INTEGRATED DNA PURIFICATION AND AMPLIFICATION USING FTA® PAPER AND PCR REAGENT ENCAPSULATION

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ABSTRACT

This paper reports the development of a combined microfluidic system for purification and amplification of DNA from FTA® paper. Using FTA® paper eradicates the need to elute nucleic acids from a solid-phase matrix prior to downstream processing, enabling both purification and amplification to be easily performed in a single chamber on the microfluidic device. Following optimization of the methodology and associated control systems, successful amplification of the D21 S11 locus from human buccal swab samples stored on FTA® paper was achieved. Such a system offers advantages in terms of a simple sample introduction interface and the ability to process archived samples in an integrated microfluidic environment with minimal risk of contamination.

KEYWORDS

Archived sample processing, Real-world interface, FTA® paper, DNA purification, DNA amplification.

INTRODUCTION

The use of FTA® paper, which contains chemicals for cell lysis and protein denaturation, is commonly used for the long-term storage of biological samples, e.g. forensic casework. Such paper also protects nucleic acids from oxidative and UV damage, thus enabling long-term storage of biological samples [1].

For many years, microfluidic devices have enabled processes associated with genetic analysis to be performed, but it has only been relatively recently that such developments have started to focus on the integration of processes within a single device rather than a more modular approach. Both nucleic acid purification and amplification using the polymerase chain reaction (PCR) are often at the core of such genetic analyses. Challenges associated with the integration of these techniques include reagent compatibility and confinement of any solid-phase matrix utilised.

Inclusion of FTA® paper within a microfluidic system has been reported only once, where the FTA® paper was used in filtration mode, to accommodate relatively large sample volumes, for the detection of HIV-1 from oral fluids with subsequent reverse transcription – loop mediated isothermal amplification [2]. This work differs in that it uses the FTA® paper in its conventional form allowing the analysis of archived biological samples to be carried out. In addition the use of electro-osmotic flow (EOF) and wax-encapsulated PCR-based DNA amplification reagents provides a reduced foot print and reduced operational complexity for the device.

The development of integrated microfluidic systems for genetic analysis is detailed in an excellent review by Njoroge *et al.*[3]. While it is clear that significant advances have been made, one aspect of the methology which has yet to be fully addressed is the development of robust sample introduction methods to enable the easy introduction of biological samples into a microfluidic devices for analysis, the so-called "real-world interface". The work presented here exploits the use of FTA® paper as both a means of simple sample introduction and as a conduit for the analysis of archived biological samples.

EXPERIMENTAL

Glass microfluidic devices were produced using standard photolithography and wet-etching techniques based on the design shown in Figure 1a. Silanisation of the PCR chamber was performed using trichloro-(1*H*,1*H*,2*H*,2*H*-perfluorooctyl)silane in order to minimise DNA polymerase adsorption.

Human genomic DNA samples were collected using Omni Swabs [Whatman, UK] which were scrapped along the inside cheek of volunteers. Simulated archived biological samples were produced by depositing the buccal swab samples onto FTA® paper [Whatman, UK] while still wet and allowing to air dry for a minimum of 1 hour before being placed in a desiccated storage environment until required. The proprietary chemicals within the paper used to achieve this high degree of protection have inhibitory effects on DNA amplification processes and so require removal prior to downstream analysis.

PCR reagent storage on the microfluidic device way facilitated by adding 1.5 µL of a 10 x concentrated PCR reagent solution to the recess in central chamber, enabling the correct working concentrations (1x GoTaq® buffer, 2 mM $MgCl_2$, 1 unit GoTaq® Hot Start DNA polymerase, 10 mg ml^{-1} bovine serum albumin, 0.01% (w/v) poly(vinylpyrrolidone), 0.1% (v/v) Tween-20, 200 µM each deoxyribonucleotide triphosphates and 0.1 µM D21 S11 forward and reverse primers) to be achieved upon release and mixing with the TE buffer. The PCR reagents were then covered in a thin layer, approximately 150 µm, of low melting temperature eicosane wax.

In order to perform DNA purification on the microfluidic system, the etched channels and chambers were filled with FTA® purification reagent [Whatman, UK]. Next, additional FTA® purification reagent and TE buffer (10 mM Tris, 0.1 mM EDTA, adjusted to pH 8.0) were loaded into the appropriate wells as shown in Figure 1a. A 2 mm disc was removed from the FTA® paper using a Micro-Punch [Harris, UK] and placed within the central chamber of the microfluidic device (Figure 1b). Platinum electrodes, connected to an external Paragon 3B Power Supply Unit

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[Kingfield Electronics, UK], were then placed into the reagent and waste wells in order to facilitate EOF.

