating glass fibre filters or silica-gel membranes,[10] and microchips with channels or chambers containing (i) silica beads held in place by weirs and/or immobilised using sol–gel chemistry,[8,11–13] (ii) arrays of micro-fabricated pillars,[14,15] or (iii) monolithic materials.[16,17] Alternative approaches include renewable-surface affinity microcolumns[18–20] [where derivatised 60 μm beads were removed and replaced using sequential injection analysis[21] (SIA)-based fluid handling], and microchips with single aminosilane-modified open channels[22] or arrays of open channels derivatised with chitosan,[23] where binding and release of DNA were controlled by changes in solution pH. Many of these systems were discussed in a recent review by Horsman *et al.*[24]

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For DNA purification within the APDS, we have constructed a microfluidic device containing a well-defined bed of silica-coated pillars (which we will henceforth refer to as the 'pillar chip').[25] Compared to columns or channels packed with particles, pillar arrays constructed using micrometer- or nanometer-scale fabrication technology offer superior structural homogeneity, greater versatility and lower flow resistance.[26,27] Furthermore, as a suitable surface can be created during construction, this approach can circumvent problems associated with filling channels with binding materials after the fabrication processes have been completed.[14] Over the past few years, a range of devices incorporating pillar structures have been developed for biomolecule separation,[28,29] cell isolation,[30] and DNA fractionation[27,31,32] and purification.[14,15,33] Of particular relevance to our investigation, Christel *et al.*[15] constructed a device with micro-fabricated silica-coated pillars for the purification of DNA. The internal surface area of the device (*ca.* 0.36 cm^2) was six times greater than a similar chip without pillars, and an equivalent increase in the quantity of captured DNA (from a standard solution of fluorescein-tagged plasmid digest) was reported. Experiments with lambda DNA revealed a 50% capture efficiency and ten-fold concentration effect using an input volume of 500 μL (5×10^4 copies) and an elution volume of 25 μL. However, purification of DNA from lysed cells or real sample matrices was not investigated.

Cady *et al.*[14] used a similar approach to fabricate an array of silica-coated square pillars in a microfluidic channel, which had an effective surface area of 2.1 or 4.2 cm^2, depending on the etching depth. The binding capacity for bacteriophage lambda DNA was estimated at 82 ng cm^{-2} and around 10% of the loaded DNA was recovered in the first 50 μL elution. The device was also used to purify chromosomal DNA from *Escherichia coli* cells that were lysed by prior incubation in a binding buffer. Approximately 87% of the protein from the cell lysate was removed by the device.[14]

Hashioka *et al.*[33] recently described an approach to integrate DNA purification and detection on a single chip for clinical diagnosis applications. The purification step within this device involved the immobilization of the DNA on alumina-coated micro-fabricated pillars under acidic conditions and elution with alkaline solution. However, in their preliminary report, crucial parameters such as reproducibility, DNA recovery and carryover were not quantified and the detection of DNA from real samples was not examined.[33]

The pillar chip described in this paper was designed for the purification of DNA within autonomous instruments that continuously monitor the environment for the presence of biological threat agents. The chip was therefore integrated into an SIA-based fluid-handling module to automate the DNA purification process. DNA from spiked samples was extracted, washed, eluted then analyzed using a quantitative real-time PCR assay. Important autonomous system parameters such as capture flow rate, sample input and elution volumes, carryover, and the effect of debris from lysed *Bacillus anthracis* (*B. anthracis*) spores were determined. A mass balance was conducted to determine the fate of input DNA not recovered in the eluent. Aqueous suspensions of an aerosol standard reference material that inhibits PCR were spiked with *B. anthracis* genomic DNA and used to demonstrate the purification capability and sensitivity of the pillar chip device.

Experimental

Automated fluid-handling module

The sequential injection analysis (SIA) system was a FloPro-4P (Global FIA, Fox Island, WA) fitted with a syringe pump (1 mL, Cavro, Sunnyvale, CA) and two multi-position selection valves (10- and 14-port, Cheminert, VICI, Houston, TX). A schematic diagram of the SIA manifold is shown in Fig. 1. PFA tubing (0.8 mm I.D., 1.6 mm O.D., Cole-Parmer, Vernon Hills, IL) was used throughout the SIA manifold. Clean tubing cuts were obtained with a rotating blade (Upchurch Scientific, Oak Harbor, WA). Tubing connections were made using flangeless ¼-28 and 10-32 nuts and ferrules (VICI). A laptop computer running a LabVIEW (National Instruments, Austin, TX) -based graphical user interface controlled the instrument. The carrier liquid used to propel fluids throughout the SIA manifold was deionized water (18 MΩ from a Milli-Q system, Millipore, Billerica, MA).

