Toward a microchip-based solid-phase extraction method for isolation of nucleic acids

A silica-based solid-phase extraction system suitable for incorporation into a microchip platform (μ-TAS) would find utility in a variety of genetic analysis protocols, including DNA sequencing. The extraction procedure utilized is based on adsorption of the DNA onto bare silica. The procedure involves three steps: (i) DNA adsorption in the presence of a chaotropic salt, (ii) removal of contaminants with an alcohol/water solution, and (iii) elution of the adsorbed DNA in a small volume of buffer suitable for polymerase chain reaction (PCR) amplification. Multiple approaches for incorporation of this protocol into a microchip were examined with regard to extraction efficiency, reproducibility, stability, and the potential to provide PCR-amplifiable DNA. These included packing microchannels with silica beads only, generating a continuous silica network via sol-gel chemistry, and combinations of these. The optimal approach was found to involve immobilizing silica beads packed into the channel using a sol-gel network. This method allowed for successful extraction and elution of nanogram quantities of DNA in less than 25 min, with the DNA obtained in the elution buffer fraction. Evaluation of the eluted DNA indicated that it was of suitable quality for subsequent amplification by PCR.

Keywords: DNA purification / Microchip / Nucleic acids / Solid-phase extraction

1 Introduction

While the Human Genome Project approaches successful completion, the applications for high-speed, high-throughput DNA sequence analysis are just beginning. Genomic analysis for mutations and polymorphisms in individuals will represent the next level of diagnostic and clinical approaches for detecting and treating disease states. Traditional diagnostic and clinical assays relying on DNA sequence information involve purification of the DNA from whole blood, amplification by the polymerase chain reaction (PCR), and subsequent separation using slab gel electrophoresis. This is a lengthy procedure and, as such, is not directly applicable to high-throughput analysis. In addition to time savings, high-throughput methods require smaller reaction volumes to reduce the cost of reagents and the amount of waste generated. Decreasing user intervention, to decrease labor costs and reduce the risk of contamination, is also important for high-throughput analyses.

The most time-consuming step in the traditional analysis is the electrophoresis step. Initial efforts to increase the speed of electrophoretic analysis and provide direct computer quantitation saw the development of the capillary electrophoresis (CE) instrument. This has since been miniaturized into a microchip format allowing the separation of DNA fragments in a matter of minutes compared to tens of minutes for CE and hours for slab gels. Parallel processing further reduces analysis times, as shown by the simultaneous separation of 96 samples on a single microchip device [2]. Clinically relevant separations carried out on these microchips show no loss of diagnostic capability [3–5]. While a decrease in the time required for the analytical separation provided a significant reduction in total analysis time, the net effect on the entire process is less significant. Clearly, the greatest potential would be reaped by integration of all three steps in a single microchip, forming what is known as a μ-TAS. Realization of such a device requires miniaturization of the pre-electrophoresis steps in forms amenable to microchip-based methods. Recent efforts on adapting the PCR procedure to a microchip have demonstrated several successful methods [6–9]. The time saving potential of microchip PCR was best demonstrated using infrared (IR)-mediated heating [10], where rapid thermocycling resulted in successful PCR amplification in under 240 s.

Historically, the time necessary for DNA purification was greatly reduced by the shift from phenol extraction-based procedures to those using solid-phase extraction on silica.

Correspondence: Dr. Jerome Ferrance, Department of Chemistry, McCormick Road, University of Virginia, Charlottesville, VA 22904, USA
E-mail: jpf3p@virginia.edu
Fax: + 804-982-3048

Abbreviations: TE, Tris-EDTA buffer; TEOS, tetraethoxysilane; μ-TAS, μ-total analysis system
or ion exchange resins [11]. These methods also provided a method more amenable to miniaturization, as shown by the work of Tian et al. [1] who developed a DNA purification system with a volume of 500 nL. This system, using a capillary packed with silica beads, showed successful solid phase extraction (SPE) of PCR-amplifiable DNA directly from whole blood in less than 30 min. Using a similar procedure, Christel et al. [12] reported the first purification of DNA using a microchip. In this case, pillar-like structures, fabricated in a silicon microchip using reactive ion etching, increased the available surface for adsorption. While this shows some potential, the procedure for creating the silica support is complex and the distance between each pillar is relatively large – both of these limiting the efficiency of the extraction procedure.

