NUCLEIC ACID PURIFICATION ON A LAB-ON-A-DISC WITH TIME-CONTROLLED INCUBATION

David J Kinahan, Lourdes AN Julius, Mark Tutty, Faith Mangwanya, Rohit Mishra, Damien King and Jens Ducrée**
School of Physical Sciences, Dublin City University, IRELAND

ABSTRACT

Here we present integrated Lab-on-a-Disc (LoaD) cartridges which are applied to the purification of nucleic acid using the silica bead based method. We utilize a novel combination of 'event-triggered' dissolvable film (DF) valving [1] and a centrifugo-pneumatic siphon valve (CPSV) [2] to permit timing of sample incubations, washes and DNA elution. We present two systems; one in with a conventional 'wash through' elution which achieves a purification yield of 32.3% ± 5.4% (n = 4). The second system, which uses a CPSV to enable extended wash and elution steps, provides an increased efficiency of 58.4 ± 7.5% (n = 3).

KEYWORDS

Lab on a Disc; DNA Purification; Dissolvable Films; Event-triggered valving.

INTRODUCTION

The centrifugal microfluidic platform [3] has been widely used for integrating and automating a range of bioanalytical protocols in a sample-to-answer fashion for applications in biomedical point-of-care diagnostics, cell analysis, environmental monitoring and quality control of industrial processes. The LoaD platform comprises of a disposable cartridge, typically of a similar geometry as optical data storage media (e.g. CD or DVD), on which reagents are stored and Laboratory Unity Operations (LUOs) are implemented through the interplay of the programmable spin rate with microfluidic structures.

The LoaD platform offers a number of advantages for use at point-of-care or point-of-use. The use of the centrifugal force, via a low-cost spindle motor, for pumping and LUOs such as metering, aliquoting and mixing eliminates the need for costly pumps and their error-prone fluidic interfaces. The availability of the centrifugal field also makes the LoaD platform well compatible with various bench-top methods, e.g., for plasma separation. The system also has offers the benefit that it can be loaded and operated while open to atmosphere; a simple disposable syrette can be used to introduce sample as opposed to a cumbersome 'world-to-chip' interfacing based on liquid connectors.

Th key enabling technology for higher-level process automation on the LoaD platform is the valving. Most valves are either externally actuated, for instance by an instrumentation-based IR laser, or rotationally controlled using the interaction between the centrifugal field and the capillary force.

Nucleic acid purification is an important technology area for LoaD with its diverse applications spanning cancer diagnostics and food quality monitoring. Silica-bead based purification, where the sample is incubated with beads, washed and then DNA eluted from the beads, is particularly popular in microfluidics systems.

As the common, normally-closed valving techniques on the centrifugal platform are not reversible, most groups follow a 'flow-through' strategy [4]. Often, the deficiencies of this approach can be compensated by presenting results after subsequent on-disc DNA amplification (i.e. LAMP). Others perform DNA purification by incubating the reagents with beads for an arbitrary time; however, these platforms are based on complex ancillary instrumentation [5]. Recently, CPSVs manufactured with high precision have shown potential towards sample incubation and routing; however, they have not been applied to nucleic acid purification [6].

In this work we present two silica-bead based DNA purification systems (Figure 1), both of which leverage our dissolvable-film (DF) valving technology, and compare their performances. The first concept uses a 'flow-through architecture' utilising rotationally actuated pulse valves [7] to trigger release of reagents. The time the reagents are in contact with the silica beads scales with the inverse of their flow rate, which, in turn, depends on the spin rate and the hydraulic resistance of the conduit. As these conditions are bound by manufacturing and operational aspects, the resulting incubation time cannot easily be varied.

The second method for 'timing incubation' employs event-triggered DF valves to control reagent release; in addition, a centrifugo-pneumatic siphon valve (CPSV) controls the transfer of reagents through a bead-filled incubation chamber. The siphon thus keeps reagents in contact with the beads for user-defined intervals and prevents the release of subsequent reagents until the liquid is passed through to waste via a reduction in the (programmable) spin rate.

We compare these two approaches by processing a DNA standard through this disc. First our DNA standard is incubated with silica beads, then washed with concentrated ethanol (75% EtOH) to remove chaotropic salts, and finally eluted from the silica beads using buffer adapted from a commercial kit. By comparing the fluorescence of processed to the initially loaded DNA samples, we estimate the recovery efficiency of out methods.

