

Abstract

Due to an increased public concern for food safety and quality, food processing industries have an urgent need for fast and reliable contaminant detection technologies. Conventional pathogen detection methods are time consuming, cost intensive, require skilled laboratory workers and are inappropriate for in-field operation. Microfluidic bio-analytical systems can effectively approach this challenge by automated and rapid pathogen detection in lab-on-a-chip (LOC) set ups. This thesis is focused on the development of a flow cytometry based integrated microfluidic sensing platform comprised of an enrichment and a detection module. To establish bacterial enrichment within the LOC set up, both conventional in-tube and microfluidic on-chip methods were investigated and coupled with an optical detection system.

For this purpose, a model system for immunomagnetic separation (IMS) of bacteria as a cell enrichment technique has been developed. The method comprised preparation of paramagnetic particles coupled with specific antibodies, enrichment by IMS and finally flow cytometric detection of captured bacteria after viability staining. Several poly-and monoclonal antibodies were investigated with the aim to create efficient immunomagnetic beads (IMBs). The most suitable antibody was chosen by applying an enzyme linked immunosorbent assay (ELISA) and flow cytometry analysis. The key parameters for IMB preparation, magnetic separation and flow cytometric studies were optimized where carboxyfluorescein diacetate (CFDA) was used to evaluate the viability of the cell. Under final optimized condition, the developed method showed 98 % capture efficiency towards the specific antigen *Salmonella* Typhimurium and very low (< 5 %) binding with non-target bacterial strains.

Subsequently, IMS was implemented into the microfluidic environment and a magneto-microfluidic set up was developed to achieve that. The magnetic section was a combination of two electromagnets controlled by a DC-DC converter and a magnetic brick. A disposable PDMS chip fabricated by standard photolithography was used for both immuno-capture and separation steps. The resulting alternating magnetic field created by electromagnets guided the functionalized paramagnetic beads in a sinusoidal path inside the microchannel and thus enhanced the probability of interaction between the reaction partners. The optimum channel geometry, flow rate, alternating magnetic field frequency and bead size were determined. It was possible to obtain a capture efficiency of about 68 % by using a channel width of 100 μm with a

flow velocity of about 0.8 mm/s (flow rate 0.5 $\mu\text{L}/\text{min}$). A detection limit of about 10^3 cells/mL was obtained.

Finally, a design for an integrated microfluidic set up is proposed where three different laboratory functions, namely microbial enrichment and separation via IMS and optical detection through flow cytometry can be performed in a single disposable PDMS chip. The suggested lab-on-a-chip microfluidic system allows to isolate, concentrate and detect pathogens in food, feed or beverage industries in real-time and has the potential to offer significant advantages compared to conventional systems.