Following removal of inhibitors, thermal cycling was carried out using a thermoelectric Peltier element, which provided both the heating and cooling required. The following program was used: initial denaturation at 94 °C for 5 minutes, 35 cycles of 94 °C for 30 seconds, 60 °C for 30 seconds and 72 °C for 30 seconds, with a final extension step of 60 °C for 7 minutes. Amplified DNA samples were subsequently analysed off-chip by capillary gel electrophoresis using a 3500 Genetic Analyzer [Applied Biosystems, UK].

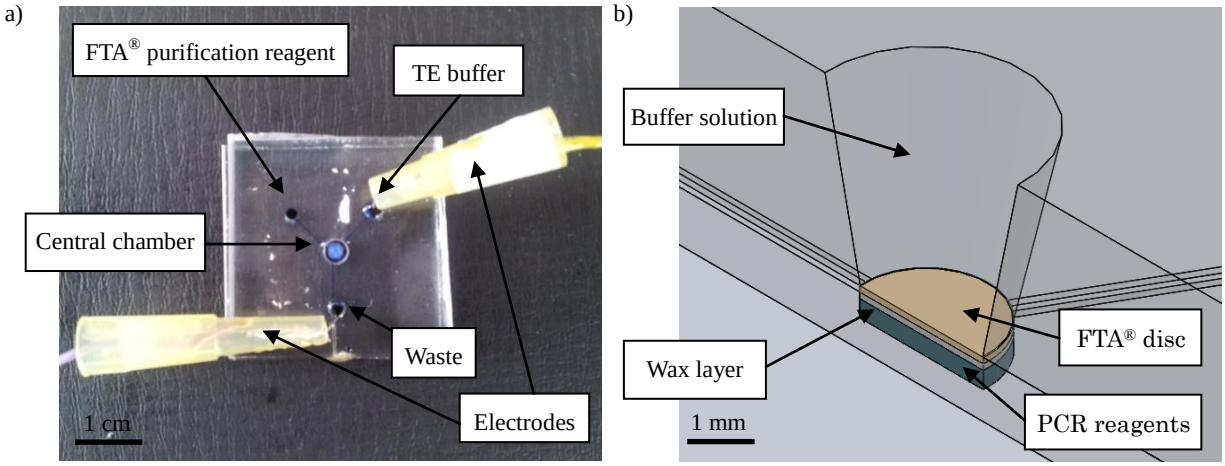

Figure 1: *a) Photograph showing the microfluidic device used to perform integrated DNA extraction and amplification experiments. The channels connecting the buffer wells to the central chamber in the 1 mm base plate were etched to a depth of 100 µm and the central chamber drilled to a total depth of 600 µm. Access ports (1.5 mm diameter) were drilled in the 3 mm top plate to accommodate reagents and electrodes for EOF. The central chamber inlet was tapered to facilitate insertion of the FTA® paper disc; b) Schematic cross-section showing how the FTA® paper discs are placed in the central chamber on top of a layer of wax encapsulated PCR reagents.*

RESULTS & DISCUSSION

Preliminary experiments demonstrated the feasibility of manipulating the reagents for DNA purification using EOF. A detailed evaluation showed that the average mobility due to EOF for the FTA® purification reagent and TE buffer were 7.9 x 10^{-6} cm^2 V^{-1} sec^{-1} and 1.1 x 10^{-5} cm^2 V^{-1} sec^{-1}, respectively.

In the conventional FTA® purification procedure, a drying step is included following washing with TE buffer, prior to addition of DNA amplification reagents. The introduction of a drying step in a microfluidic system can be problematic as air bubbles can arise, causing issues with uneven heat and reagent distribution for subsequent steps in the process. In particular, for the methodology proposed here, the removal of the drying step would allow a continuous liquid phase to be maintained throughout the procedure enabling EOF to be utilized. It was demonstrated that the drying step could be eliminated with no significant difference being observed (One-Way Analysis of Variance at a 0.05 significance level) between the mean and variance from samples which did and did not undergo a drying step. It was found that by omitting the drying step the microfluidic procedure could be dramatically simplified.