MATERIALS AND METHODS

Disc Manufacture

The microfluidic cartridges (Fig. 1a) used in this study were assembled using multi-lamination methods from four layers of Poly(methyl methacrylate) (PMMA) and four layers of PSA (Pressure Sensitive Adhesive, Adhesives Research, Limerick, Ireland). Voids in the 86-µm thick PSA layers representing microchannels and other small features were defined by a commercial knife-cutter

(Graphtec, Yokohama, Japan). Larger features such as reservoirs for reagent storage and sample collection were created in 1.5-mm thick PMMA layers using a laser cutter (Epilog Zing, USA). These layers were aligned on a custom assembly jig. Between each alignment step, the attachment of layers was reinforced using a hot-roll laminator (Hot Roll Laminator, Chemsultant Int., US). Each DF was mounted on a PSA tab using previously described methods and then manually positioned within the disc during manufacture.

Experimental Test Stand

All discs in this study were imaged using a test and development tool commonly referred to as a "spin stand". Discs are mounted on a computer controlled motor (FESTO, Germany) and spun at user-defined rates (Fig. 1) using a custom control program (LabVIEW, National Instruments, USA). The discs were imaged by a stroboscopic light source (Drelloscop 3244, Drello, Germany) and a sensitive, short-exposure time camera (Pixelfly, PCO, Germany) synchronous with the motor. The motor generates one digital pulse per revolution upon passing a software-selectable angle.

For the wash, the disc was spun at 30 Hz and valves were actuated by pulsing to 70 Hz for 12 seconds. For the time-controlled strategy, incubations occurred by rapidly acceleration and decelerating the disc between 50 Hz and 30 Hz. The valve was primed by decelerating (at 1 Hz s^{-1}) to 10 Hz while the siphon primed and the chamber emptied. All other acceleration and deceleration was implemented at 12.5 Hz s^{-1}.

DNA Purification and Quantification

The DNA purification disc uses reagents adapted from QIAquick PCR Purification kit (Qiagen, Hilden, Germany); the extracted DNA in the eluate and was quantified using a Quant-iT™ PicoGreen® dsDNA (ThermoFisher). First, the Lamda DNA standard provided with the PicoGreen® kit was diluted to a concentration of 2 µg mL^{-1} using the method and buffer provided (TE Buffer). A working solution of Quant-iT™ PicoGreen® reagent is also created as per supplier's protocol.

DNA sample is first prepared by dilution the 2 µg mL^{-1} DNA sample 5:1 using buffer PB (provided in the QIAquick kit). PE Buffer is also prepared (by dilution with Ethanol (EtOH) according to the kit instructions) as wash reagent. Finally, EB buffer (again provided in the QIAquick kit) is used for elution. A 200-µl aliquot of each buffer is loaded onto each of the disc.

For quantification, 30 µl of the sample recovered disc and from the reference DNA (2 µg mL^{-1} which is further diluted in buffer 5:1 to match the dilution of the original sample by buffer PB) are each added to 30 µl of the working solution of Quant-iT™ PicoGreen®. Fluorescence is measured using a commercial plate reader (Tecan) per the Quant-iT protocol and, for reporting, fluorescent signal is normalised relative to the reference (2 µg mL^{-1} DNA) signal.

