

# Purification of PCR-Inhibitory Components by Cellulose Acetate Membranes

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## ABSTRACT

Conventional methods for extracting DNA from blood results in the presence of heme, which is an inhibitory factor decreasing PCR sensitivity. Current techniques for removing the inhibitors are laborious, costly, and/or sample-specific. We investigated using electrophoresis and a biopolymer membrane to separate DNA from heme. These membranes are biocompatible, can be cast directly onto silicon wafers using a standard microfabrication process without use of adhesives or introduction of exogenous contaminants. We have characterized the membranes' properties and altered their casting conditions yielding a filter with a low molecular weight cut-off: 350Da.

Purified genomic DNA was mixed with hemoglobin, and the mixture was boiled to denature the DNA and dissociate the hemoglobin releasing heme into solution. The solution was immediately cooled, and placed in a chamber on one side of the membrane. By applying an electric field across this membrane, and running the experiment on ice, we were able to pass the single-stranded DNA while restricting heme since its formula weight (614Da) is larger than the membrane cutoff. The DNA was purified and enriched as observed by spectrophotometry.

These membranes are easy to make, inexpensive, and can be incorporated into existing DNA chips, thereby adding a purification module to create a lab-on-a-chip design.

**Keywords:** Cellulose Acetate, Lab-on-a-chip, PCR, DNA, Hemoglobin.

## 1 BACKGROUND

The polymerase chain reaction (PCR) is a method to copy and amplify specific deoxyribonucleic acid (DNA) sequences. PCR is a powerful tool for nucleic acid analysis and is extensively used on blood samples to diagnose genetic diseases, characterize microbial infections, perform forensic analysis, and test blood prior to banking [1]. A

major limitation to PCR-based tests is the presence of substances that inhibit the Taq polymerase enzyme activity [2]. Hemin, leukocyte DNA, and added anticoagulants such as EDTA or heparin have been identified as PCR inhibitors [1]. Hemin, a prosthetic group of the hemoglobin released from red blood cells following hemolysis, binds to the Taq enzyme and inhibits the polymerase reaction.

Different methods have been developed to remove the inhibiting compounds from blood prior to sample testing. Published reports have used MgCl<sub>2</sub> to improve the PCR reaction to overcome the effect of inhibitors [3,4]. In addition inhibitors have been reduced by using Bovine serum albumin (BSA), Proteinase K-phenol, phenol chloroform, Chelex 100 chelating resin, DNazol, silica membranes, sodium acetate, glass-milk, magnetic beads, and other proprietary reagents [5-9]. While many of these reagents have resulted in robust PCRs, they can be laborious, time consuming, and assay specific [10]. While methods to avoid difficulties with PCR inhibition have been developed, the mechanism of inhibition remains largely unknown [11]. The goal is to develop DNA extraction methods that are quick, simple, inexpensive, and not assay-specific.

In this report, we introduce the use of cellulose acetate membranes combined with electrophoresis as a filtering technique to purify DNA from heme.

## 2 MATERIAL AND METHODS

Polymer solutions were prepared by dissolving 25% cellulose acetate(CA) (39.8 wt% acetyl content, M<sub>n</sub>=30,000), in N,N-dimethylacetamide (DMAc) (w/v) (Sigma Aldrich, St. Louis, MO). The membrane solution was spun cast onto Corning Transwell<sup>®</sup> inserts (Corning, Acton, MA). The Transwell<sup>®</sup> inserts have permeable membranes (3μm pore size) and were used as frits to cast the CA membranes (Figure 1). Inserts were removed from the wells, turned over, taped to a blank wafer to secure it to the vacuum chuck on the spinner, and the polymer solution was spun at 2000 rpm for 5 sec on top. The inserts were

then quenched in deionized water for 10min at room temperature and dried with a nitrogen gun.