Pillar chip fabrication

The pillar chip (Fig. 2) was fabricated from silicon wafers using standard photolithographic techniques and deep reactive ion etching. The silicon pillars were oxidized to yield a 150 nm silica surface layer suitable for DNA capture in the presence of chaotropic agents.[34] The chip had an elliptical flow channel that featured a bed (3.5 × 3.5 mm) of pillars with height, width and center-to-center spacing of 200, 15, and 30 μm, respectively. The surface area available for DNA capture within the chip was approximately 3 cm^2.

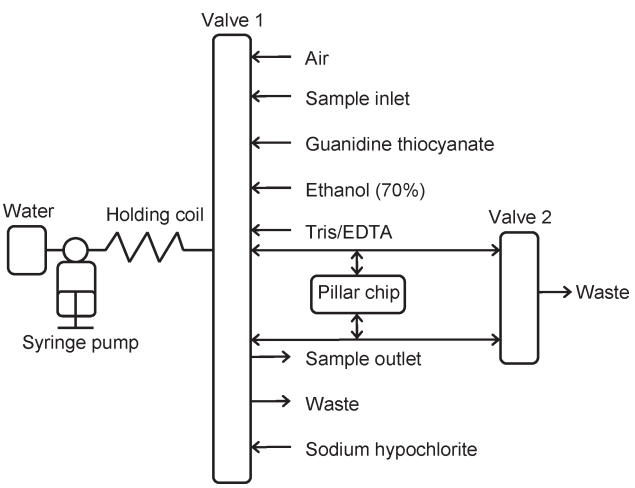

Fig. 1 Schematic diagram of the sequential injection analysis (SIA) manifold used to perform automated DNA purification with a micro-fabricated pillar chip. The manifold consisted of a bidirectional syringe pump, a holding coil and two multi-position selection valves. The pillar chip was connected to two outer ports on each valve, *via* two T-pieces. This H-type configuration enabled the pillar chip to be flushed from either direction using positive pressure. The holding coil and waste line were connected to the center port of valve 1 and valve 2, respectively.

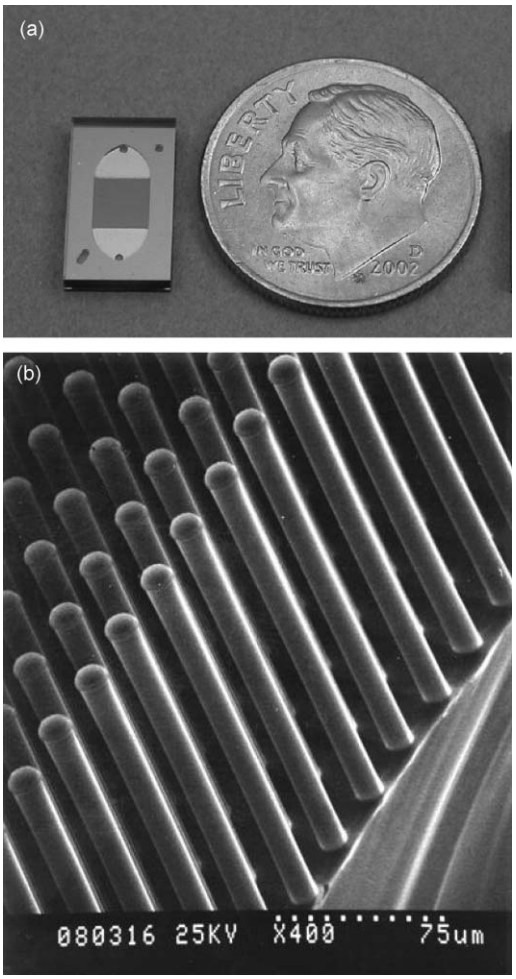

Fig. 2 (a) Micro-fabricated pillar chip for the purification of DNA. (b) Scanning electron micrograph of the pillar structure.

