SB^{3}C2016
Summer Biomechanics, Bioengineering and Biotransport Conference
June 29 –July 2, National Harbor, MD, USA
SB^{3}C2016-1067

INCREASED CAPTURE OF MAGNETIC MICROBEADS DUE TO SWITCHING OF ELECTROOSMOTIC FLOW

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INTRODUCTION

Detection and isolation of bio-molecules for immunoassays is crucial to efficient and accurate performance of micro total analysis systems (μTAS). To accomplish this, magnetophoretic separation techniques are commonly used in these microfluidic platforms. This technique utilizes magnetic microbeads coated with epitomes targeted to specific pathogens as part of an antigen-antibody binding complex to tag specific bio-molecules [1]. Separation is then accomplished through the application of a magnetic field to isolate the microbeads/bio-molecule complex from the continuous flow in the device. The continuous flow in microfluidic devices can be either pressure driven or electrokinetically driven. Electrokinetically driven mechanisms, such as electroosmotic flow (EOF) has advantages over pressure driven flow due to the absence of mechanical pumps, which can be inefficient [2], as well as the ability to operate at atmospheric pressure.

The difficulty with magnetophoretic immunoassays is producing a reliable and efficient capture of microbeads to ensure accurate results with the highest possible sensitivity using dilute samples. The capture efficiency (CE), or ratio of number of particles captured to number of particles injected into the channel, relies on the attractive magnetic force overcoming the viscous forces of the liquid moving the particles with the flow. However, previous studies have shown that magnetic beads can escape the area with high magnetic field strength [3], resulting in uncaptured beads and decreased sensitivity. Thus, a switching method is implemented to increase the bead residence time in the area of higher magnetic field strength. This switching is accomplished by alternating the external voltage driving the electroosmotic flow through the microchannel. The goal of this study is to assess increase in capture efficiency using the switching flow mechanism as compared to the constant flow mechanism.

METHODS

The microchannel device used during testing is shown in Fig. 1. The channel is produced using standard soft lithography with the final device made from PDMS bonded to a glass slide, resulting in a channel that is 50mm long with a cross section of 50 μm x 50 μm. A 1/8" x 1/8" x 3/8" tall neodymium (NdFeB) magnet is placed above the channel, 25 mm from either well. The channel is pre-treated with NaOH to induce surface charge on the walls and then PBS with Tween20 to prevent the microbeads from sticking to the wall when not captured.

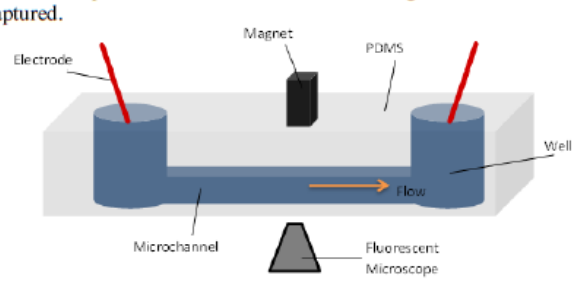

Figure 1: Schematic of microchannel device.

The beads used were Dynabead® M280 Sheep anti-Rabbit IgG tagged with Alexa Fluor® 488 Rabbit anti-Mouse IgG by slow tilt mixing for 24 hours at 8°C. The beads were suspended in PBS buffer to achieve the desired concentrations for the experiments (C1 = 2×10^6, C2 = 4×10^6 beads/mL).

The capture zone was determined for the device by running tests at the lowest flow rate followed by immediately running the buffer to clear out any uncaptured beads before determining the distance from

the front and far edge of the magnet beads were visualized. The electroosmotic flow is governed by equations 1 and 2

Poisson's equation: $\nabla^2\phi = 0$ (1)

Helmholtz-Smoluchowski equation: $U_e = \varepsilon\zeta E/\mu$ (2)

These two equations are coupled so that the EOF velocity, U_e corresponds to the applied potential, ϕ. For the experiments, voltage was applied for 20 min total, with the time breakdown shown in Table 1.

Table 1: Applied voltage conditions as function of time. V for case 1 is 650 volts; V for case 2 is 750 volts.

