ABSTRACT

Organic-aqueous liquid (phenol) extraction is one of many standard techniques to efficiently purify DNA directly from cells. With effective mixing, the cell components naturally distribute themselves into the two fluid phases in order to minimize interaction energies of the biological components with the surrounding solvents. The membrane and protein partition into the organic phase while the DNA stays in the aqueous phase. This work presents two phase microflows for use to effectively extract DNA from cells. Electrohydrodynamic instability is used to mix the two phases in order to increase the surface area over which biological component partitioning may occur.

KEYWORDS: Two Phase Flow, Electrohydrodynamic mixing, Organic Aqueous Liquid Extraction, Phenol Extraction

INTRODUCTION

A major research thrust in microfluidic science and technology is the development of autonomous platforms for the extraction and purification of biological material from cells. Batch fabricated disposable diagnostic and medical treatment units hold great potential to enable both research and healthcare advances. This work presents the initial steps towards miniaturizing one of the fundamental DNA preparative techniques used in molecular biology: organic-aqueous liquid extraction also termed phenol extraction. This extraction technique will be a fundamental component of highly integrated biological analysis systems by allowing efficient DNA purification.

Genomic or plasmid DNA extraction using phenol extraction is one of many standard techniques commonly performed in biology laboratories [1]. Briefly, the procedure consists of lysing cells in a protease solution, and adding a phenol:chloroform 1:1 by volume mixture to the aqueous solution. A vortexing step mixes the two phases and allows the different cellular components to partition into either the aqueous or organic phases. With effective mixing, the cell components naturally distribute themselves into the two phases in order to minimize interaction energies of the biological components with the surrounding solvents. The cell membrane components and proteins partition into the organic phase while the DNA stays in the aqueous phase. This partitioning occurs over molecular dimensions at the aqueous-organic interface. Thus, effective mixing maximizes the surface area over which this partitioning occurs. Therefore, the smaller the discrete phase domains, the more effective the DNA extraction procedure is. After the mixing step, the two immiscible phases are allowed to separate and the aqueous phase is removed using a micropipette. The DNA is then concentrated...
by precipitation in ethanol and resuspended in an aqueous buffer. However, this technique has only been useful for a large number of cells and uses a large volume of liquid (~ 1 ml) because of DNA loss during the aqueous phase removal and the size of micropipette tools. In recent years phenol extraction has been somewhat replaced by DNA preparative kits in which the DNA is adsorbed onto the surface of silica beads and eluted after other cell components are passed through a separation column. However, these DNA preparative kits are thought to be less effective at producing protein-free DNA samples and can have a lower DNA yield than phenol extraction. These contaminating proteins may interfere with biochemical processing reactions. Phenol extraction is considered the gold standard in producing clean, purified DNA samples with little protein contamination.

EXPERIMENTAL PROCEDURES

This work studies two phase fluidic interactions to promote the mixing of two fluid phases consisting of an aqueous and organic (phenol/chloroform 1:1) phase for a miniaturized organic-aqueous liquid extraction μTAS device for DNA processing from whole cell samples. A microfluidic channel (85 μm x 30 μm x 2 mm) has been constructed in glass using standard processing and thermal bonding techniques similar to those employed by Mathies [2] to form two converging channels into which the aqueous and organic samples are introduced. During flow experiments, the phenol phase is labeled with a lipophilic dye, DiI (1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) for phase contrast of the organic phase during flow using an epifluorescent microscope with a TRITC filter cube (Olympus IX71). The mixing of the two phases may also be enhanced by patterning coplanar electrodes on a glass plate prior to thermal bonding to promote electrohydrodynamic instability.

EXPERIMENTAL RESULTS: PRESSURE DRIVEN FLOW

The Phenol and aqueous phases were co-infused through the microfluidic structure using a syringe pump at a variety of flow rates from 0.1-10 μl/min. The flow profile was very difficult to control due to the large interfacial tension between the two immiscible fluid phases. Under some conditions stable stratified co-infusion was achieved (Fig. 1). However, more often the two phases would produce a slug flow profile in which slugs of each phase would alternatively flow through the channel. Depending on the fluid

**Figure 1:** Co-infusion of water and phenol/chloroform solution showing a stable stratified flow profile. The phenol is dyed with a lipophilic dye and is bright under epifluorescent microscopy.
velocity these slugs could break off from the inlet very slowly or very rapidly where they could hardly been seen using standard video (Fig. 2). The slug flow profile minimizes the interfacial interaction area and is therefore more stable than the stratified flow. Under certain conditions a transition from stratified to slug flow is seen. This flow profile is similar to those seen when a hydrostatic pressure difference between the two phases has been shown to produce droplets [3,4].

**Figure 2**: (a-c) Three video frames showing the water phase breaking into a slug of fluid during a slug flow profile. (d) many alternating slugs at high velocity.

**EXPERIMENTAL RESULTS: ELECTROHYDRODYNAMIC MIXING**

The mixing of the two phases was accomplished by patterning electrodes to promote electrohydrodynamic EHD instability. EHD mixing has been demonstrated by other researchers [5]. In order to understand how the mixing will proceed, it is important to understand the stability criteria of the interface. A classic problem in electrohydrodynamic instability was solved by Melcher [6]. In EHD stability analysis, the aqueous phase is assumed to be infinitely conducting due to dissolved ions, while the organic phase is assumed to be non-conducting. As electrodes are biased, charges accumulate at the organic-aqueous interface in the electric field. At a critical voltage the interface becomes unstable. In order to produce an EHD instability interdigitated electrodes (10 μm wide, 10 μm spacing) were biased with a 30 V peak to peak voltage bias at low frequency (1-10 Hz) AC excitation. Here the phenol phase aligned along the electrodes and was pulled towards the electrode at maximal excitation and moved away at 0 V in phase with the applied bias (Fig. 3). Next two electrodes located at the ends of the channels were DC biased at voltages ranging from 0-1000 V. At a critical voltage of 30 V an EHD instability is seen which becomes more violent at the bias is increased. This instability is chaotic and produces vortices between the two phases (Fig. 4). This increases the surface area over which the partitioning of biological material may occur.

**DISCUSSION**

This work has shown that hydrostatic flow control in combination with electrically promoted mixing should be able to promote an elongated interface between the organic and aqueous phases for efficient on-chip phenol extraction of DNA. However, due to the complex slug flow profiles seen it was difficult to effectively characterize the EHD effects. Future work will focus upon redesigning the microfluidic interface into a self contained mixing chamber so that the fluid flow profiles may be better controlled to avoid slug flow profiles and keep the different phases separate, redesigning electrodes so that a more uniform electric field may be produced to better control the EHD instability mixing, determining a method to effectively separate the phenol from the aqueous phase.
after DNA extraction has occurred in order to move the aqueous phase downstream for further DNA processing, and investigating the nanoscale interaction between the biological components with the different fluid phases.

Figure 3: (a) brightfield microscopy showing the 10 μm wide interdigitated electrodes. (b) Phenol phase aligning with the electrodes at maximal voltage biased at ±15 V, 1 Hz (c) The same electrodes ½ of a second (90° phase) later at 0 potential

Figure 4: (Left) An EHD instability promoted at 150 V bias. (Right) the same instability ½ a second later.

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REFERENCES