Dielectrophoresis Based Micro-Devices for Separation and Analysis of Micro-Particles

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Abstract

Miniaturized micro-channel devices have been developed for use in a Micro-Total-Analysis Systems (μTAS). These devices serve one integral function of μTAS – separation and manipulation of micro-particles. Dielectrophoresis (DEP) has been used to perform this function. DEP can be used in the manipulation, enrichment, and separation of micro-particles. Polystyrene beads with ~4.8μm diameter were used as the model micro-particles. The beads were collected at the electrodes of the micro-channel at 10V and100Hz AC voltage. The optimum flow rate for the collection of micro polymer beads was obtained by using the DEP micro-channel. Collection efficiency of the micro-particles was measured as a function of the flow rates. Flow rates of 0.01-3.5 ml/min were used in this analysis. The collection efficiency of the micro-channel was determined using standard hemacytometry and by measuring the number of micro polymer beads in each flow stream before and after collection. The collection efficiencies were 81% at 0.3ml/min, 40.75% at 0.7ml/min, and 11.85% at 3.5ml/min. The size of the polymer beads, which are of the same order of magnitude as typical bacterial cells, suggest that these micro-channels can be used in the separation and manipulation of microbial particles.

Introduction

The separation and manipulation of cells is a topic of much interest. Presently, cell separation can be used for biomedical and biological functions to distinguish individual cells from a mixture, to purify cell sub-populations, and to separate dead cells from living ones. Bacteria such as *E. coli* need to be identified and isolated in water, food, and the environment. As a result, the need for a micro-total analysis system arises. Several applicable areas are pharmaceuticals, food processing, and water treatment. *E. coli*, a bacterium that is present in the intestinal tract of humans and animals, can be a threat to food and water safety. Dielectrophoresis, which is utilized as the separation technique in our work, can be defined as the motion of particles due to the effect of an inhomogeneous electric field [1]. This paper describes specifically how DEP micro-channels can be fabricated and used in manipulation of micro-particles. We can extend this concept to manipulation of *E. coli* though actual experiments with the bacteria have not been performed as part of this work. We have demonstrated that dielectrophoresis can be further integrated with MEMS-based sensors to complete a μTAS system.

Micro-Total Analysis System and Dielectrophoresis

A μTAS is a lab on a chip concept that was introduced in the 1980s. The original vision was to develop miniaturized devices where all necessary parts and methods to perform a chemical analysis are integrated. Portability, automation, low volumes of sample usage, low volumes of waste produced, and rapid detection are all factors that motivate the usage of the total analysis system [2]. Some of its applications include field analysis of environmental samples and biowarfare agent detection (Figure 1).

Figure 1: Lab on a chip applications

DEP is the motion of particles caused by electrical polarization effects in non-uniform electric fields, usually alternating current (AC) electric fields as illustrated in Figure 2. The induced motion is determined by the dielectric properties of the particle, and, unlike electrophoresis, where motion of the particle is determined by the magnitude and polarity of the net electrical charge, alternating current (AC) rather than direct current (DC) electric fields are employed [3]. It should however be noted that DEP is not necessarily an AC phenomenon. Motion of particles also occurs at very low frequencies indicating that DEP is also observed in "almost" DC like conditions.

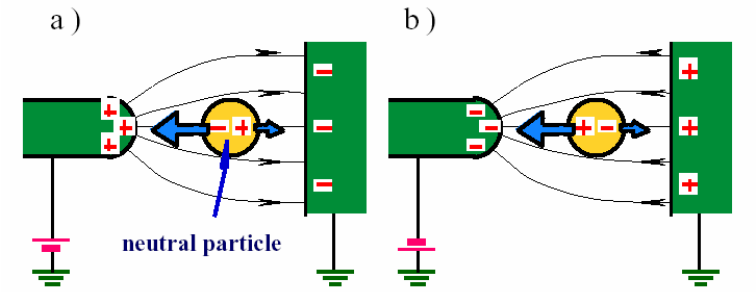

Figure 2: Positive and Negative DEP

Because there are both positive and negative DEP, dielectrophoresis forces can be used in the characterization, handling, and manipulation of microscale and nanoscale bioparticles [4]. This includes the sorting, trapping and separating of cells, viruses, and bacteria etc.

DEP Micro-Channel

There are both micro fabricated components and electronic controls that go into the miniaturized devices that perform chemical or biological analysis The μTAS contains channel and fluid connectors; pumping, dosing and injecting devices; and reactors, mixers, and valves; and sensors. Within the concept of a μTAS, DEP is one of the techniques that can be utilized to perform initial manipulation, sorting and separation of micro-particles [5], nanowires [5] and biological agents [4]. A novel DEP based micro-channel, was developed and used in the experimentation reported here. Stereolithography [5] was used for microchannel fabrication. Standard IC fabrication techniques [6] were used in fabricating interdigitated gold electrodes on a glass substrate. The microfabrication process is described in the next section.

Fabrication Process

Stereolithography (SLA) is a rapid-prototyping technique that enables designers to go directly from a 3D model to parts. SLA is used in the fabrication of the micro-channel. A computer controlled laser cures resin in a layer by layer manner to fabricate the part. (Figure 3).