Time (min)	Steady State		Switching	
	Inlet	Outlet	Inlet	Outlet
0-8	+V	Ground	+V	Ground
8-11	+V	Ground	-V	Ground
11-14	+V	Ground	+V	Ground
14-17	+V	Ground	-V	Ground
17-20	+V	Ground	+V	Ground

The beads were visualized and images were captured using inverted fluorescent microscopy, with example images of captured beads shown in Fig. 2. A calibration curve of fluorescence as a function of concentration is shown in Fig. 3. Beads located in the starting well or before the capture zone were not considered, those located in the capture zone were considered captured, and those located in the channel after the capture zone or in the ending well were considered uncaptured.

Figure 2: Fluorsecent images of beads captured under switching protocol at concentration of 4×10^6 beads/mL and 750 V (left) and 650 V (right).

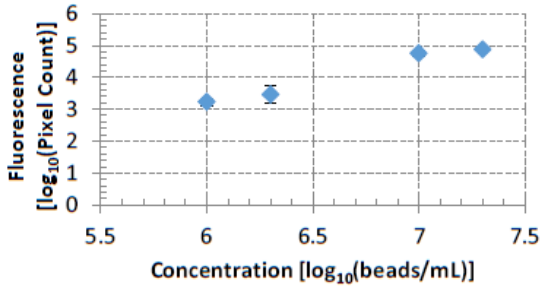

Figure 3: Calibration curve of fluorescence as function of bead concentration.

The images were analyzed using MATLAB to determine the fluorescent intensity based on Red-Green-Blue (RGB) values to determine percentage of beads captured based on pixel count (PC) of captured and uncaptured beads according to

$$Capture\,\% = \frac{PC\,captured}{PC\,captured + PC\,uncaptured} * 100\% \quad (3)$$

The relative percentage difference between switching and constant flow capture was determined by

$$Rel\,\%\,Diff = \frac{Capture\,\%\,Switch - Capture\,\%\,Constant}{Capture\,\%\,Constant} * 100\% \quad (4)$$

RESULTS

Capture percentage for both switching and constant flow as well as relative percentage difference for voltages of 750 V and 650 V and concentrations of 2×10^6 beads/mL and 4×10^6 beads/mL are shown in Table 2.

Table 2: Capture percentage for switching and constant flows and the relative percentage difference.

Concentration (beads/mL)	Voltage (V)	Switching Capture %	Constant Capture %	Relative % Diff
2×10^6	650	85.0 ± 5.8	41.7 ± 5.5	99.2 ± 30.0
2×10^6	750	83.6 ± 6.7	37.6 ± 6.6	122.5 ± 42.1
4×10^6	650	80.6 ± 4.1	37.3 ± 4.6	112.6 ± 29.5
4×10^6	750	71.1 ± 6.2	31.7 ± 5.8	122.8 ± 44.7

The main result is seen in the difference between capture percentages of the switching and constant flow protocols. The drastic increase in capture percentage in the switching situation, around 2 times the capture percentage, is due to the increased residence time in the higher magnetic field capture zone. The different concentrations and voltages had very little effect on the capture percentages. There was a relative difference of less than 20% for each permutation in voltage or concentration, keeping all variables (voltage, concentration, and switching versus constant flow) constant. This difference is insignificant compared to the drastic improvement in capture percentage when incorporating the switching protocol.

The maximum capture zone was determined to be 12 mm before the front of the magnet and 15 mm beyond the back of the magnet.

DISCUSSION

The change in capture efficiency as a result of the switching flow protocol was examined and compared to the capture efficiency using constant flow. The major finding of this study shows that switching causes a relative difference of 2 times the capture percentage for all cases as compared to the constant flow protocol. This is due to the switching function returning beads that would be lost under constant flow conditions back to the capture zone and the influence of the higher magnetic field strength.

Future examination of this device through variation of the exact switching protocol could optimize the capture efficiency and residence time in the capture zone. In addition, varying the switching protocol could also result in more beads entering the capture zone, potentially increase the sensitivity. Further studies consisting of beads capturing bio-molecules would also provide vital information concerning the performance of the device in its intended application as a portable magnetophoretic immunoassay.

ACKNOWLEDGEMENTS

This research study was supported by the National Institute for Occupational Safety and Health Grant #1011206.

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