The aim of the work described in this report was to examine alternatives to microfabricated pillars to create a low-cost and highly efficient method for SPE-mediated extraction of DNA that could be easily implemented on a microchip. While the dimensions of the capillary device used by Tian et al. [1] promise adequate extraction when transferred to a microchip, in practice, this is difficult to achieve due to constraints with retaining silica beads within the microchip. One solution to this problem is to use a weir structure, as employed by Oleschuk et al. [13] for the extraction of fluorescein and BODIPY. This requires an additional step in the fabrication process, however, and can also lead to unexpected flow patterns. One potentially viable alternative to the use of particles, is to construct a continuous silica network in the channel via sol-gel chemistry. Sols are liquid colloidal suspensions which, when catalyzed, gel to form solid structures [14–16] (hence the term sol-gel). Since the time for condensation can be controlled, the sol-gel precursor can be flowed into a microchamber as a liquid then gelled in place to form the necessary solid matrix. Although the ease of incorporating these silica materials into micro-devices is evident, their ability to serve as extraction media for DNA was unknown.

Here we present the results of a preliminary study on the potential of introducing a silica matrix into a microchip to serve as a solid phase for DNA extraction. Using both capillary and microchip systems, various matrices including silica beads, continuous silica networks created from sol-gel chemistry, and combinations of the two, have been examined with regard to extraction efficiency, reproducibility, stability, and the ability to produce PCR-amplifiable DNA. We demonstrate that it is possible to use nanoliter bed volumes with the appropriate solid phase secured in a microchannel and effectively extract DNA in a pressure-driven system.

2 Materials and methods

2.1 Reagents

Silica beads, 15 μm and 5 μm diameter, were a gift from Mallinkrodt-Baker. Carbowax Sentry polyethylene glycol (PEG) 3350 was purchased from Union Carbide (Danbury, CT, USA). Propytrichlorosilane, tetraethoxysilane (TEOS), guanidine HCl, HNO₃, KOH, NaOH, Tris, EDTA, 2-propanol, and HinD III digested λ-phage DNA were purchased from Sigma-Aldrich (St. Louis, MO, USA). PicoGreen dsDNA intercalating dye was purchased from Molecular Probes (Eugene, OR, USA). HCl was purchased from Fisher (Fairlawn, NJ, USA). Taq polymerase and other PCR reagents were purchased from Perkin Elmer (Santa Clara, CA, USA). Fused-silica capillary tubing, 200 μm ID, was purchased from J&W Scientific (Folsom, CA, USA). Borofloat glass for cover plates was purchased from S.I. Howard Glass (Worcester, MA, USA) and borofloat glass coated with chrome and photoresist, for production of microchip devices, was purchased from Nanofilm (Westlake Village, CA, USA). All solutions were prepared in Nanopure water (Barnstead/Thermolyne, Dubuque, IA, USA). TE buffer (10 mM Tris, 1 mM EDTA, titrated to pH 7.6 with HCl), 6 M guanidine-HCl in TE buffer, and 80% 2-propanol were used for the SPE procedure. Stock solutions (0.5 μg/mL) of the HindIII digested λ-phage DNA were prepared in 6 M guanidine-HCl solution and TE buffer. These were diluted as needed in the appropriate buffer for DNA adsorption experiments and for use as standards in the DNA quantitation assay.

2.2 Device preparation

Sols were prepared by hydrolyzing a 27% v/v solution of TEOS in water by the addition of 0.1% v/v HNO₃ and heating to 60°C for 10 min then 80°C for 60 min with stirring at 200 rpm. Gelation of the sol was carried out by the addition of 5.0% v/v of a 1 M solution of hydroxide in water for base-catalyzed condensation, or by increasing the temperature to 100°C for condensation under acidic conditions. For slurry-type devices, the silica beads were added to the sol before condensation. All base-catalyzed sol-gels were allowed to age 24 h at room temperature and all sol-gel/bead slurries were aged at 100°C for 1 h to facilitate ethyl alcohol evaporation and promote condensation. Capillaries were filled using positive pressure, with the packing material, either sol-gel or sol-gel/silica bead slurry, being injected from a 1 mL syringe. Microchambers were filled with sol-gel using a 1 mL syringe attached to a microchip interface. Devices incorporating silica beads and sol-gel/silica bead mixtures were filled using vacuum to draw material into the microchamber.
The microdevices themselves were prepared from boro-
float glass using standard lithographic and wet etching
procedures. Etched wafers and cover plates, through
which access holes had been prepared, were thermally
bonded at 680–695°C.

