

CAPTURE-AND-RELEASE CONCENTRATION OF BACTERIA USING FREE-FLOW ZONE ELECTROPHORESIS

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ABSTRACT

A microfluidic free-flow zone electrophoresis device was developed for the purpose of concentrating biological particles in buffer. This device utilizes the novel technology of "capture-and-release," a semi-continuous process that improves upon previous filtration procedures. Low and high throughput designs of this device were tested with solutions of bacteria in various buffers. Concentration factors as high as 4.5 were achieved.

Keywords: bacteria, capture-and-release, microfluidics, zone electrophoresis

1. INTRODUCTION

The detection of biological warfare agents is requisite for military and civil authorities. The sensitivity of detection is partially dependent on sample preconditioning, or increasing target analyte concentration while removing interferents. Preconditioning techniques involve maximizing the concentration of the target analyte followed by sample fractionation. Sample fractionation divides the original sample into two or more fractions of different composition; this facilitates analyte concentration as well as preliminary separation from interferents. We have developed a microfluidic transverse electrophoretic device for concentrating biological particles in buffer upstream of a detection stage. This sample preconditioning unit is a key component of a portable system for collecting and detecting biological particles from the air [1].

Electrokinetic concentration and/or separation methods based on ZE or isoelectric focusing (IEF) have been employed by other research groups [4-6]. These methods each have their distinct advantages, but differ significantly from the method described here. Non-microfluidic electrokinetic methods can take 10-24 hours to complete separation [4], while this microfluidic ZE device being presented takes minutes. Capillary electrophoresis methods [5,6] are faster, but do not offer the ability to continuously concentrate bacteria that our method does. Operating under semi-continuous flow offers increased throughput and the ability to integrate this device with other technologies. Moreover, this microfluidic ZE device is exceptional in that the electrodes are placed in close proximity, allowing a strong electric field to be applied perpendicular to the direction of flow.

2. EXPERIMENTAL

Microfluidic devices shown in Figure 1a were fabricated by cutting Mylar laminates with a CO₂ laser, followed by stacking the layers, alternating Mylar and adhesive. Palladium foil electrodes were used as the side-walls of the channel [2, 3]. Bacteria in solution, flowing between the electrodes, were imaged using epifluorescence microscopy. Devices capable of higher throughput were designed with electrodes located above and below the channel (Figure 1b), as well as with pneumatically controlled valves. The placement of the electrodes in this system increases the exposed wetted area of the electrodes, allowing increased concentration of bacteria at the surface. Furthermore, the on-chip valves allow automated collection of the concentrated sample.

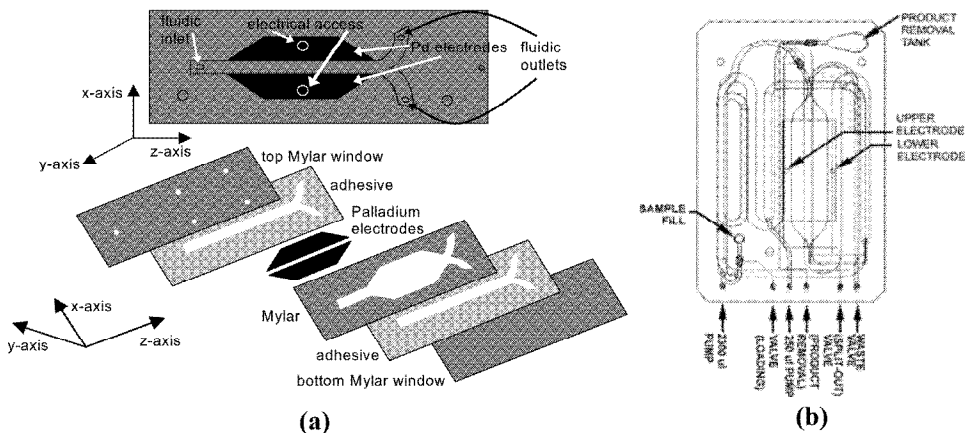


Figure 1. a) Schematic drawing of side-by-side electrode device. The channel is 2.5 cm long, 1300 µm wide, and 25 µm deep. **b)** Schematic drawing of high-throughput card. Channel dimensions are 10 mm wide, 30 mm long, and 1300 µm-500 µm deep.