Using the EOF mobilities determined previously it was possible to calculate the optimum time required to flow the solutions over the disc at a given voltage, ranging from 50 V cm^{-1} to 150 V cm^{-1}, to ensure removal of potential PCR inhibitors but also to maintain DNA on the membrane. Following EOF in the presence of either the FTA® purification reagent or TE buffer the amount of DNA present at both the anode and cathode was quantified in order to determine the optimum applied voltage for both reagents. It was demonstrated for both the FTA® purification reagent and TE buffer that no detectable DNA, based on the limit of detection of the PicoGreen® assay of 250 pg mL^{-1}, was removed from the FTA® disc at any of the applied voltages. No quantifiable DNA was detected at either the anode or cathode wells, indicating that the DNA on the FTA® disc suffered no adverse effects from either EOF or electrophoresis. In addition, the FTA® discs were subjected to conventional DNA amplification following washing. Evaluation of the relative PCR efficiency, established by capillary electrophoresis, showed a slight increase in fluorescence signal intensity at the higher voltages (Figure 2). Therefore, an applied voltage of 150 V cm^{-1} was chosen for washing which enabled a 10 x bed volume to be completed within 77 seconds for each solution.

Successful encapsulation of concentrated PCR reagents enabled both DNA purification and amplification to be performed in a single chamber on the microfluidic device. PCR reagents were located in the recess of the central chamber under a layer of eicosane. The FTA® disc was placed on top of the wax layer, in plane with the microfluidic channels, allowing washing of the FTA® disc with both FTA® purification reagent and TE buffer. The PCR reagents were released during the initial denaturing step due to melting of the eicosane layer and dissolution of the DNA amplification reagents in the TE buffer. After thermal cycling, the PCR products were analysed by conventional capillary gel electrophoresis which confirmed that successful DNA amplification had taken place (Figure 3).

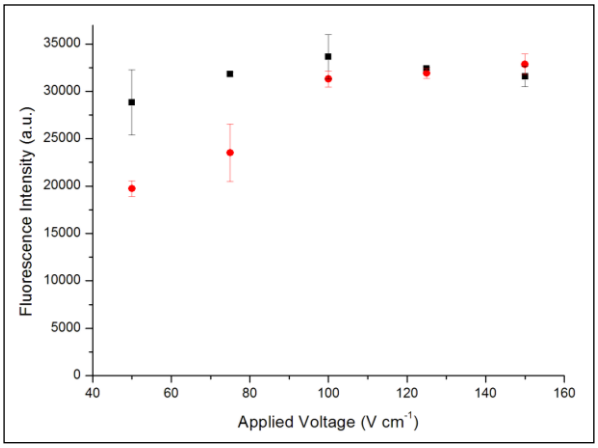

Figure 2: *Graph showing relative fluorescence intensity of the PCR products produced when FTA® discs were washed with FTA® purification reagent (■) and TE buffer (●) at different applied voltages (n=3).*

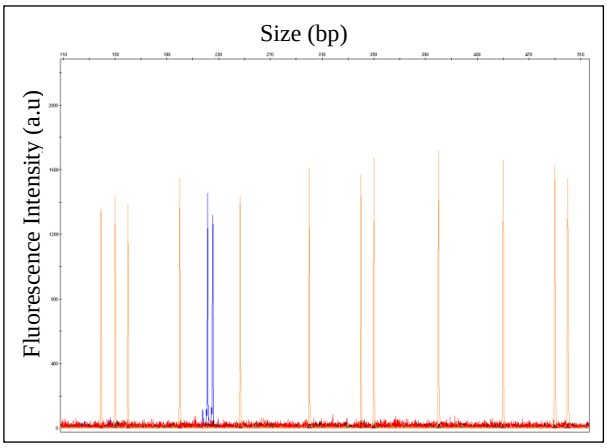

Figure 3: *Electropherogram showing PCR products from the amplification of the D21 S11 locus, using the microfluidic system for integrated DNA purification and amplification, as confirmed on an ABi3500 Genetic Analyser using a GeneScan™ 500 LIZ® Size Standard (n=3)*

CONCLUSIONS

The work presented here demonstrates the successful integration of DNA purification and amplification processes on a single microfluidic device. Direct inclusion of FTA® discs within the system provides a simple "real-world interface" that exploits the Micro-Punch to facilitate addition of the biological samples into the microfluidic device. The inherent advantages of using FTA® paper also make the proposed system ideal for the analysis of archived biological samples. Using EOF in place of more traditional hydrodynamic pumping mechanisms eliminates any moving parts from the reagent transport mechanism and thus simplifies the complexity of design and footprint of the overall microfluidic system. By combining both DNA purification and amplification techniques on a single device the risk of sample contamination was greatly reduced.

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