2.3 Apparatus

The SPE apparatus consisted of a Harvard Apparatus
Model 22 Syringe Pump (Harvard Apparatus, Holliston,
MA, USA), and a 100 μL Hamilton gas-tight syringe
(Hamilton, Las Vegas, NV, USA). For capillary experi-
ments the syringe was connected with a Micro-tight PEEK tub-
ing sleeve and mini-tight fittings (Upchurch Scientific, Oak
Harbor, WA, USA) to a 40 mm long section of fused-silica
capillary tubing. For microchip-based experiments the
syringe was connected using 0.75 mm ID. PEEK tubing
and mini-tight fittings to a noncommercial Teflon/plexi-
glass microchip interface.

2.4 SPE procedure

All extraction devices were rinsed with MeOH for silica
activation before use. Between extractions, the device
was flushed with TE and 6 M guanidine solutions before
loading the sample. A 25 μL aliquot of the λ-phage DNA
in guanidine stock solution (12.5 ng of DNA) was passed
through a device at a flow rate between 100–200 μL/h. A
25 μL aliquot of 80% 2-propanol was passed through the
device to remove the guanidine and contaminating sub-
stances. The DNA was then eluted from the solid phase
by passing TE buffer through the device. The load and
wash solutions, along with aliquots of the eluent were col-
lected in microcentrifuge tubes and analyzed using Pico-
Green intercalating dye in a TD-700 fluorometer (Turner
Designs, Sunnyvale, CA, USA). Extracted DNA samples
were PCR-amplified using a Perkin Elmer Thermocycler
and a standard PCR protocol [17]. All amplified samples
were analyzed on the Bio-Analyzer 2100 (Agilent Technol-
ogies, Palo Alto, CA, USA) using the DNA 500 kits accord-
ing to the manufacturer's instruction.

3 Results and discussion

3.1 Extraction using silica beads in a
microchamber

Transfer of the DNA SPE procedure described by Tian et
al. [1] to a microchip is difficult because the beads must
be retained within the chamber. Although complicated
and time-consuming, Oleschuk et al. [13] showed it was
possible to use differential etching to create a structure
within the microchip to retain the particles. To examine
the potential of using such a structure to retain silica
beads for DNA extraction, a microdevice was prepared
having a 30 μm deep inlet and extraction chamber, while
the outlet channel was etched only 12 μm deep. This
formed a weir to prevent loss of the 15 μm silica beads
used to fill the chamber. Although clogging of the inlet
channel often occurred, beads could be successfully
packed as a slurry in a 5% v/v glycerol/water solution
using vacuum. Figures 1a and b are images of a micro-

Figure 1. Solid-phase media in microfabricated chambers. (a) Silica
beads; (b) silica beads + 10 x magnification; (c) base-catalyzed sol-gel;
d) two-step silica beads + sol-gel; (e) two-step silica beads + sol-gel –
10 x magnification.
chamber packed with silica beads. Once packed, extraction efficiencies using these devices were found to be as high as 80%—however, extractions on the same microchip and between microchips were highly irreproducible. This is illustrated in Fig. 2 showing the percent of DNA eluted from consecutive extractions on three microchip devices. The first row of Table 1 presents the mean extraction efficiency and the high coefficient of variance observed in this system. In addition, Fig. 2 shows that only one microchip was still functional after four extractions, and that microchip failed after the fifth extraction. It was observed that as the microchip was used, the beads began to pack more tightly, causing increased backpressure, decreased flow, and varied flow patterns through the system. This led to decreased extraction over time, and was believed to be a main cause of irreproducibility. Eventual destruction of the devices was also observed as the pressure increased to the point at which shattering of the glass microchip substrate occurred. Even given the limitation of performing only a single extraction per microchip, this approach was found to be unsuitable due to the variable performance between microchips, which is believed to result from variations in the amount and arrangement of the silica beads within the packed bed.