Pillar chip packaging

The package (Fig. 3) precisely aligned the fluid connections to the pillar chip and enabled inbuilt temperature control over the device. Heating and temperature sensing were accomplished using a printed-circuit board with standard surface mount resistors and a surface-mounted resistance temperature detector (RTD), respectively. A plate held the pillar chip in precise alignment with the heaters and fluid connections, which penetrated through the circuit board. A thermally conductive pad compressed between the pillar chip and the circuit board components ensured good thermal contact. A foam rubber pad in the bottom of the package applied compliant pressure to the component stack and provided insulation. The fluid interconnects have been described elsewhere.[35] During the DNA elution step, the chip was heated to 80 °C under PID control (Watlow Electric Manufacturing Company, St Louis, MO). Two T-pieces (PEEK, ZT1FPK 10-32, 0.75 mm through hole, VICI) were connected to each fluid line from the chip that enabled the pillar bed to be flushed, using positive pressure, from either direction.

Automated extraction procedure

The chip was primed with guanidine thiocyanate solution (60 μL, 2 M, 3.3 mM Tris, pH 6.5, Teknova, Hollister, CA).

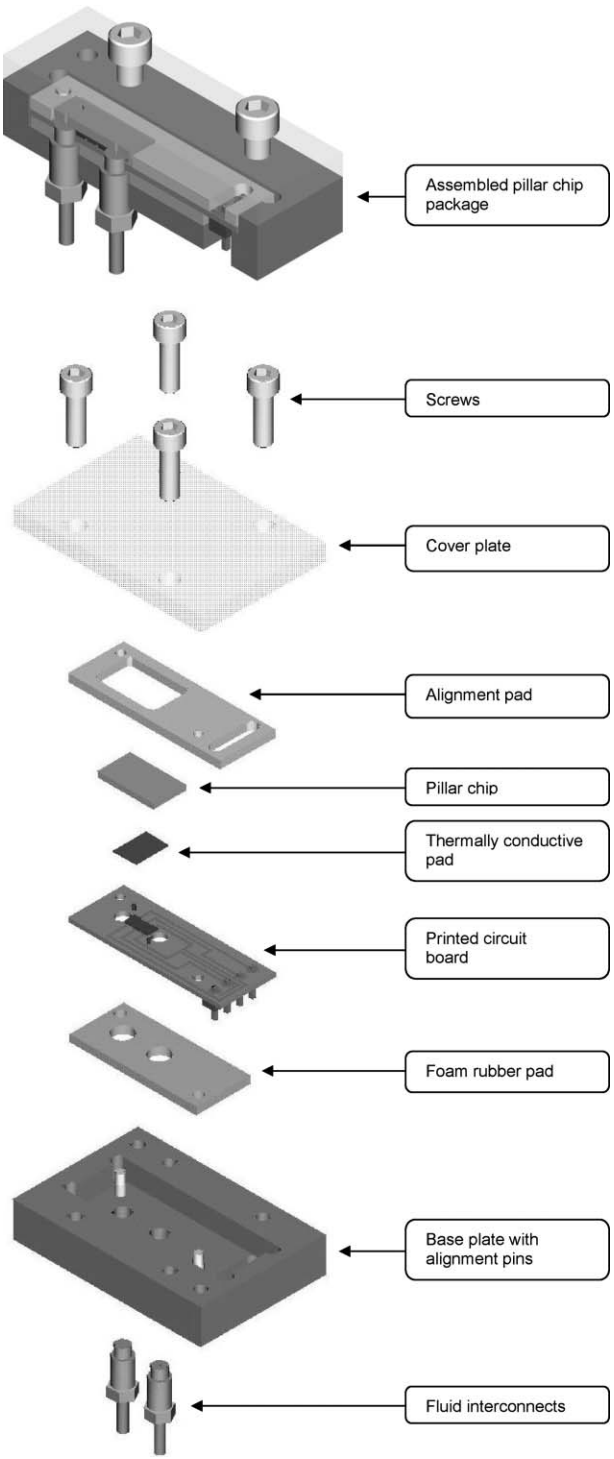

Fig. 3 Pillar chip package that housed the micro-fabricated pillar chip used for DNA purification. The printed-circuit board incorporated the surface-mounted resistors for heating and an RTD temperature sensor.

