

MICROFLUIDIC DEVICES FOR THE DETECTION OF SEXUALLY TRANSMITTED INFECTIOUS AGENTS IN A URINE-BASED MATRIX

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ABSTRACT

This paper describes work delivered under the Innovation R&D Programme (National Measurement System, UK Department for Business, Innovation and Skills) to develop a microfluidic Lab-on-a-Chip (LOC) platform facilitating nucleic acid-based detection of sexually transmitted infections (STIs) from a urine-based matrix. Optimisation of DNA extraction methodologies, utilising silica-based monoliths on a microfluidic device, enabled direct processing of clinically-relevant large volume samples of artificial urine in reduced time, by maximising sample loading flow rate and minimising chaotropic salts added to drive DNA binding. Extracted DNA was successfully amplified by quantitative polymerase chain reaction (qPCR) demonstrating potential for process integration in LOC formats.

KEYWORDS: DNA extraction, qPCR, sexually transmitted infections, urine

INTRODUCTION

An estimated 1 million new cases of curable bacterial STIs occur daily worldwide [1]. As such, STIs present a major challenge to global healthcare, highlighting the need for development of novel technologies offering rapid point-of-care (POC) detection and diagnosis, to improve disease control and inform therapeutic intervention. Emerging microfluidic LOC analytical technologies offer advanced alternatives to conventional diagnostic methods, as they facilitate automated integration of multiple sample processing and analysis techniques. In addition, the potential for system miniaturisation using microfluidic technologies offers a number of advantages for development of POC platforms, including increased speed of analysis, reduction in sample and reagent volumes used, reduced power consumption compatible with battery operation and minimal user intervention.

For the detection of STIs, urine provides a favourable choice as patient specimen, as it can be easily self-collected and is non-invasive. Urine, however, constitutes a highly diluted sample and has not been widely investigated as a sample matrix for microfluidic LOC analysis. Ideally, little or no prior processing of clinical samples at the bench should be required in order to provide simple and cost-effective POC diagnostics. Here, we present an efficient sample loading methodology for performing DNA extraction directly from an artificial urine (AU) matrix on a microfluidic device supporting successful processing of specimen volumes of up to 1ml, with reduced processing time. This system differs significantly from others reported, since it provides processing of clinically relevant large volumes on a microfluidic device without pre-concentrating the biological matrix [2] or adding buffering reagents [3].

THEORY

This study explores the development of a rapid DNA extraction methodology utilising silica-based monoliths on a microfluidic device. Solid-phase nucleic acid extraction relies on the binding of nucleic acids to a silica support in the presence of a chaotropic salt at $\text{pH} \leq 7.5$; this is below the pK_a of the surface silanol groups and so reduces the negative charge at the surface thereby decreasing electrostatic repulsion and facilitating nucleic acid adsorption. A high capacity solid phase is categorised by a high surface area for binding and several approaches are available to achieve this, including the generation of porous monolithic silica structures or the high density packing of silica beads [4]. Removal of proteins and other contaminating macromolecules is achieved using an alcohol wash and subsequent retrieval of purified nucleic acids from the solid phase is brought about by elution in a low ionic strength medium.

Purification of DNA from biological specimens by solid-phase extraction on microfluidic devices is commonly performed by addition of a chaotropic salt, such as guanidine hydrochloride (GuHCl), prepared in solution, directly to the sample, increasing the total sample volume for loading and hence increasing processing time. Through optimisation of the microfluidic DNA extraction system reported here, novel methodologies have been developed which minimise sample volumes for processing in addition to increasing loading speed, without loss of DNA extraction efficiency.

EXPERIMENTAL

Glass microfluidic devices were manufactured using standard photolithography and wet etching techniques to produce the design shown in Figure 1. Thermally activated silica monoliths were prepared in DNA extraction chambers by curing a mixture of potassium silicate and formamide at 90°C overnight [5]. All DNA extractions were performed using hydrodynamic pumping, allowing sample loading and recovery via inlet and outlet channels. Silica monoliths were activated using 10mM TE buffer (10mM Tris, 1mM EDTA, $\text{pH} 6.7$) for 30 minutes at $5\mu\text{l min}^{-1}$. An AU matrix was prepared in order to assess compatibility of the proposed system with direct analysis of urine specimens [6]. Standard DNA extractions were performed using samples comprising $5\text{ng } \mu\text{l}^{-1}$ human genomic DNA (gDNA) prepared in either water or AU. This was then added to a 5M GuHCl solution prepared in either 10mM TE buffer or AU. The sample was then loaded onto the silica monolith at a flow rate of $2.5\mu\text{l min}^{-1}$ followed by a washing phase with 80% (v/v) isopropanol ($5\mu\text{l min}^{-1}$). Finally, the bound DNA was eluted with water at $1\mu\text{l min}^{-1}$. Throughout the extraction process, $2\mu\text{l}$

fractions were continuously collected and double-stranded DNA (dsDNA) content was quantified using a Quant-iT™ Picogreen® assay [Invitrogen, UK] and FLUOstar Optima Plate Reader [BMG Labtech, UK].