### 3.2 Extraction using a sol-gel matrix

To overcome problems associated with the use of silica beads in a weir-controlled microchamber, silica sol-gel structures were created and tested for their DNA extraction ability. The condensation reaction, which produces the final network structure, can be catalyzed by the addition of base. Acid-catalyzed condensations can also be used to form the sol-gel matrix, but these reactions occur more slowly. The simplicity of this approach avoids many of the problems associated with packing particles into the microchip, but the ability of this matrix to extract DNA had to be investigated. Initial efforts to examine this approach for DNA extraction focused on filling microchip chambers with sol-gel, as illustrated in Fig. 1c. However, since the gel matrix cannot be removed from the device once formed, capillary devices provided a more cost-effective vehicle for initial tests. Using capillary devices, two types of TEOS-based sol-gels were investigated—hydrophilic gels, formed by the base catalyzed condensation of the sol, and more hydrophobic gels, modified by addition of propyltrichlorosilane (PTS) during gel formation. As expected, better efficiencies were obtained with the more hydrophilic material, however, neither matrix produced extraction efficiencies comparable to what could be achieved using silica beads in a comparable capillary format [1]. This may be due to the small pore size in the sol-gel matrix (>100 nm), which would inhibit liquid flow. Attempts to increase the size of the pores by the addition of polyethylene glycol to the sol-gel precursor solution did not significantly improve flow in the capillary devices. Table 1, lines 2 and 3 provides the mean extraction efficiencies calculated for capillary devices filled with these two types of sol-gels.

A further disadvantage of the sol-gel matrices was the large coefficients of variance determined for these materials. This very poor extraction reproducibility was seen both within a single capillary segment and also between multiple segments of capillary cut from a long sol-gel filled capillary. One possible explanation for this is related to the stability of the gel. As the sol-gel ages and dries, it forms a xerogel that is not mechanically stable, with cracks forming within the structure as well as between the gel and the capillary/microchamber walls (Fig. 1c). At this point, flow through the matrix increases, but most liquid passes through the cracks with very little of the sur-

<table>
<thead>
<tr>
<th>Resin type</th>
<th>Extraction efficiency (%)</th>
<th>Standard deviation (%)</th>
<th>Coefficient of variance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica beads</td>
<td>57.1</td>
<td>43.1</td>
<td>75.5</td>
</tr>
<tr>
<td>TEOS, PTS sol-gel</td>
<td>19.2</td>
<td>15</td>
<td>78.1</td>
</tr>
<tr>
<td>TEOS, sol-gel</td>
<td>33.2</td>
<td>20.2</td>
<td>60.8</td>
</tr>
<tr>
<td>Acid-catalyzed sol-bead slurry</td>
<td>60.9</td>
<td>12.4</td>
<td>20.4</td>
</tr>
<tr>
<td>Base-catalyzed sol-bead slurry</td>
<td>8.7</td>
<td>5.5</td>
<td>63.2</td>
</tr>
<tr>
<td>Two-step bead-sol system</td>
<td>70.6</td>
<td>2.15</td>
<td>3.05</td>
</tr>
</tbody>
</table>
face area expected to be involved in extraction of the DNA. Acid-catalyzed hydrophilic TEOS gels were created in capillaries and microchips, however, as with the base-catalyzed gels, these gels did not allow liquid flow through the gel matrix except when cracked. Therefore, acid-catalyzed matrices were not investigated for DNA extraction capabilities as they did not solve the problems associated with base-catalyzed gels (cracking, fluid flow).

3.3 Silica bead/sol-gel capillary devices

Silica beads alone are problematic due to compression of the particles during use, and sol-gels are problematic due to poor mechanical stability. It seemed likely, however, that a combination of the two approaches might provide a workable system. This approach has already proven successful in capillary electrochromatography (CEC) where a sol-gel matrix is used to immobilize chromatographic particles with the particles imparting mechanical strength to the gel [18]. To examine the potential of this approach for DNA extraction, 15 μm silica beads were added to the sol-gel precursor mixture to form a slurry. However, a stable suspension was not obtained due to the size of the particles and their proclivity for settling out in the devices before the matrix had completely gelled. In an effort to improve the stability of the suspension, the 15 μm beads were replaced with 5 μm silica particles, which we had determined to yield similar extraction efficiencies (data not shown).

The hybrid sol-gel/silica bead matrices were created in capillaries using either a base-catalyzed or acid-catalyzed condensation step. While it was expected that both methods would perform similarly, it was surprising to find that the acid-catalyzed media performed substantially better than the base-catalyzed material. Extraction efficiencies for the acid-catalyzed matrices were comparable to that found using silica beads alone (Table 1), while the base-catalyzed slurry extracted very poorly. An additional benefit of using the acid catalyzed media was the improved reproducibility over using silica beads alone, with a decrease in the variance from 75 to 20%. The exact reason for the large difference between the acid- and base-catalyzed slurry mixtures is not known, but we believe it is related to the rate of condensation and the subsequent variation in the surface area available for extraction.