Aqueous sample solution (typically 100 μL) spiked with *B. anthracis* genomic DNA was mixed in the holding coil with an equal volume of guanidine thiocyanate (2 M, 3.3 M Tris, pH 6.5, Teknova), then dispensed to the chip at a flow rate of 1 μL s^{-1}, unless specified otherwise. The pillar chip was washed with ethanol (70% v/v, 3 mL, 100 μL s^{-1}). Ethanol was

displaced from the chip by pumping a zone of air (100 μL, 10 μL s^{-1}) across the pillar bed. The DNA was eluted from the bed (10 μL, 10 mM Tris, 1 mM EDTA, pH 8.0, Teknova) with heating to 80 °C. The eluent (10 μL) was collected and then analyzed using a *B. anthracis* real-time PCR assay. After each extraction, the pillar chip, sample input and output lines were cleaned of residual DNA with aqueous sodium hypochlorite (100 μL, 50 μL s^{-1}, 1.2% m/v), followed by rinsing with deionized water (1 mL, 100 μL s^{-1}). Finally, the chip, sample input and output lines were primed with air (150 μL, 10 μL s^{-1}). Zones of air (10 μL) were used to minimize dispersion during certain fluid manipulations, for example, to prevent solutions aspirated into the holding coil from mixing with the carrier. This approach to fluid handling, termed zone fluidics, has been described elsewhere.[36]

Real-time TaqMan® PCR

A TaqMan® PCR assay specific for *B. anthracis* was used to determine the DNA concentrations of extracted samples. PCR master mix (15 μL, AccuPrime SuperMix I, 5 mM $MgCl_2$, Invitrogen, Carlsbad, CA), forward and reverse primers (0.2 μM, Integrated DNA Technologies, Coralville, IA) and a fluorescence resonance energy transfer (FRET) probe (0.4 μM, 5′ FAM, 3′ Black Hole Quencher, Integrated DNA Technologies) and sample (5 μL) were combined in a polypropylene reaction tube (25 μL, Cepheid, Sunnyvale, CA). Real-time PCR was performed in a commercially available thermal-cycler (SmartCycler, Cepheid). The cycle threshold was calculated automatically using a fixed threshold, set at 30 mV with background subtraction enabled. The thermal cycling protocol used was 95 °C for 120 s followed by 45 cycles of 95 °C for 15 s, 60 °C for 30 s, 72 °C for 15 s. A fluorescence measurement was made for each cycle at the end of the 60 °C hold. A stock solution of *B. anthracis* genomic DNA was obtained from Dugway Proving Grounds (Dugway, UT). The stock concentration (4 ng mL^{-1}) was determined using a PicoGreen® dsDNA quantitation kit (Molecular Probes, Eugene, OR) and was diluted prior to use with deionized water as required.

Real-time PCR quantitation

Quantitative real-time PCR has been described elsewhere.[37,38] We utilized a real-time PCR assay with *B. anthracis* specific primers and TaqMan® probe. A real-time PCR calibration curve was obtained from ten-fold serial dilutions spanning five orders of magnitude, from 16 fg up to 1.6 ng, with cycle thresholds that ranged from 18.5 to 35, respectively. A plot of mean (n = 3) cycle threshold *vs.* log mass (fg) yielded an equation of $y = -3.28x + 41.2$ ($r^2 = 0.9998$), in agreement with previously published results.[38] This calibration function was used to convert cycle threshold to recovery (in mass) for purified DNA in the pillar chip eluent.

Mass balance DNA recovery from guanidine thiocyanate solutions

B. anthracis genomic DNA was recovered from guanidine thiocyanate solutions using ProbeQuant G-50 microcolumns (Amersham Biosciences, Piscataway, NJ), according to the manufacturer's protocol. The columns were calibrated for DNA recovery using standard solutions of *B. anthracis* genomic DNA prepared in guanidine thiocyanate (2 M, 3.3 mM Tris, pH 6.5). An aliquot (50 μL) of a DNA standard solution (3.2–3200 pg input mass, ten-fold dilutions) was passed through a column, then a fraction (5 μL) of the eluent (50 μL) was subjected to real-time PCR. Triplicate measurements (three individual columns) were made for each input mass of DNA. The amount of input DNA not captured by the pillar bed during each extraction was determined by collecting the entire guanidine thiocyanate volume at the waste line of the SIA system, processing an aliquot (50 μL) through a ProbeQuant column, and then subjecting 5 μL of the eluent to real-time PCR analysis.

Sample preparation and analysis

Urban particulate matter (UPM) was an aerosol standard reference material (1648) from the National Institute of Standards and Technology (NIST, Gaithersburg, MD). Aqueous suspensions of UPM were prepared daily as required by mixing a weighed amount of the solid with known volume of deionized water spiked with *B. anthracis* genomic DNA. Certified killed (γ-irradiated) *B. anthracis* (Ames strain) spores were obtained from Dugway Proving Grounds (Dugway, UT).

