Lab-on-a-chip Flow Cytometry

Fast Analysis of Microbial Contaminants

Even a very small number of pathogens can cause severe threat towards food safety. Due to increased safety concerns, there is a high demand for fast and sensitive methods for the detection of microbial contaminants in the food processing industries. Specific shortcomings of traditional culture based methods are highly time consuming. This includes enrichment, growing on selective differential agar plates, and consequently biochemical confirmation [1] and labor-intensive sample preparations. Therefore, it is necessary to deliver specimens to and subsequent analysis at typical laboratory while food processors have to wait several days to prove or disapprove a possible contamination, which could cause disposal of goods produced in the meantime. Moreover it significantly underestimates the number of microorganisms because of viable but non-culturable (VBNC) population.

Many human pathogenic strains such as *E. coli*, *L. monocytogenes* and *Salmonella sp.* belong to such a group. Flow cytometry provides a direct, rapid and accurate way of real-time monitoring of cell populations in the industrial product line and is an ideal tool for the assessment of microbial contaminations. But commercial flow cytometers are bulky, costly and require skilled personnel and thus are often non-suitable for the application in the industrial production line. An integrated lab-on-a-chip approach combining immunomagnetic separation (IMS) and flow cytometry has been developed for the enrichment and detection of microbial contaminants in food samples.

**Principles of Lab-on-a-Chip Flow Cytometry**

Commercial flow cytometry involves the combination of fluidics, optics and electronics to analyze different properties of a large number of particles individually suspended in a fluid. After injection particles are distributed three dimensionally, which are compressed to form a single-file stream of cells by hydrodynamic focusing. Later the cells pass through a laser beam generating
optical signals (scatter and fluorescence) that are eventually processed. A similar approach has been implemented into the microfluidic flow chip that could be more accessible and affordable for industries.

Hydrodynamic focusing has been achieved in flow chip by controlling two parallel stream of sheath flow that focuses the sample flow.

A novel method [2] for producing chips with flexible layout has been developed that includes optical lithography, PDMS molding and PDMS chip sealing. After production, the chip is interfaced and integrated with the remaining setup. This is achieved with the novel chip-chuck device (fig. 1).

The main advantage of the flow chip is the achievement of a significant size reduction and a simple fabrication procedure. The on-chip detection module is composed of two optical fibers that include input and output fiber. The excitation light is generated by an argon laser (488 nm). The laser light is passed through a single-mode fiber directly into the chip where the cells are excited and the scattered or emitted light is collected by a multimode fiber and transferred to the detection system. To avoid complicated adjustment several parallel channels were designed into the chip structure. To collect scattered and emitted light into multimode detection fiber, distance between fiber and channel and also the detection angle have been optimized similar to conventional flow cytometer. Signals from detectors are processed electronically and analyzed by using simple software.

**Enrichment by Using Microfluidic IMS**

In the event of a contamination the number of undesired microorganisms is very low but sufficient to cause food safety hazards. Therefore a specific and efficient enrichment step such as Immunomagnetic Separation (IMS) is required prior to analysis. Paramagnetic particles can be grafted with antibodies against the target pathogen. The ability to vary the specificity of the antibodies, bead type and size
make IMS a versatile and flexible technique. A specific antibody captures the target organism from a food matrix and background microflora and the pathogens are concentrated into a smaller amount of sample volume. Since the beads turn into super-paramagnetic in the presence of an external magnetic field, the bead-bacteria complex can easily be separated by using a magnet.

Microfluidics offers a powerful way to automate and miniaturize sample preparation for evaluation of biological samples. Therefore on-chip IMS using micron sized fluidic channels have been demonstrated in the study. Compared to conventional in-tube IMS, lower amount of beads is required in the chip system and therefore the costs per assay get reduced. Immuno-magnetic beads were immobilized by using the permanent magnet fixture (fig. 2) in chips consisting of long fractal meanders while contaminated samples were flowed over them.

After incubation the beads with captured bacteria can be released for detection into the flow-cytometry chip. Different space-filling Peano curve designs (fig 3) have been investigated varying channel cross-section dimensions, total channel length and volume, number of turns etc. in order to determine the optimal conditions for maximum interactions between paramagnetic particles and bacteria inside the chip. Preliminary results showed that number of turns is crucial (fig. 3 B) and the reason could be the generation of turbulent flow in every turns. Irregular wave motion of the bacteria inside the sample fluid results in better access to come in contact with beads which are immobilized in the bottom of the channel. To further improve the capture efficiency we are working on the development of an electromagnet with rotational magnetic field.

**Detection of Bacterial Contamination**

In contrast to other methods flow cytometry not only allows the rapid detection of individual cells but also determines their viability. The determination of viable cells in food is performed by using a live cell marker. CFDA (Carboxy fluorescein di-acetate) is a non-fluorescent esterase substrate which serves as viability probe showing enzymatic activity and cell membrane integrity. Upon hydrolysis by intracellular esterases CFDA releases carboxy-fluorescein, a green fluorescent product. Therefore metabolically active cells show green fluorescence after excited by a specific laser. The bead-bacteria complex (fig. 4) eluted from IMS chip can be detected by observing an increased green fluorescence signal compared to that of free magnetic particles. Alternatively, it is possible to use other viability dyes.

**Combination of Lab-on-a-chip Flow Cytometry and On-chip IMS**

Lab-on-a-chip (LOC) technology has strong potentials for use in the production line since they are miniaturized and can be performed autonomously. They are also
potentially fast and highly sensitive. Such an experimental microfluidic set up for LOC flow cytometry has recently been developed in cooperation with Mads Clausen Institute (MCI) at University of Southern Denmark. The setup is a combination of an enrichment and detection chip which, after further investigations should be able to detect pathogens inside the processing facilities in real-time. This enables the industries concerned to avoid the needs of retracting massive amounts of products from the market once analysis results are back from typical labs dealing with traditional culture methods.

Literature

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