

EVALUATION OF MICROFLUIDIC DNA EXTRACTION SYSTEM USING A NANOPOROUS ALUMINUM OXIDE MEMBRANE

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ABSTRACT

A nanoporous membrane was integrated into a microfluidic system designed for extraction of gDNA (genomic DNA) from lysed whole blood. The effectiveness of this system was tested by passing known concentrations of gDNA through nanoporous membranes of varying sizes and measuring the amount of gDNA deposited on the membrane as related to varying salt concentrations. DNA extraction efficiency increased as concentrations of salt increased and nanopore size decreased. It is anticipated that small pore sizes will present clogging problems due to the complex composition of lysed blood. Based on this result, gDNA was extracted from whole blood successfully followed by PCR which was completed successfully without inhibition. With the overall goals of high extraction rates, purification levels and DNA retrieval, it was determined that 200nm sized pores and high salt concentrations yielded the most favorable results.

Keywords: aluminum oxide membrane (AOM), microfluidic, DNA extraction, salt concentration, nanopore effect, lab-on-a-chip.

1. INTRODUCTION

In a micro TAS (Total Analysis System), sample preparation plays an important role in system success. DNA retrieval from biological samples has included methods such as silica, magnetic silica for solid phase extraction, chitosan for ionic interaction and micro/nanopillar sieve separation. [1, 2, 3] Silica bead extraction uses sol-gel-immobilized silica particles in a microchannel for purification of DNA on a microchip SPE device.[3]

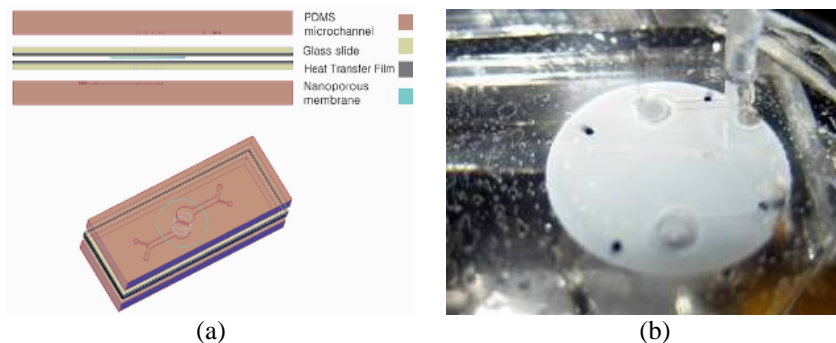


Figure 1. (a) Fabrication of microfluidic DNA extraction system. (b) Microfluidic DNA extraction system integrated with nanoporous membrane.

Chitosan coated microfluidic channels make use of pH-dependent protonation, with pH 5 leaving chitosan protonated for negatively charged DNA interaction and pH 9 leaving chitosan neutral for DNA release. This concept is sufficiently robust to extract DNA from complex biological systems such as lysed whole blood. [1] Such methods require various reagents, washing steps and have varying levels of effectiveness. Nanoporous membrane gDNA extraction is especially favorable because of its simplicity. [4] In preparation for use in a clinical setting, quantification and qualifying tests will be performed.

2. MATERIALS AND METHOD

The chip that incorporates the nanoporous membrane was fabricated with an SU-8 mold, heat transfer film and glass slides. The nanoporous membrane was embedded and sealed with heat transfer film between glass slides. The microfluidic channel was made with PDMS bonded to the top and bottom of the glass. Samples with approximately 500ng of gDNA were passed through using varying salt concentrations. The amount of gDNA in the waste solution was measured using a spectrometer (Nanodrop N-100), therefore suggesting an amount of gDNA left captured on the membrane. Results relating pore size (200nm, 100nm, 20nm) with salt concentration and gDNA capture were obtained by spectrometry. To verify this system, gDNA was extracted using whole blood mixed with lysis buffer and then Real-Time PCR was performed on the gDNA. Initial denaturing at 95°C for 15 seconds followed by 45 cycles of 95°C for 0 seconds, 60°C for 0 seconds, and 74°C for 8 seconds. The temperature transition rate for all steps was 20°C/sec. A 192bp amplicon size of VKORC1 gene was amplified with forward primers and reverse primers. After finishing the PCR step, DNA melt analysis was performed at a rate of 0.3°C/s.