Results and discussion

Module design and operation

An SIA-based fluid-handling module enabled complete automation of the fluid-handling operations required for the purification of DNA using guanidine thiocyanate extraction chemistry and a silica surface in the form of a micro-fabricated pillar chip. For this SIA system, the reagents required for extraction, including guanidine thiocyanate, ethanol (70% v/v), Tris/EDTA elution buffer, and sodium hypochlorite (1.2% m/v) were positioned on the multi-position valve and drawn from as required. The device was then programmed to mimic the operations that would typically be performed manually in the laboratory.

The pillars on the bed were spaced 15 μm apart. During the purification of DNA from unfiltered environmental samples, the pillar bed also acted as a filter leading to the accumulation of the particulates at the leading edge of the bed. Each entry port to the pillar chip was connected to both selection valves *via* a T-piece, which enabled the pillar bed to be flushed from either direction under positive pressure. This H-type configuration was essential for removing particulates from the pillar bed that accumulated when attempting to purify DNA from spiked environmental samples. Ethanol (70% v/v, DNA has limited solubility in this solvent) was pumped to the bed in the reverse direction, which washed the surface-bound DNA and removed any particulates that accumulated during the sample adsorption step. The smallest volume of elution buffer that could be reproducibly positioned, heated and recovered from the pillar chip bed was 10 μL. Recovery increased at least six-fold when the device was heated to 80 °C, compared to 25 °C, during the DNA elution step (data not shown). The sample

input and output from this DNA extraction module have already been interfaced with an aerosol collector and a flow-through PCR thermal-cycler, respectively.[3]

Carryover from previous samples

The pillar chip was designed to be reusable and capable of processing single samples in series. Therefore, carryover of target DNA from one sample to the next must be avoided. However, owing to the inherent sensitivity of the PCR assay (*i.e.* single copy detection), carryover of a single copy of template DNA could potentially lead to a false positive result on a subsequent negative sample. We have previously shown that aqueous solutions of sodium hypochlorite are effective for rendering residual template DNA inactive for PCR in automated fluid-handling systems.[6]

To prevent carryover on the present system, the pillar chip, sample inlet and outlet lines were automatically decontaminated after each extraction by flushing lines with sodium hypochlorite. Since residual sodium hypochlorite can inhibit the PCR reaction, the system was then flushed with the appropriate volume of deionized water. No carryover of *B. anthracis* DNA was detected ($n = 20$) by real-time PCR after extractions where the input mass of DNA was $\leqslant 3.2$ ng (*i.e.* subsequent extraction with deionized water as the sample yielded no cycle threshold). The time required for the automated sample (100 μL) extraction and clean-up routines were approximately 10 and 5 min, respectively.

PCR interference from extraction solutions

Given that the PCR is susceptible to interference from certain chemical species, the effect of extraction solutions, including guanidine thiocyanate and ethanol, on our real-time PCR assay was examined. PCR reactions (25 μL) were prepared containing varying concentrations of either guanidine thiocyanate (from 1×10^{-5} to 0.1 M) or ethanol (from 3×10^{-4} to 1.7 M) with a constant DNA concentration (16 pg per 25 μL reaction). Complete PCR inhibition (*i.e.* no cycle threshold detected) was observed at guanidine thiocyanate and ethanol concentrations greater than 1×10^{-2} and 1 M, respectively. As a guanidine thiocyanate concentration of 2 M was used for the extraction, these experiments revealed that low-level guanidine contamination of the elution buffer could interfere with the real-time PCR assay. To prevent this contamination, the pillar chip was flushed with a sufficient volume (>300 μL) of ethanol. Precautions were also taken to prevent contamination of other fluid lines with guanidine thiocyanate during switching of the multi-position valve. By comparison, the PCR was tolerant of ethanol; small residual volumes (*ca.* 1 μL) on the pillar bed prior to loading the chip with elution buffer did not interfere with the PCR detection.

DNA mass balance

Understanding the amount of input DNA that is recoverable from the pillar chip is critical in defining system performance. Using a sample input volume of 100 μL and an elution volume of 10 μL, the recovery of DNA from the chip was typically between 10 and 20%. This translates to a concentration factor, from the sample to the eluent, of between one and two. To determine the fate of DNA not recovered in the Tris/EDTA elution buffer, the other extraction solutions that had passed through the chip were collected and analyzed. Knowing that guanidine thiocyanate inhibited the PCR assay, these fractions were first processed through desalting (ion-exchange) columns. A calibration function of recovery *versus* input DNA concentration was established for the desalting columns; typical recovery was approximately 80%. Knowing the PCR inhibition concentration for ethanol, these fractions were collected, diluted ten-fold with deionized water, then subjected to PCR analysis.