Genomic DNA was extracted and purified from the blood of a healthy donor using Puregene kit (Gentra Systems, Minneapolis, MN). The DNA purity and concentration were determined using optical absorbance at wavelengths 260 and 280nm using a Beckman DU600 spectrophotometer [11]. Human hemoglobin (Sigma Aldrich, St. Louis, MO) was mixed in Tris Borate EDTA (1X TBE) at a concentration of 12.7mg/mL. The final test solution consisted of DNA (57.43 $\mu$ g/mL) and hemoglobin (101.4mg/mL) in TBE and was boiled at 95°C for 5min to denature the DNA. The solution was flash cooled by placing it on a cold block (4°C) immediately after boiling.

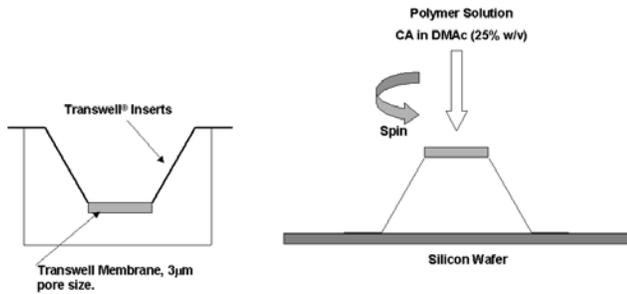


Figure 1: Schematic for casting the CA membrane on the Transwell® insert.

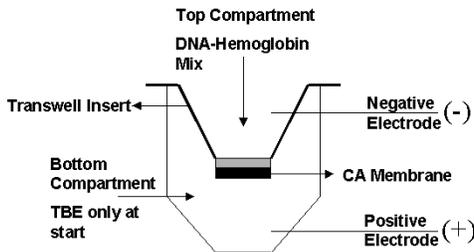


Figure 2: Experimental set-up for DNA-heme separation.

Experiments were performed using the bottom portion of a 50mL centrifuge tube, placed on ice, with the CA membrane separating the top and bottom compartments (Figure 2). Two mL of the DNA-Hemoglobin mix was loaded into the top compartment while the bottom compartment was filled with 2 mL TBE. Eighty volts were applied across the membrane (positive to the bottom compartment) for approximately 40min. Absorbance readings (240 to 680nm) were taken from the original mix prior to loading, the bottom, and the top compartments.

### 3 RESULTS

The resulting CA membranes were 25 to 40 $\mu$ m thick. Scanning electron microscopy revealed two 20-30nm thick skin layers, one on each side, containing an internal

structure with large voids (up to 30 $\mu$ m long and up to 10 $\mu$ m wide) (Figure 3). The CA membrane used was characterized previously and was found to have a molecular weight cut-off (MWCO) of 350Da [12]. This membrane was selected from the range of membranes described in [12] since its MWCO is less than the heme (614Da) and it passes negatively charged compounds without significant losses of material.

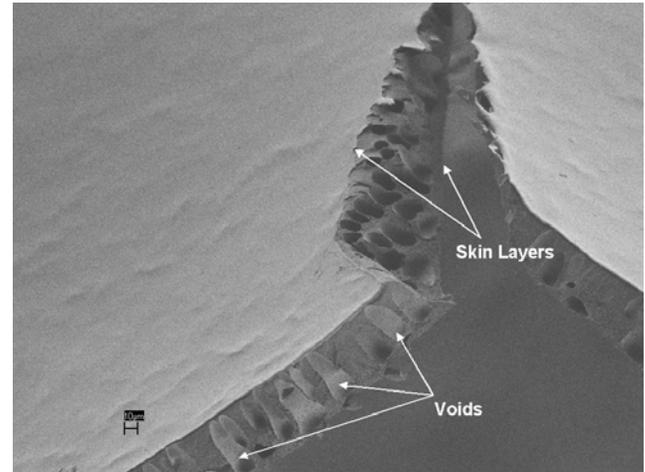


Figure 3: SEM of fractured membrane showing the internal structure.