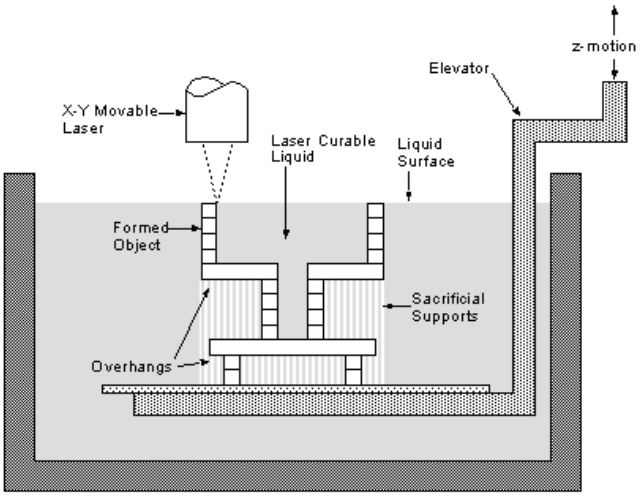

Figure 3: Stereolithography Process

Micro-electrodes under the channel are also required to complete the DEP micro-device. In order to do that, Pyrex glass is used as a substrate. Photolithography is used to transfer the shape of the electrodes onto the glass substrate. The separately fabricated SLA flow channel is then bonded to the electrodes using an epoxy and curing. The bond pads for electrical connections are then wire bonded (Figure 4). For more details on the fabrication process one is referred to S.Rajaraman et al. [5].

Manipulation and Separation Process

As stated above, dielectrophoresis is used in the separating technique of this work, and is defined as the motion of particles due to the effect of an inhomogeneous electric field. Most biological particles have similar electrophoretic (EP) mobilities and therefore dielectrophoresis is preferred over EP as it easier to separate two different particles from a solution as the electrophoretic mobilities do not come into play. EP relies on the electrophoretic mobility of

particles, hence distinguishing between the biological particles (or in this case polymer beads) become difficult.

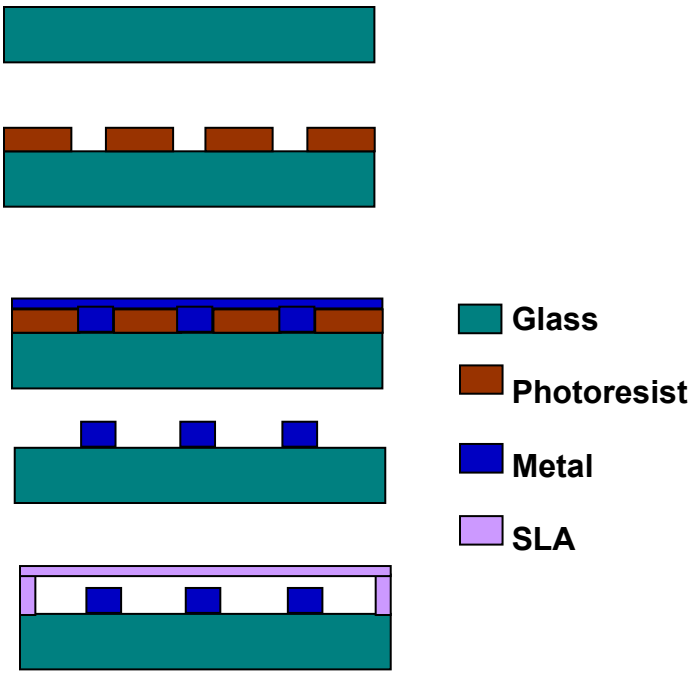

Figure 4: Fabrication process flow of DEP micro-channel

In order to be able to manipulate the particles, standard polystyrene beads (4.8 μm mean diameter, *Bangs Laboratories Inc*., Fishers, IN) were suspended in DI water and were flown through the DEP device that was connected with inlet and outlet ports. The flow was controlled with a syringe pump (Orion Model M362, *Expotech USA Inc.*, Houston, TX). The device was first checked for leaks at various flow rates. Once it was ascertained that the device is leak-free, AC voltage was applied to the electrodes of the DEP device using a standard function generator (*Stanford Research Systems Inc.*, Sunnyvale, CA). Various voltages were applied to determine the characteristic AC voltage (amplitude and frequency) for the polystyrene beads. It was determined that the beads were experiencing positive DEP (attracted towards the electrodes) at 10V and 100Hz. They were experiencing negative DEP at higher frequencies with the same voltage, which is in conformity with the theory of DEP. Once the characteristic frequency was determined, the next step was to optimize the flow rate for maximum collection efficiency. These are described in the next sections. Separation of beads was achieved by using a mixture of two different beads in solution and using the same characteristic voltage to collect one of them while the other is not affected by the voltage and just flows through the system. For more details on this, one is referred to S.Rajaraman et al. [5].