A plot of DNA recovery from the Tris/EDTA eluent, guanidine thiocyanate and ethanol wash solution at two different input DNA concentrations is shown in Fig. 4. Approximately 21% of the input DNA was captured, retained, and recovered in the Tris/EDTA elution buffer. Between 17 and 24% was accounted for in the guanidine thiocyanate solution; this represents DNA that was not captured by the pillar bed. Only 3–9% was lost from the pillar bed during the ethanol wash step. The remaining 43–48% was not recovered and remained bound to the pillar chip surface. During routine operation, DNA not recovered from the surface in the Tris/EDTA elution buffer would be lost to waste, as the chip is flushed with sodium hypochlorite and deionized water between extractions.

Our findings indicated that a substantial amount (43–48%) of the input DNA was not recovered from the pillar chip when the elution volume was only 10 μL. For an input mass of 320 pg, we found that increasing the elution volume to either 25 or 50 μL increased the mean recovery ($n = 3$, $\pm 1\sigma$) to 37% ($\pm$ 6%) and 43% ($\pm$ 3%), respectively. However, these higher recoveries were accompanied by reduced concentration factors. For elution volumes of 10, 25 and 50 μL, the concentration factors were 2.1, 1.5 and 0.7, respectively, and

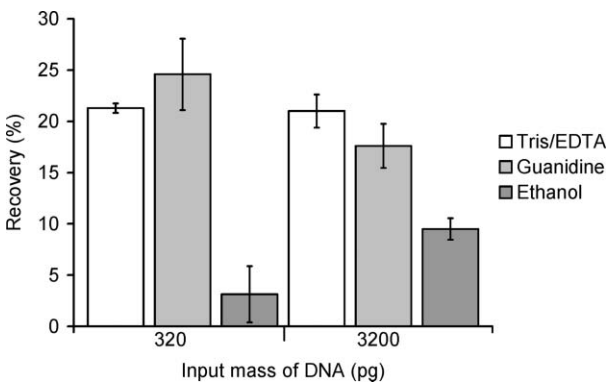

Fig. 4 Mass balance determination of *B. anthracis* genomic DNA in the extraction solutions used by the automated pillar chip module at two different input masses (320 and 3200 pg). Guanidine thiocyanate was used for DNA adsorption, ethanol for washing and Tris/EDTA for elution of the purified sample. Error bars indicate ± 1 standard deviation of the mean ($n = 3$) recovery from replicate extractions. DNA not accounted for in these solutions remained bound to the pillar chip surface after the Tris/EDTA elution, and was lost to waste during the automated clean-up procedure. Sample input and elution volumes were 100 and 10 μL, respectively.

therefore an elution volume of 10 μL was used for subsequent experiments.

Effect of input volume on concentration factor

One potential advantage of this method is the ability to concentrate nucleic acid out of a complex clinical or environmental sample. The effect of input volume on concentration factor was investigated at two different flow rates (Fig. 5). This plot shows that concentration factors increase significantly with sample input volume. Relatively large sample volumes (10 000 μL) can be processed using a high flow rate, to realize a concentration factor of *ca.* 80 while maintaining reasonable sample processing times (*ca.* 20 min).

DNA from unlysed and lysed *B. anthracis* spores

To confirm that the automated pillar chip module was suitable for the purification of genomic DNA from samples containing lysed *B. anthracis* spores (*i.e.* that the process was not impeded by the presence of cellular debris), PCR calibrations for standard solutions containing lysed and unlysed spores were prepared both with and without the purification procedure (Fig. 6). In the case of unlysed spores, the relatively low measured concentrations primarily represent extra-cellular DNA. The slight off-set between the values obtained with and without the extraction procedure was attributed to a small portion of extra-cellular DNA that was still attached to the spore surface and therefore not able to bind to the pillar chip surface (*i.e.* not captured during the adsorption step). It is possible that some of the unlysed spores added directly to the PCR reaction were lysed during the initial 95 °C hold and became available for subsequent amplification, but *B. anthracis* spores are not typically lysed by temperature alone. As expected, the measured DNA concentration increased (by approximately two orders of magnitude) when the spores were lysed off-line by bead beating. Most importantly, the measured