The DNA-hemoglobin solution was boiled to denature the DNA and facilitate its passage through the membrane. Boiling also dissociates heme from hemoglobin. Electrophoresis was performed on ice to minimize annealing of the single-stranded DNA. Figure 4 shows the relative concentrations of DNA and heme in the test solution prior to boiling and loading onto the membrane. The peaks at 260nm and 409nm represent DNA and heme.

Only DNA was present in the in the bottom compartment after 40 min of electrophoresis (Figure 5). This experiment was repeated three times under same conditions and results were identical.

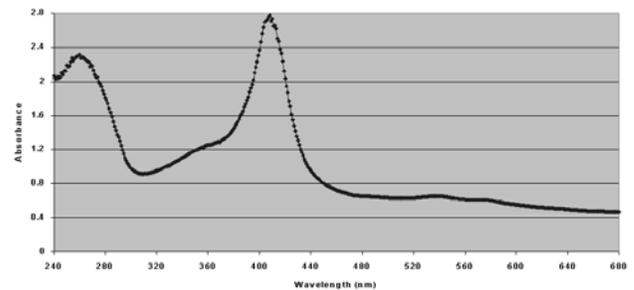


Figure 4: Optical absorbance spectrum for the DNA (260nm) and hemoglobin (409nm) mix.

### 4 DISCUSSION AND CONCLUSIONS

Membranes, made of 25% cellulose acetate dissolved in DMAc, were cast successfully onto Transwell® inserts and showed structural integrity and good adhesion to the substrate. These membranes have been cast similarly on other substrates including silicon wafers and commercial filters. The CA membranes adhered well to these substrates also, thereby permitting their use on multiple materials.

Our previous membrane binding experiments, [12], showed that negatively charged and neutral small molecules with molecular weights smaller than the MWCO passed without significant binding to the membrane. In contrast, small positively charged molecules bound to the membrane. Although single-stranded DNA is nominally much larger, its small cross-sectional dimensions and high degree of flexibility allow it to pass through the membrane, while its negative charge minimizes loss within the membrane. The electric field pulls both heme and DNA to the membrane, but only single stranded DNA passes through while heme is retained at the surface.

Future work will implement use of the membrane in a 96 well plate format. These experiments will use smaller volumes of DNA-heme (100-150µL), which are more comparable to volumes used in standard PCR diagnostic laboratories. Future experiments will use dried blood spots as the starting DNA heme material to test the power of the membranes in a more clinical setting. DNA purification will be assessed using PCR and gel electrophoresis in addition to spectrophotometry data.

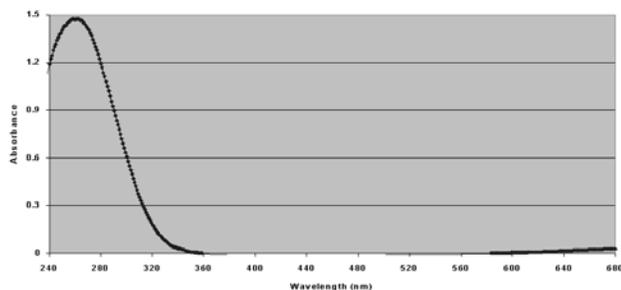


Figure 5: Optical absorbance spectrum for the filtrate (bottom compartment) after separation; only the DNA peak is evident.

## ACKNOWLEDGMENTS

This material is based upon work supported by the STC Program of the National Science Foundation under Agreement No. ECS-9876771.

The authors acknowledge the assistance of the Biochemistry Core and the Electron Microscopy Core at the Wadsworth Center, which is supported in part by the National Science Foundation Grant DBI-0116551.

This work was performed in part at the Cornell Nanofabrication Facility (a member of the Nanotechnology Infrastructure Network) which is supported by the National Science Foundation under Grant ECS-9731293, its users, Cornell University and Industrial Affiliates.

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