Hemacytometry

Hemacytometry was used in evaluating the collection efficiency and ultimately finding the optimum flow rate. A hemacytometer is a device used in manually counting particles or

cells, consisting of a microscopic slide with a depression, the base of which is marked in grids (Figure 5). A measured volume of the beads in the deionized water solution is placed in the hemacytometer and covered with a glass slide. The number of beads in the squares are counted under a microscope and standard sheets (from the manufacturer) are used to calculate the concentration of a particular sample. This technique is a simple way of determining the collection efficiency of the DEP process as we can find the concentrations of the samples before and after the process. There are other complex and expensive ways of performing the same analysis but hemacytometry offers a simple technique to do the same though it might not be as accurate as some other techniques.

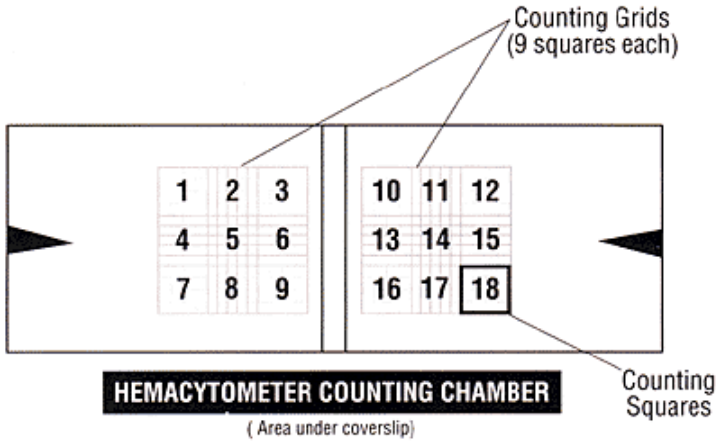

Figure 5: Hemacytometer

Results

First, a sample of the polymer bead solution is taken, and a known volume applied to the hemacytometer. The device is then carefully placed under the microscope to count the number of beads prior to applying the voltage. This is the standard to which the post-process bead counts (at different flow rates) are measured and compared. So several measurements are made for this solution and the average concentration is calculated. Second, a known volume of the standardized solution is taken and is run through the micro-channel and voltage is applied. Some of the polymer beads are collected at the electrodes. Then fresh DI water (of known volume) is sent through the channel and the collected particles are separated (voltage is removed to help this process) and then counted using the hemacytometer using the technique described in the last section. In this way we can run experiments at various flow rates (inlet) and calculate the collection efficiencies at these rates using the same initial solution (standard known concentration). Collection efficiency is defined as the number of particles in the separated sample divided by the number of particles in the standard solution, expressed as a percentage. The optimum flow rate is defined as one where the efficiency is maximum. The flow rates of the polymer beads, that were used range from 0.1-3.5 ml/min. In this experiment, the efficiencies were evaluated to be: 81% at 0.3ml/min, 40.75% at 0.7ml/min, and 11.85% at 3.5ml/min. This follows the expected trend whereby a higher flow rate lowers the efficiency. This is determined by the difference between DEP force applied and drag forces

of the fluid. The drag force of the fluid is expected to increase with an increase in flow rate. Figure 6 shows a plot of the same.

There were other experiments that were performed at different flow rates. There was difficulty in correlating collection efficiency vs. flow rate in the some of the experiments, which can be attributed to contamination in the channel, age of the micro-device, particles getting stuck in the device etc. The best collection efficiency was determined to be 81% at 0.3 ml/min. An ideal flow rate of 0.3-0.5 ml/min could be envisioned for future experiments with polymer beads as well as *E.coli* for good collection.

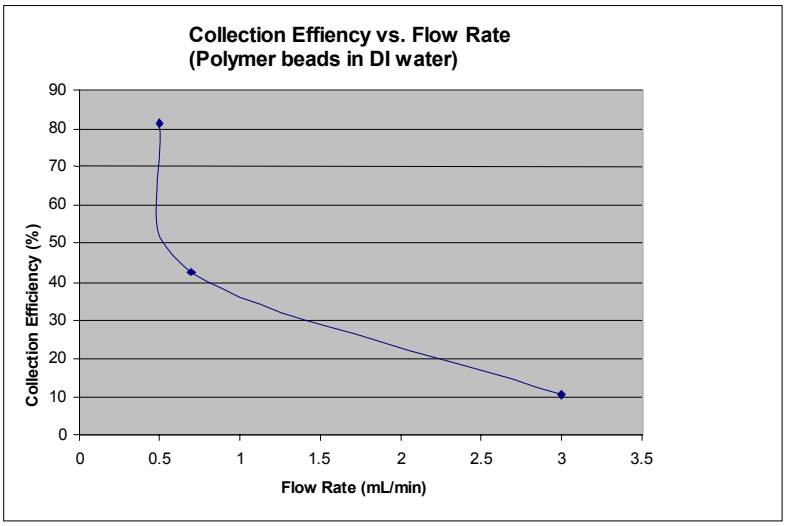

Figure 6: Collection Efficiency as a function of the flow rate

Conclusion

DEP-based micro devices and technologies can provide a simple, rapid, and economical method for separation and concentration of particles and microbial agents. This technology is applicable to the areas of pharmaceuticals, food processing, and water treatment. In the fabrication of the devices, the use of clean room facilities is minimized. Results with micron size polymer beads described here provides as a starting point for further tests using bacterial particles.

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