Optimisation of the system to achieve rapid, large volume sample processing was performed through a series of DNA extractions investigating the effects of varying the quantity of 5M GuHCl solution added to the sample prior to loading and also the rate of sample loading onto the monolith. Validation of the proposed system was performed by processing model samples simulating concentrations of patient and pathogen nucleic acids found in clinical urine specimens. For the model sample, 1ml AU was spiked with 25ng human gDNA plus 10^5 copies of a plasmid-based multi-STI pathogen target (pSTI). The pSTI contained cloned sequences from *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium*, providing targets for amplification and detection by qPCR. Following DNA extraction, eluted samples were analysed by both PicoGreen® assay, to determine the total dsDNA content, and by qPCR, to determine plasmid copy number. qPCR was carried out using custom TaqMan® probes and primers [Applied Biosystems, UK]. Thermal cycling was performed on a StepOnePlus™ Real-Time PCR instrument [Applied Biosystems, UK] using an initial denaturation step of 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds and 60°C for 1 minute.

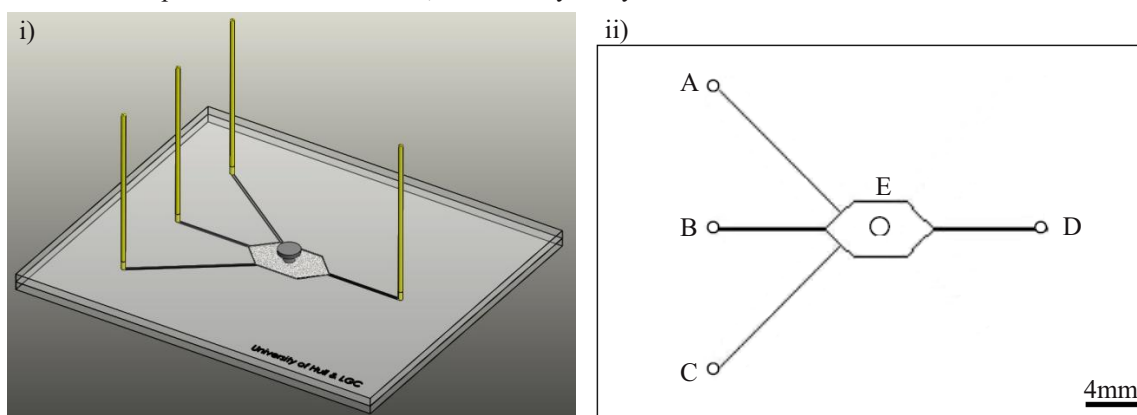


Figure 1: 3D representation (i) and schematic (ii) of the DNA extraction microfluidic device. The DNA extraction chamber was etched to a depth of $100\mu\text{m}$ resulting in a volume of $2.4\mu\text{l}$. Channels were connected, via holes in the top plate, to $360\mu\text{m}$ diameter PEEK tubing enabling hydrodynamic pumping (A-D). A 1mm port was incorporated in the centre of the DNA extraction chamber to permit addition of the solid-phase reagents (E).

RESULTS AND DISCUSSION

For all experiments the efficiency of DNA extraction performed was assessed by calculating the quantity of DNA eluted as a percentage of the quantity of DNA initially loaded onto the system. Investigations comparing DNA extraction using either water or AU as the sample matrix with added 5M GuHCl solution prepared in either TE buffer or AU (1 volume sample : 9 volumes GuHCl) revealed comparable DNA extraction efficiencies (Table 1). The demonstration that DNA extraction efficiency was not adversely affected by using an AU sample matrix suggests the proposed system is compatible for direct processing of clinical urine specimens.

Table 1: DNA extraction efficiencies using various sample/GuHCl matrices ($n=6$).

Sample Matrix	GuHCl matrix	DNA extraction efficiency (%) \pm SD
Water	TE buffer	21.7 ± 7.8
AU	TE buffer	22.0 ± 4.2
AU	AU	21.6 ± 6.2

In order to minimise sample volumes for loading onto the device, experiments were performed using the standard conditions described but varying the volume of biological sample : volume of GuHCl solution (5M in TE buffer). Little variation in DNA extraction efficiency was observed for ratios of 1:9, 1:7, 1:6 and 1:4 (Figure 2). A dramatic reduction in efficiency was found to occur, however, once the ratio was reduced to 1:3, indicating insufficient quantity of chaotrope to achieve successful DNA binding to the silica-based monolith. Subsequent experiments revealed that GuHCl could be directly dissolved in the urine-based sample to a final concentration of 5M without compromising DNA extraction efficiency. Samples were therefore prepared using this technique in all further experiments allowing sample volumes to be minimised by eliminating addition of GuHCl in solution.