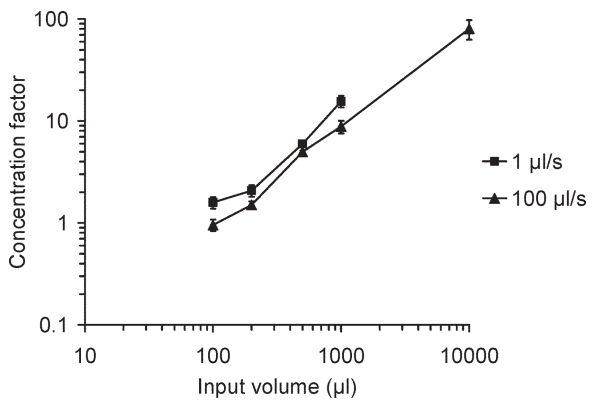

Fig. 5 Increasing the sample input volume to the automated pillar chip module increased the concentration factor at two different input flow rates. A large sample input volume (10 000 μL) captured at a high flow rate (100 μL s^{-1}) realized a concentration factor of 80. Extractions were performed using single input *B. anthracis* genomic DNA concentration of 3.2 pg μL^{-1}. The sample elution volume was constant at 10 μL. Each data point represents the mean concentration factor of replicate extractions (n = 3), error bars indicate ±1 standard deviation of the mean.

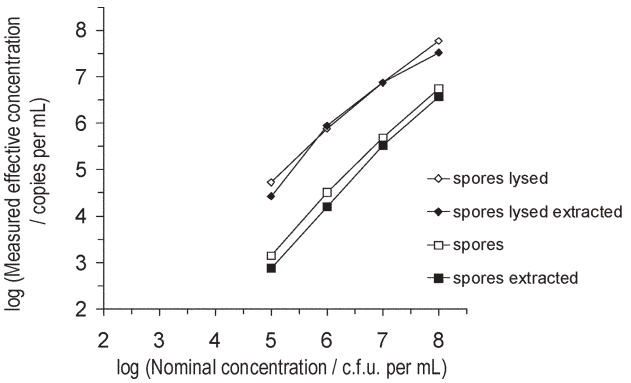

Fig. 6 PCR calibration for standard solutions containing lysed and unlysed *B. anthracis* spores, with and without the purification procedure, which shows that the presence of cellular debris does not impede DNA extraction in the pillar chip module.

concentrations of DNA for the lysed spores with and without the purification procedure were comparable and therefore the presence of cellular debris does not prevent DNA extraction from samples containing lysed spores, even at concentrations of 10^8 c.f.u. mL^{-1} (10^7 c.f.u. entering the pillar chip in a 100 μL sample). A flow-through ultrasonic lysis module based on previously reported designs[39,40] has recently been developed for the APDS.

Purification standard

A NIST standard reference material (urban particulate matter, UPM) was used to evaluate the performance of the DNA purification module. This material was a time-integrated (12 months) sample of natural atmospheric particulate matter collected in an urban location (St Louis, MO). We prepared aqueous suspensions of urban particulate matter over a concentration range from 1 to 20 mg mL^{-1}. These suspensions were spiked with *B. anthracis* DNA and then analyzed by real-time PCR. Complete inhibition of the real-time PCR was defined as the case when the fluorescence signal did not exceed the preset threshold after 45 cycles and no cycle threshold was obtained. For these unfiltered samples, complete inhibition of the PCR reaction occurred at a UPM concentration $\geqslant$2.5 mg mL^{-1}; these samples were black and turbid. Given the availability of UPM, this standard reference material may find use as an environmental PCR inhibition standard, to characterize the performance of other DNA purification devices.

Analysis of spiked urban particulate matter samples

To demonstrate that the pillar chip was reusable when challenged with real sample matrices, we performed replicate extractions (n = 24) of *B. anthracis* genomic DNA (320 pg) from spiked UPM samples (unfiltered, 2.5 mg mL^{-1}) (Fig. 7).