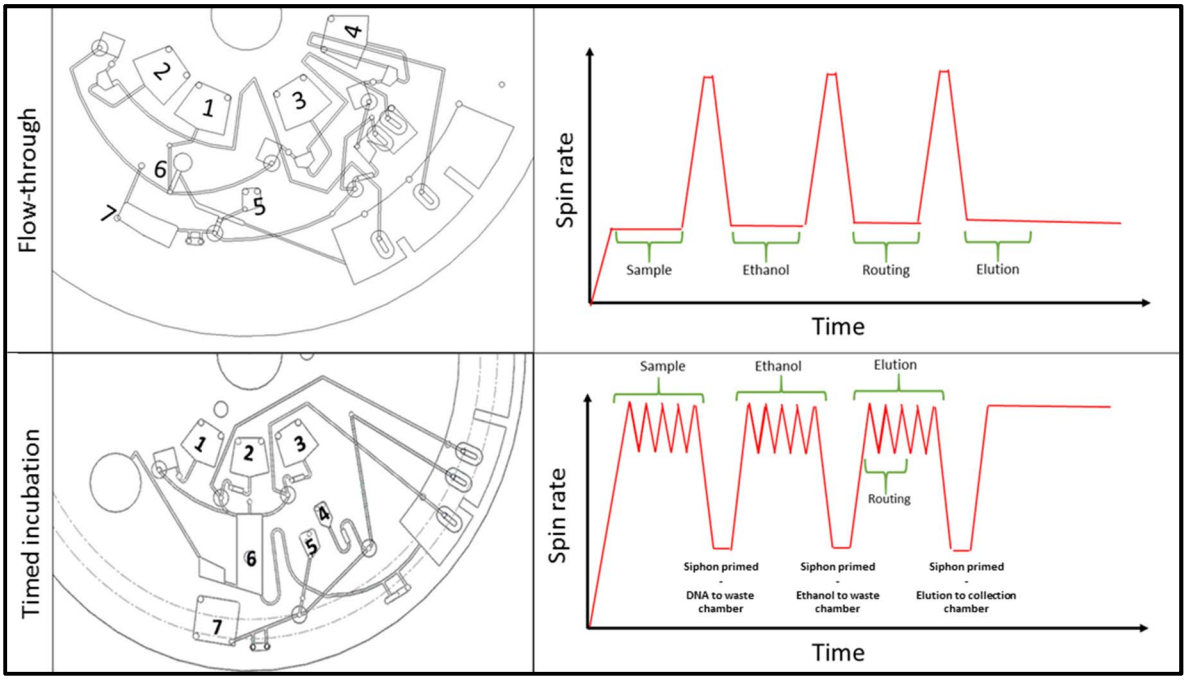

Figure 1: Disc geometries presented with their respective spin protocols. In the 'flow-through' architecture the release of reagents is controlled by pulses in spin rate; however, the time the reagents are contact with the beads is statically defined by the hydraulic resistance and thus turns out to be less reproducible. In the 'timed incubation' architecture, sample / wash / elution buffers are mix with the beads; the transfer in induced by the user programmable reduction of the rotational frequency via a CPSV. (1) sample reservoir, (2) wash reservoir, (3) elution buffer, (4) routing liquid, (5) FC-40 loading port, (6) bead chamber and (7) collection chamber.