Concentration of bacteria in this system utilizes continuous free-flow zone electrophoresis (ZE) of particles transverse to the direction of flow [2,3]. A charged particle in solution will migrate at its terminal velocity toward the electrode of opposite charge. Negatively charged bacteria will, therefore, migrate toward the anode. To demonstrate this, 3 ml of sample solutions containing 4×10^8 colony forming units/ml (CFU/ml) vegetative bacteria (*Erwinia herbicola*) suspended in 50 mM histidine buffer with 0.02%(w/v) Tween-20 were loaded into the microfluidic channel and pressure-driven by syringe pumps at a flow rate of 5 µl/s. A voltage of 2.3 V was applied across the electrodes, causing the bacteria to migrate toward the anode. In this “capture” phase, a 9 min 30 s time period based on calculated mean residence time in the channel, bacteria migrated toward the electrode and were continuously accumulated at the electrode surface (Figure 2a). Subsequently, flow was stopped for 5 seconds while simultaneously reversing the polarity of the applied electric field. This “release phase” causes the

bacteria to move away from the now negative electrode surface in a concentrated band (Figure 2b). Voltage was then turned off, flow was restarted, and the concentrated band of bacteria was collected downstream (“collection” phase), with a typical output volume of 200 μl . The concentration factor is increased by splitting the outflow, retaining only the fraction of fluid flowing nearest the capture electrode.

Two different electrode gaps were tested, 500 μm and 1300 μm . The performance of the devices, based on concentration factor, was quantified by measurement of the optical density of bacterial suspensions before and after passage through the device. A quantitative relationship exists between optical density and bacterial concentration (CFU/ml) (data not shown).

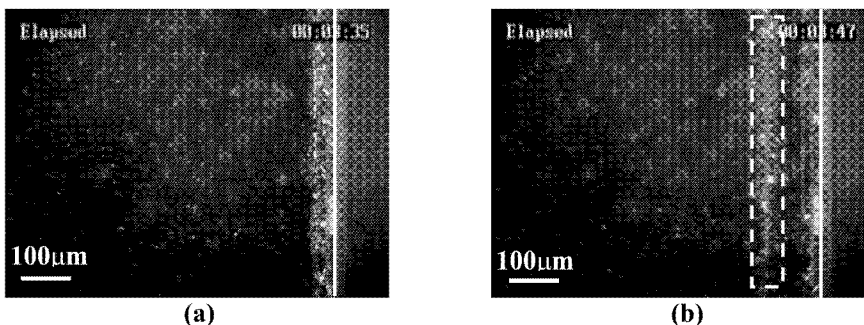


Figure 2. The capture (a) and release (b) phases of the technique described for concentrating bacteria. The surface of the electrode is denoted by a solid white line. The band of concentrated bacteria is boxed by a dashed white line in (b), 12 seconds after switching the polarity of the electrodes. Note that not all bacteria responded to the electric field for various reasons, including adhesion to the channel walls.

4. RESULTS AND DISCUSSION

Preliminary experiments demonstrated enhanced ability of the 1300- μm gap device to concentrate bacteria over the 500- μm gap device with a concentration factor of 1.80 ± 0.14 versus 1.44 ± 0.28 . Subsequent experiments performed with *Bacillus globigii* spores and a more recent device design produced concentration factors as high as 4.5.

Each phase of the process imposes limitations on the extent of possible concentration. During the capture phase, the concentration factor is primarily affected by electrode gap width, exposed electrode surface area, and channel length. Under flow, the bacterium feels a shear force, tending to move it in the direction of flow. When an electric field is applied, electrostatic forces attract the bacterium to the electrode surface. If the attractive force is larger than the shear force, the bacterium will be arrested on the electrode surface. However, if shear force is greater than attractive force, the bacterium will roll along the

electrode surface toward the outlet. Therefore, the ratio of the gap width to channel length should be, at most, equal to the ratio between the average electrophoretic and convective velocities. The concentration factor is limited in the release phase due to temporal control of the switching of the electric field polarity. The polarity is switched for long enough to move the bacteria off the electrode surface, but brief enough to prevent dispersion of the bacteria back into the channel. However, because there is no flow at this stage, the fluid filling the channel would still represent a substantial concentration of the initial sample even if bacteria were uniformly distributed through the channel. Finally, during the collection phase, the concentration factor is enhanced by splitting of the flow volume into discrete product and waste streams at the channel exit. Precise control over relative flow rates at each outlet during this phase is crucial to obtain the desired concentration effect. Increased concentration at this stage will be no greater than the ratio of product flow to waste flow.

5. CONCLUSIONS

The proposed concentration scheme exhibits all of the advantages of a filtration procedure for sample preconditioning. This is substantially more beneficial than other electrophoretic concentration systems because it can be used to semi-continuously process an arbitrary sample size while actualizing increased concentration factors. This is especially advantageous for overcoming detection sensitivity limits and significantly improving the efficacy of the detection technology. This process is superior to filtration because the collection phase is very rapid; it also avoids the high pressure drops present across filters with small pore sizes. Furthermore, it is capable of discriminating between positive and negative particles while simultaneously concentrating and separating them.

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