SOLVENT-SELECTIVE MEMBRANES FOR AUTOMATING SEQUENTIAL LIQUID RELEASE AND ROUTING OF NUCLEIC ACID PURIFICATION PROTOCOLS ON A SIMPLE SPINDLE MOTOR

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ABSTRACT

By incorporating a set of membranes which selectively dissolve upon contact with aqueous or organic solvents at strategic locations on a disc cartridge, we succeeded to fully automate the solid-phase extraction of nucleic acids by varying the spin rate of a low-cost spindle motor. A solvent- and phase-selective graphene oxide (GO) membrane [1] governs the routing of flows to designated elution and waste chambers. The serial release of on-board sample and reagents is centrifugo-pneumatically controlled by our previously introduced, event-triggered valving scheme [2]. The entire process of extraction completes in less than 8 minutes.

KEYWORDS: Graphene oxide membranes, Automation, Nucleic acid purification.

INTRODUCTION

Centrifugal microfluidic platforms can offer significant benefits for nucleic acid testing, in particular the highly-labour intensive process of nucleic acid purification can be incorporated and integrated into these systems. In order to allow for more condensed handling of fluids, new and more dynamic materials can offer diverse benefits for flow control. As previously shown graphene oxide (GO) membranes allow aqueous solutions to pass with very little flow resistance but are completely resistant to organic solutions and air [1]. This holds true even at significantly high pressure heads. Building on previous work [3], we show the development of a fully automated system incorporating a GO router which utilises an event-triggered [2] flow control element and incorporates multiple functional materials to enhance the flow control capabilities of this system. The work described here shows the development of an automated Event-trigger Graphene Oxide Router (EGOR) and uses it to purify DNA.

EXPERIMENTAL

The architecture of our EGOR system (Fig. 1) features three loading reservoirs, connected via a channel to the pneumatic chamber. Through a combination with an adhesive film, a GO membrane is placed within the siphon exhibiting an open outlet to a waste while the vertical passage to the collection chamber is only permitted for the aqueous DNA elution at the elevated spin frequencies applied in the final phase of the extraction protocol [1].The loading chambers for the organic (L_O) and aqueous (L_E) solution are at first sealed by films selectively dissolving in organic (ODF) and aqueous elution buffers (ADF), respectively. Each of these chambers is air locked by a channel connected to the waste chamber, which are sealed by another ADF and ODF tab to initially retain the liquids in their corresponding loading reservoirs.

Figure 2 shows the working principle of sequential routing of aqueous and organic solutions. Under centrifugation, the angular orientation and position of the siphon valve directs liquid into the pneumatic chamber where it traps and compresses the enclosed air pocket. Upon reduction of the spin rate, the air expands to centripetally pump liquid past the crest point of the siphon. The first aqueous solution triggers the release of the second organic solution by the dissolution of the ADF in the waste chamber. Triggered by the arrival of the organic solution to open the ADF, the aqueous elution buffer is released and penetrates at elevated spin rate through the solvent-selective GO membrane within the vertical via into the collection chamber.

RESULTS AND DISCUSSION

The capability of the EGOR system to purify DNA was analysed against the gold standard spin column method (Fig. 3). The EGOR system was capable of purifying the DNA consistently, with an efficiency of ~10% versus the gold standard method.

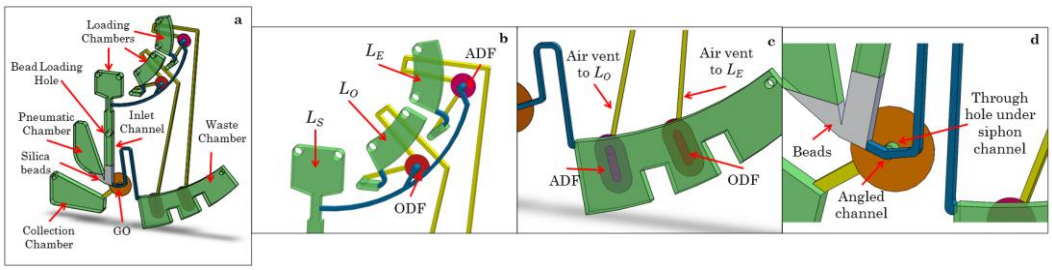

Figure 1: a) Design of EGOR system and its functional components. b) Three different loading chambers, and tab placements. c) Waste chamber, showing the position of another ADF and ODF tab, also the airtight channels connecting to the loading chambers. d) Silica bead's and GO tab's positions.

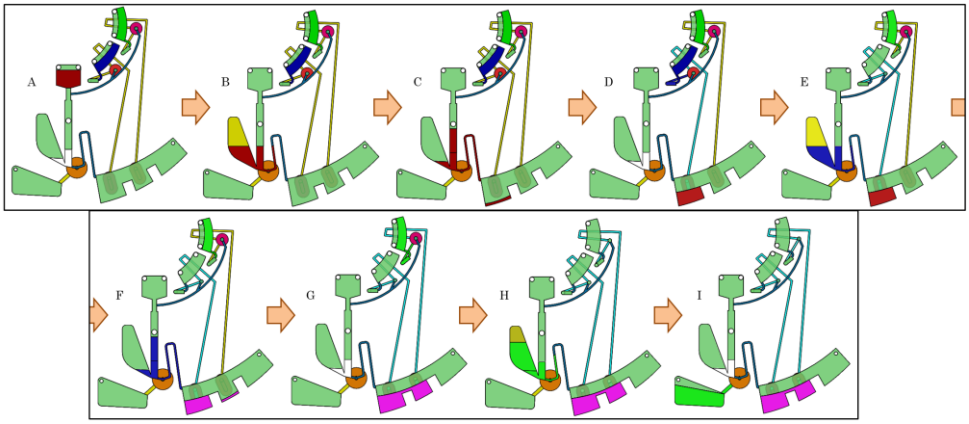

Figure 2: Working principle of the EGOR. A-C) At a high frequency, the sample (red) compresses the air within the pneumatic chamber (yellow). Upon deceleration, the entrapped air expands to pump the fluid over the siphon to waste. D-F) The dissolution of the first film in the waste chamber triggers the flow of the organic wash buffer. It is also routed to the waste chamber. G-I) In the final stage, the dissolution of the second film in the waste chamber triggers the flow of the elution buffer. The disc is spun at a higher frequency to overcome the burst frequency of the GO tab. The elution buffer is directed to the left collection chamber.

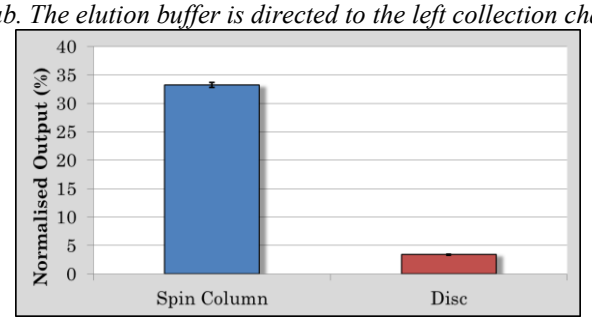

Figure 3: Comparison of normalized DNA signal from SPP using benchtop spin column setup and EGOR. 33.3% of the DNA was collected from the spin column versus the 3.4% from the disc.

CONCLUSION

While the merely rotationally automated extraction of nucleic acids with on-board stored sample and reagents through a set of disc-integrated functional membranes proved to be very robust, future work will address the optimization of the extraction efficiency.

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