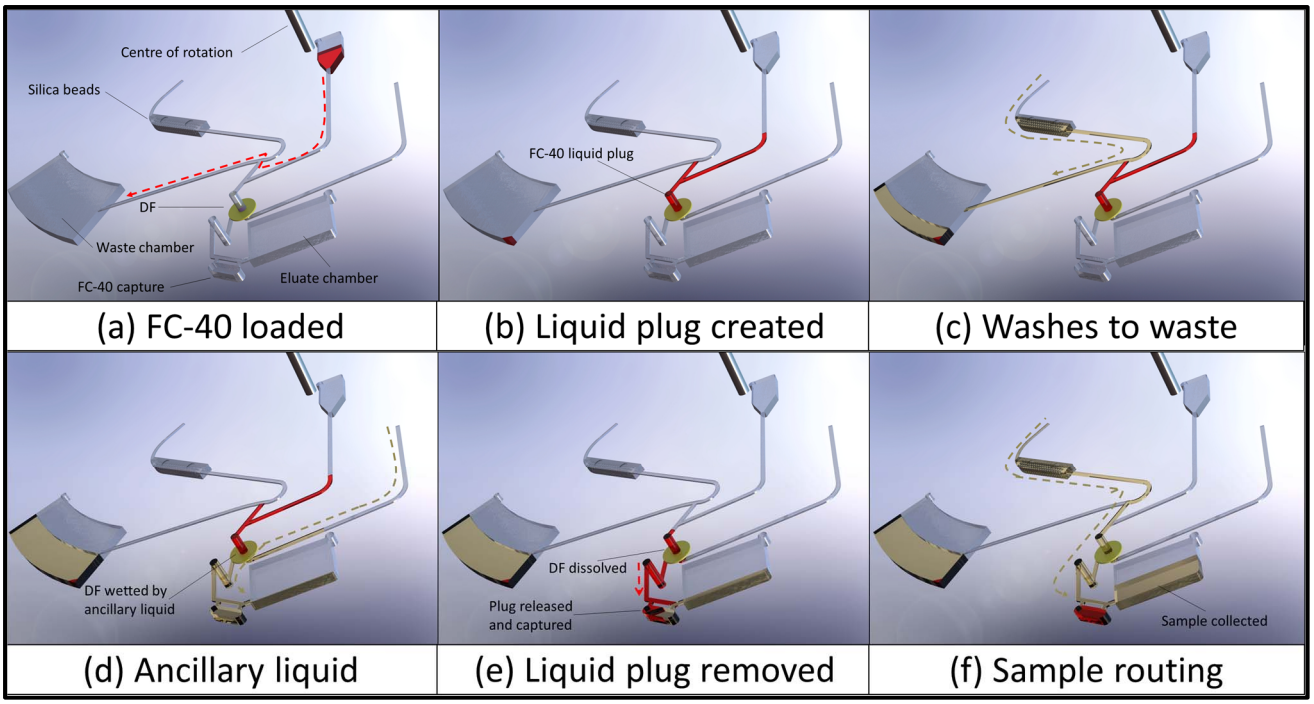

Figure 2: Operation of the routing mechanism. (a-b) The immiscible, high-density liquid FC-40 forms a plug to prevent wetting of the DF. (c) Sample and washes are routed past the immiscible plug to waste. (d-e) An ancillary liquid opens the route to the collection chamber from the 'under side' of the DF. (f) the sample can now be routed to the collection chamber.

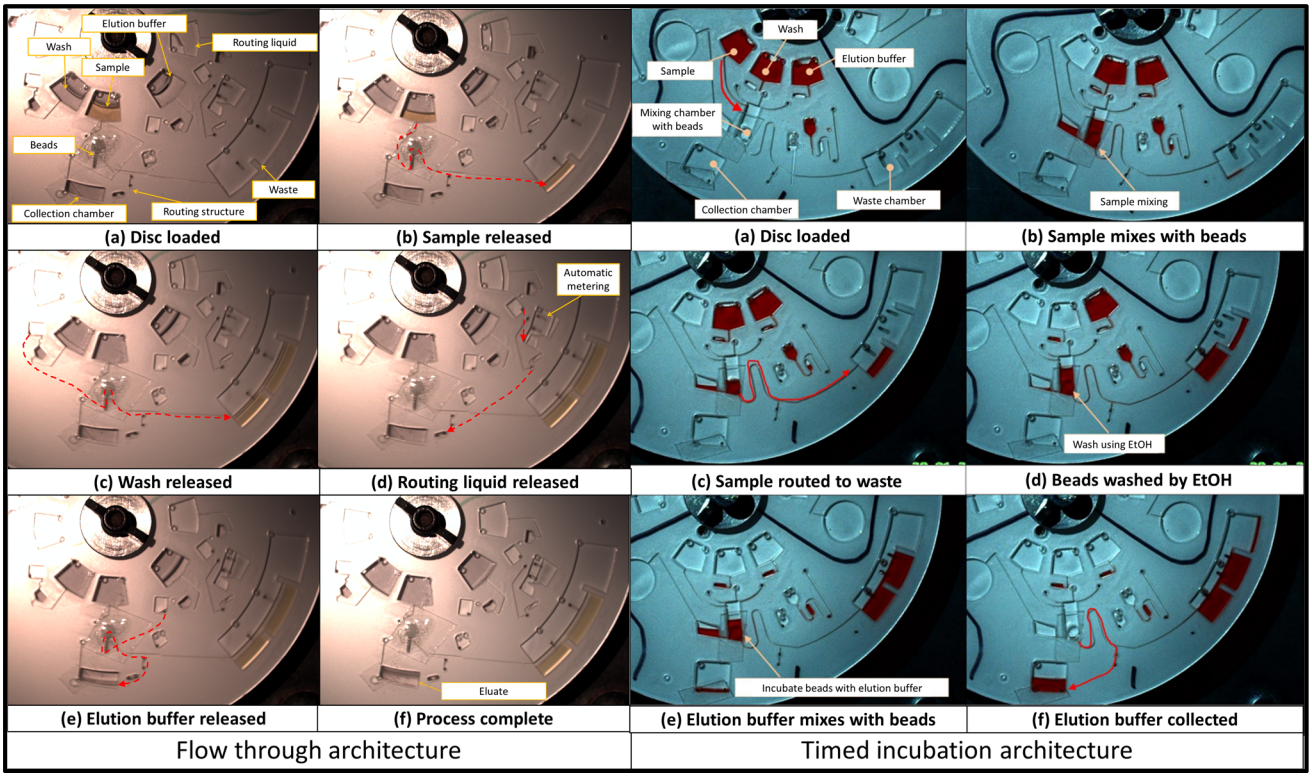

Figure 3: Frame sequences displaying the operation of the two disc architectures. For the 'flow-through architecture' actual reagents are shown while for the 'timed incubation' structure liquid handling is visualised by food dyes.