Further characterisation of the system was conducted to investigate the effects of increasing the rate of sample loading on to the monolith on DNA extraction efficiency. Experiments were carried out using the standard conditions described but with sample loading flow rates increased from $2.5\mu\text{l min}^{-1}$ to 5, 10 and $25\mu\text{l min}^{-1}$. Flow rates for DNA loading could be increased by up to 4 fold ($10\mu\text{l min}^{-1}$) without apparent reduction in DNA extraction efficiency, permitting more rapid processing of large volume samples. At a flow rate of $25\mu\text{l min}^{-1}$, however, DNA binding to the silica monolith was found to decrease markedly associated with a reduction in DNA extraction efficiency.

Validation experiments using the biological model described above were performed by dissolving GuHCl directly in the simulated urine sample to a final concentration of 5M and loading on to the monolith at $10\mu\text{l min}^{-1}$. Analysis of

extracted DNA by qPCR showed the DNA to be of sufficient quantity and quality for successful amplification to be achieved (Figure 3). In addition, these findings suggest that AU components potentially co-purified with the extracted DNA do not inhibit downstream analysis by qPCR. Comparison of results from the PicoGreen™ assay of total dsDNA and pSTI plasmid detection by qPCR showed co-elution of human gDNA with pSTI (Figure 3). Furthermore, studies varying the amount of human gDNA (25-50ng) and pSTI (10,000-100,000 plasmid copies) contained in the model sample did not affect the ability of the system to extract DNA suitable for analysis by qPCR (results not shown).

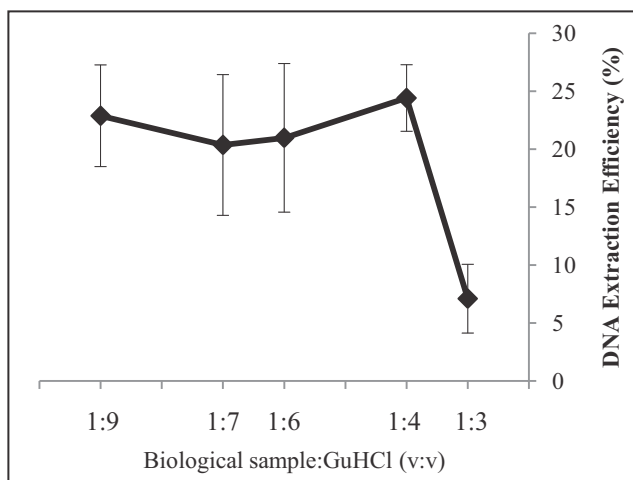


Figure 2: Effects of varying the amount of 5M GuHCl solution added to the biological sample on DNA extraction efficiency (n=6).

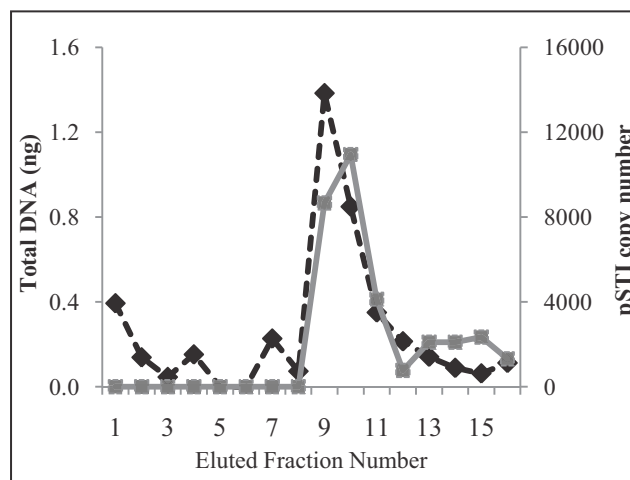


Figure 3: A typical DNA extraction profile illustrating co-elution of host (total dsDNA [-◆-]) and pathogen DNA (pSTI copy number as determined by qPCR [-■-]) from the microfluidic system.

CONCLUSIONS

A novel method for DNA extraction on a microfluidic device directly from a urine-based sample has been demonstrated, addressing issues such as requirements for processing large volume samples and speed of analysis, critical for the development of a POC diagnostic platform. Large sample volumes (1ml) can be processed using the microfluidic system described whilst sample loading times have been reduced. The proposed system successfully accommodated variations in both host and pathogen DNA quantity as occurs in clinical samples, where typical human gDNA concentrations range from 14-200ng/ml in females and 4-60ng/ml in males [7]. Since the extracted DNA was shown to be suitable for PCR amplification, there is potential for the methodology described here to be integrated with downstream processes, such as qPCR or isothermal amplification, in a single microfluidic device. Such a device could offer a POC platform for STI detection in a clinical setting enabling sample analysis, diagnosis and treatment in a single visit.

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