Owing to the high particulate load in the unfiltered samples, a larger volume (3 mL, compared to 1 mL) and higher flow rate (100 μL s^{-1}, compared to 10 μL s^{-1}) were required to wash the pillar bed prior to DNA elution. These data show that even when challenged with the high particulate load of the unfiltered UPM samples, the module

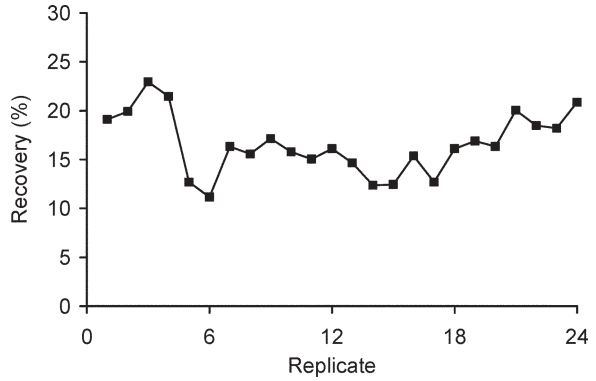

Fig. 7 Consistent recovery of purified *B. anthracis* genomic DNA from spiked PCR inhibitory environmental aerosol samples demonstrating the reusability of the automated pillar chip module. Unfiltered aqueous suspensions of urban particulate matter (a NIST standard reference material, 2.5 mg mL^{-1}) were spiked with *B. anthracis* genomic DNA (320 pg) prior to extraction. The sample input and elution volumes were 100 and 10 μL, respectively.

consistently delivered purified DNA without blockage of the device.

We also investigated the effect of input mass of *B. anthracis* genomic DNA on recovery from PCR-inhibited UPM samples (Fig. 8). The range of input masses tested was from 0.32 to 320 pg (which is equivalent to a range of 50 to 5×10^4 copies, assuming *B. anthracis* genomic DNA, including plasmids, is 5.7 Mbp/copy or 6.4 fg/copy[41]). The pillar chip consistently recovered DNA from the real sample matrices even at very low input masses. Recoveries were similar for the PCR-inhibited unfiltered spiked UPM samples, compared to DNA extracted from standard solutions prepared in deionized water. Generally, the standard deviation of the recovery increased at very low input DNA because greater variation of the cycle

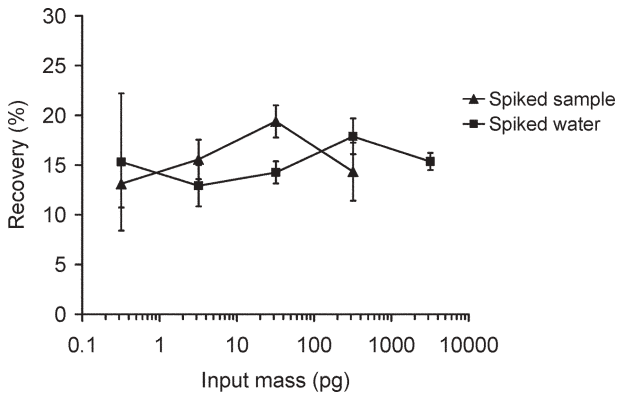

Fig. 8 The automated pillar chip module recovered purified *B. anthracis* genomic DNA, over a wide range of input masses (0.32–320 pg), from spiked PCR inhibitory environmental aerosol samples, which compared favorably to recovery data from extractions from spiked water (0.32–3200 pg). The environmental aerosol samples were unfiltered aqueous suspensions of urban particulate matter (a NIST standard reference material, 2.5 mg mL^{-1}) spiked with different masses of *B. anthracis* genomic DNA. Each data point represents the mean recovery (n = 3) of replicate extractions, error bars indicate ± 1 standard deviation of the mean.

threshold is observed for real-time PCR performed using low (<10) copies of template DNA per reaction.

Conclusions

The pillar chip module was found to be a reliable, reusable flow-through device that successfully purified and in some cases provided pre-concentration of DNA from real environmental aerosol samples. When integrated with an SIA fluid-handling module, the purification protocol was completely automated, such that an operator could load a PCR-inhibited sample and the module would deliver DNA of sufficient purity for subsequent analysis by a PCR-based assay. More so, data obtained using the pillar chip demonstrated sufficient reliability and reproducibility indicating that the device would be an exceptional candidate for DNA purification on stand-alone biological detectors such as the APDS.

Acknowledgements

The authors acknowledge Pete Meyer for the drawing of the pillar chip package. This work was performed under the auspices of the US Department of Energy by the University of California, Lawrence Livermore National Laboratory under Contract W-7405-ENG-48, with funding from the US Department of Energy Chemical and Biological National Security Program and the US Department of Homeland Security Biological and Chemical Countermeasures Program.

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