DISC OPERATION AND PERFORMANCE

A schematic of the disc architectures and the associate spin protocols is shown in Figure 1, the routing is shown in Figure 2 and a frame sequence, for both disc designs, is shown in Figure 3. The disc is manufactured from eight layers of PMMA and PSA (as described previously) and the DFs are selected to be compatible with the reagents which are loaded in each chamber. Once the disc starts rotating on the 'flow-through' disc, an FC-40 plug is created (Fig. 2) and, simultaneously, the DNA sample is pumped through the bead column into the waste chamber. The disc architecture is designed such that reagents are routed through the packed column of beads from the radial

end of the column to the inner column; this centripetal flow ensures that the beads are sufficiently wetted. Upon reaching the waste chamber, the DNA sample wets and thus dissolves the DF to put the subsequent valve in a 'live state'; thus, on the upward rotational pulse, the next valve in sequence containing the wash (PE Buffer) is opened. This process continues for the ancillary liquid, which opens a route to the collection chamber, and the elution buffer. Note that an automated metering structure ensures that the volume of ancillary liquid is both, as reproducible and small as possible.

For the 'timed incubation' variant, the disc is accelerated to 50 Hz and again, an FC-40 plug is formed to ensure that the DNA sample and PE buffer are both routed to the waste chamber. The sample flows into the incubation chamber, where it is transiently retained by a CPSV. Due to the high spin rate, centrifugal pressure displaces some liquid into the dead end pneumatic chamber (which is a constituent part of the CPSV). By rapidly cycling the spin rate between 50 Hz to 30 Hz, part of this pneumatic chamber can be alternatingly emptied and refilled; this pneumatically enhanced 'shake mode' mixing ensures that the DNA sample and the silica beads interact efficiently. After an incubation time defined by programming the spindle motor, the frequency of rotation is lowered to 10 Hz. The resulting decrease in centrifugally induced pressure expels liquid from the dead end pneumatic chamber, raising the filling level in the incubation chamber above the crest of the siphon and thus priming the siphon of the CPSV. Next, the spin rate is elevated so the content of the incubation chamber is emptied into the waste chamber and triggers the release of the PE Buffer wash. In turn, this wash is incubated with the beads and passed through to the waste chamber.

This procedure releases the elution buffer, which, again, can be incubated with the bead substrate. Siflmultaneously, an ancillary liquid is released which removes the FC-40 plug blocking the route to the sample collection chamber. Consequently, upon deceleration of the disc, the elution buffer is preferentially routed away from the waste chamber to the sample collection chamber.

The discs used in this study were characterised in two rounds of experiments using the same protocols. The 'flow-through' architecture was found to have a purification efficiency of 32.3% ± 5.4% (n = 4) normalised relative to the DNA standard (100 ± 1.3%, n = 3). We measured an extraction efficiency of 58.4 ± 7.5% (n = 3) normalised relative to the DNA standard (100% ± 7.2, n = 3) for the 'timed incubation' disc. During these experiments, we also characterised the performance of commercial Qiagen spin columns, providing an efficiency of 84.7 ± 4.2% (n = 3); we thus reach 70% of this gold standard method.

CONCLUSION

Both the disc architecture presented in this work were proved to integrate and automate DNA purification at reasonable efficiency. The CPSV enabled 'timed incubation' offered superior performance as the sample / wash / elution buffers remained in contact with the silica beads for extended periods. an incubation time of 5 minutes was chosen arbitrarily and may be adjusted for further opti-misation.

However, while it exhibited superior purification efficiency, the CPSV enabled system suffered from reduced reliability compared with the 'wash through' architecture attributed to the interaction of the siphon and the routing structure.

It is evident that further advances are possible. Improving the manufacturing method (i.e., using high fidelity milling rather than our xurography based manufacture) could improve the reliability of the CPSV enabled system. Furthermore, as the Qiagen spin columns outperform both systems, using their membrane rather than silica beads could also lead to performance improvement.

As a next step, we intend to integrate mixing with LAMP reagent and spatial multiplexing towards sample-to-answer detection of DNA based pathogens.

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CONTACT

*David Kinahan; david.kinahan@dcu.ie
*Jens Ducrée; jens.ducree@dcu.ie