3.4 PCR amplification of extracted DNA

With the ultimate goal of this work being to combine SPE, PCR and electrophoretic separation in one device, a major requirement of the SPE process is that the DNA recovered in the extraction procedure can be amplified by PCR. To examine this, amplification of a 500 bp DNA fragment was performed on λ-phage DNA extracted by each of the purification methods discussed above. The results are illustrated in Fig. 3. The 500 bp peak appears in traces corresponding to extraction using silica beads alone, and in both the acid- and base-catalyzed sol-gel slurry mixtures. It is interesting to note the absence of the 500 bp peak in trace B, which corresponds to DNA extracted using the hydrophilic sol-gel filled capillary. Repeated attempts to amplify DNA extracted using this sol-gel matrix gave similar results, while control samples using non extracted λ-phage DNA amplified as expected. It is possible that the lack of amplification in the DNA extracted using the sol-gel media may be related to the presence of ethanol in the sol-gel structure (formed during sol formation) that became trapped in the pores. Since amplification was obtained when using the base catalyzed slurry mixture, however, other explanations are being investigated.

3.5 Silica beads/sol-gel microchip devices

The above results indicate that the best approach to extract DNA for PCR amplification is to use an acid-catalyzed sol-gel precursor solution combined with silica beads. The slurry approach did not transfer well to a microfabricated device, as beads tended to quickly settle out of the slurry and clog access ports during the filling
step. Additional settling occurred as the gelation reaction took place, resulting in a two-phase system and decreasing the reproducibility of the system. This phenomenon has been noticed when using a similar approach in CEC, and has been overcome by using a two step process, in which the particles are first packed into the system and then held together by the sol-gel [19–22]. This method was adapted for use with the microchip devices, with the microfabricated chamber first being packed with 15 \( \mu \)m diameter silica beads, then filled with a silica sol solution (pH 2–4). A microchamber packed with silica beads and gelled sol-gel solution is shown in Figs. 1d and e. A redesign of the microchips allowed a small silica bead/sol-gel frit to be used to contain the beads within the microchamber in place of the weir. Both the frit slurry and the final sol were allowed to gel at 100°C for 30–60 min. The results using this approach, presented in Fig. 4 and Table 1, indicate that slightly higher extraction efficiencies can be obtained compared to silica beads alone, but the reproducibility between extractions and between microchips is greatly improved with an average %RSD of 3.05. In addition, the stability of the system is dramatically increased, with no loss of extraction efficiency as was seen with the silica bead system.

Figure 5 contains a DNA profile for an extraction carried out using the silica bead/sol-gel system. It is clear that all of the DNA is removed from the sample load solution as it passes through the matrix and is retained during the washing step. The DNA is then eluted from the solid phase in a very small volume of elution buffer (> 10 \( \mu \)L), with most of the DNA released immediately. This makes it ideal for integrating with microchip-based PCR, as only a small volume will have to be transferred between areas on the microchip. While the total time for loading, washing and eluting is approximately 24 min in this experiment, it is anticipated that this time can be reduced even further by appropriately optimizing the conditions for each step.

4 Concluding remarks

Various approaches for creating a silica support suitable for SPE of DNA on a microchip have been examined. The simplest approach for packing chromatographic particles provides high extraction efficiency, but poor reproducibility and stability, with prolonged use resulting in destruction of the microchip. An alternative approach of creating a silica network via sol-gel chemistry was found to provide poor extraction and unsuitable reproducibility due to its poor mechanical strength. Furthermore, PCR-amplifiable DNA was not eluted from this solid phase. The most suitable system involved combining both approaches, in a two-step process, where the particles are first packed into a channel and then held into place with sol-gel. This provided excellent efficiency, reproducibility, and stability while also providing PCR-amplifiable DNA. Using this approach the purification of \( \lambda \)-phage DNA was demonstrated on a microchip with a total purification time less than 25 min. Further optimization of the system will investigate shortening the extraction time, increasing the extraction efficiency, and methods to simplify on-line coupling of SPE and PCR in a \( \mu \)-TAS device.

The authors would like to thank Dr. Bouis and Mallinckrodt-Baker for the gift of silica beads, Braden Giordano for assistance with PCR amplification, and acknowledge a grant from the National Cancer Institute (#1 R21 CA78865–01) for financial support of this work.

Received September 25, 2001
5 References