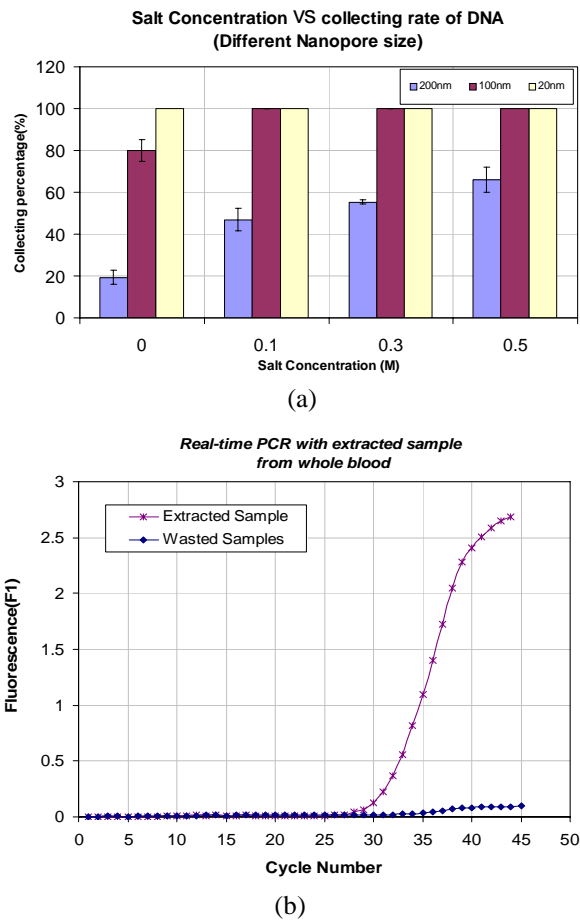


Figure 2.(a) DNA yield rate depends on salt concentration. As salt concentration increases, DNA morphology changes and aggregates more readily.(b) Real-Time PCR amplification with extracted gDNA from blood samples showing PCR compatibility.

3. RESULTS AND DISCUSSION

Membrane pore sizes of 200nm, 100nm and 20nm collected DNA at rates of 20%, 80% and 100% respectively. Implementing a high salt concentration causes gDNA to aggregate, therefore increasing the capture rate. The capture rate for 200nm sized pores increased from 20% to 70 % with this change as shown in Figure 2a. Salt concentration greater than 0.1M caused the majority of the gDNA to be captured in the 100nm pore size membrane. Small pore sizes presented difficulty in maintaining high purity, decreasing the collecting time and avoiding pore blockage due to the complex material found in lysed whole blood. It was determined that 100nm and 200nm sized pores, coupled with the effects of high salt concentration yielded the most favorable results. Downstream PCR is not inhibited by the high salt concentration because the captured gDNA is detached using a separate buffer. To validate the microfluidic DNA extraction system, a gDNA elution test was performed with blood samples and then amplified using the eluted samples, see Figure 2b. In a positive control and extracted sample, the amplification of VKORC1 gene was completed successfully without inhibition and melting peaks appeared at 88.5°C, typical of the VKORC1 gene. Nanoporous membrane gDNA extraction methods can help to increase efficiency in lab-on-a-chip applications.

4. CONCLUSION

Nanoporous aluminium oxide membrane was embedded in microfluidic system for extracting gDNA from human blood. Before use the whole blood sample, the membrane-on-a-chip was evaluated with different pore sized membrane and salt concentration. Based on this evaluation, the gDNA was extracted from whole blood sample and VKORC1 gene was amplified with the primer. The extracted gDNA was amplified effectively without PCR inhibition factor. This method is possible to use as the first part of lab-on-a-chip.

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