**A Magnetic Bead-Based Microfluidic Mixer as a Sample Preparation Module for Portable PCR-Based Biosensing**

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**Abstract**

For the purposes of rapid detection of bacterial pathogens and forensic deoxyribonucleic acid (DNA) analysis, a portable, fully-automated, polymerase chain reaction (PCR)-based detection system has been developed in our lab. Microfabricated DNA purification and real-time PCR microchips were fabricated and tested for their ability to purify and detect DNA sequences from a variety of bacteria. To further broaden the utility of this system, we are developing a sample preparation module, which would isolate and purify cells of interest. Current work has focused on the development of a magnetic bead-based microfluidic sample preparation system for the isolation and purification of target cells from a raw sample using antibody-coated magnetic beads.

**Summary of Research**

A portable, fully-automated PCR-based detection system has been developed in our lab for the rapid detection of bacterial pathogens [1-3] and forensic DNA analysis. To further broaden the utility of this system, we have developed a microfluidic sample preparation module that removes cells of interest from a raw sample by mixing with antibody-coated magnetic beads, as shown in Figure 1.

Fabrication of the microfluidic mixers employs a two-step SU-8 process to generate the polydimethylsiloxane (PDMS) mold. The PDMS-on-PDMS mixer chip (Figure 2) consists of serpentine channels with herringbone structures for passive mixing [4, 5], and the two layers of PDMS are manually aligned and adhered using oxygen plasma and 60°C baking temperatures. Bubble traps and post-mixing stray-bead-capture chambers are included, and are used in combination with permanent magnets for cell capture.

![Microfluidic mixer schematic.](image1)

![Digital photograph of the Microfluidics Desktop automated PCR system.](image2)
Bead leakage was studied at various flow rates with different methods of magnetic field application. Higher flow rates resulted in more bead leakage and weaker flexible magnets allowed for more even bead distribution throughout the mixer than the rare earth magnets. Passivation of the PDMS is required to prevent proteins and cells from nonspecifically adhering to the polymer walls. Experiments with different methods of PDMS passivation (5% Pluronic, 5% Bovine serum albumin (BSA), and no passivation) were conducted, and it was found that incubation with 5% BSA was most effective. The 5% Pluronic passivation technique requires incubation inside the PDMS device for at least 24 hrs prior to use, which introduces difficulties such as bubble formation, as opposed to the 5% BSA passivation method, which requires only 10 minutes of incubation prior to the actual experiment.

Cell samples and antibody-coated magnetic beads are simultaneously pumped into the microfluidic device for passive mixing. The external magnetic field traps the magnetic beads and captured cells within the PDMS channels, and a phosphate buffer saline (PBS) wash is pumped through the device to separate the captured cells from the raw sample. The cells are subsequently lysed open with a flow-through of 4.0 M guanidine thiocyanate (GuSCN) lysis buffer, and the cell contents are pumped into the DNA purification microchip. Bacterial cells have been selectively isolated from both pure bacteria culture samples and contaminated raw chicken samples and lysed to release DNA. The DNA purification microchip employs silica-coated microstructures to selectively bind, wash, and elute nucleic acids in preparation for real-time PCR. The Microfluidics Desktop platform (shown in Figure 3) with integrated microprocessor, pumps, valves, thermocycler and fluorescence detection modules, is used to isolate and lyse bacterial cells, purify bacterial DNA, and detect bacterial DNA using real-time PCR amplification. Between 100 and 1000 Salmonella typhi cells could be detected using this system with good repeatability and an average turnaround time of 120 minutes.

References