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FNSNF

On-chip Activation of Monocytic Cells and Immunomagnetic-based Detection of Proinflammatory Cytokines as a Response to LPS Stimulation

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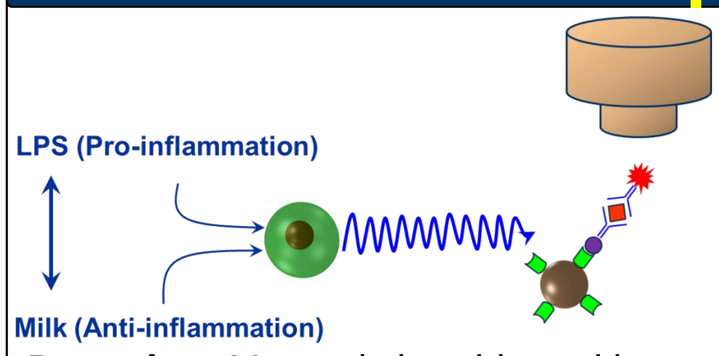
Abstract

A microfluidic experimental system is proposed to quantify the variation of pro-inflammatory cytokines as a response to lipopoly-saccharide (LPS) and milk stimulation of monocyte-derived macrophages (U937 cells). The variation of the cytokine secretion will be quantified after treating the cells with LPS and in vitro-digested milk in parallel in a magnetic bead-based immunoassay.

Concept



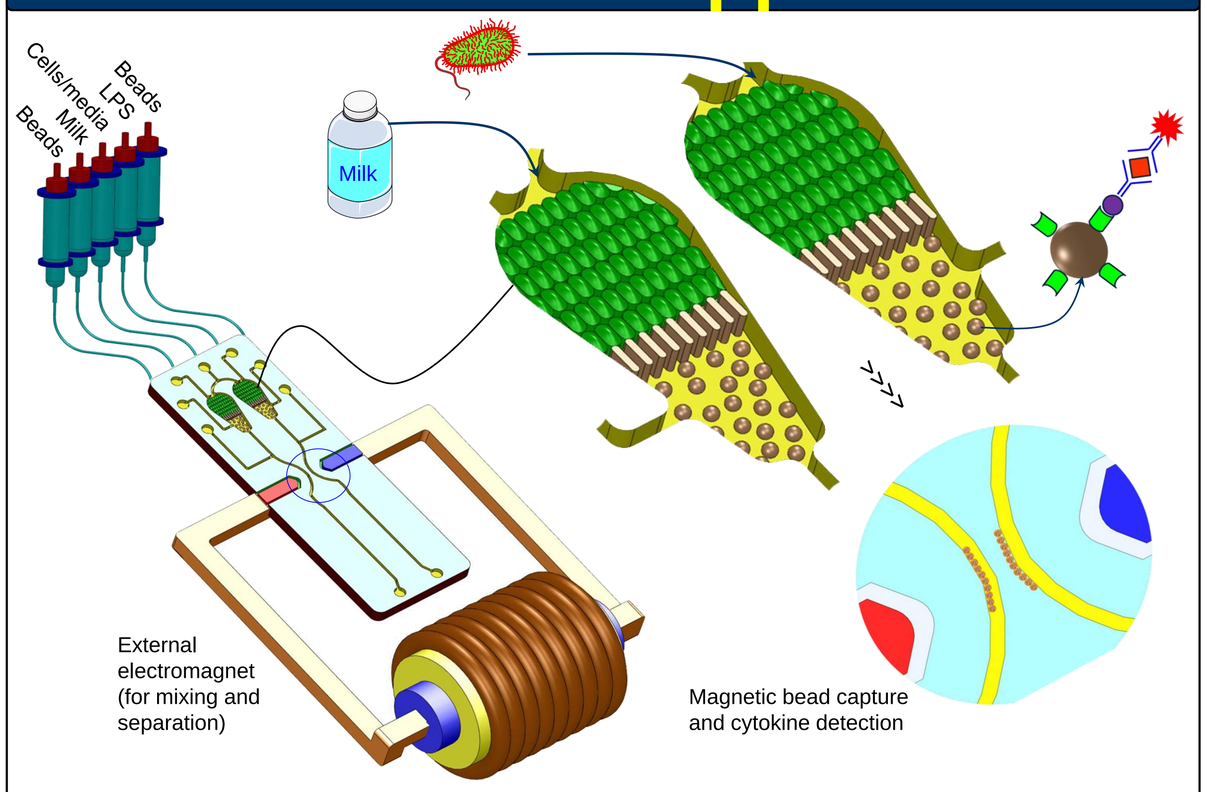
Stimuli: lipopoly-saccharide (LPS);

Cell model: Differentiated U937 (macrophages)

Biomarker: IL-6

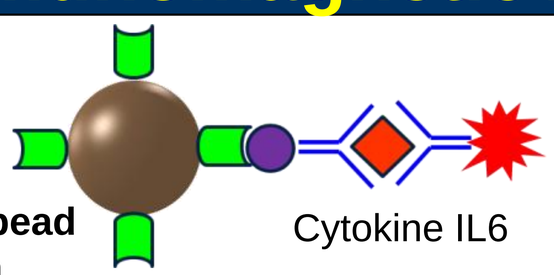
Detection: Magnetic bead-based immunoassay with fluorescence microscopy in a microfluidic system.

Microfluidic Approach



- Two cell culture chambers for differential stimulation, interfaced by immunoassay chambers with *anti-IL6* coated magnetic beads
- Cell-culture and immunoassay chambers separated by a filter
  - blocking an exchange of cells and beads
  - allowing only the cytokines to diffuse from the cell culture to the immunoassay chamber to be captured by the beads
- Continuous agitation of the magnetic beads by an external electromagnetic field to enhance the cytokine capture efficiency
- **Protocol:** (1) Cell stimulation by LPS and milk, (2) cytokine secretion, (3) cytokine capture and detection.

Immunomagnetic Assay



Magnetic bead coated with IL6 antibodies

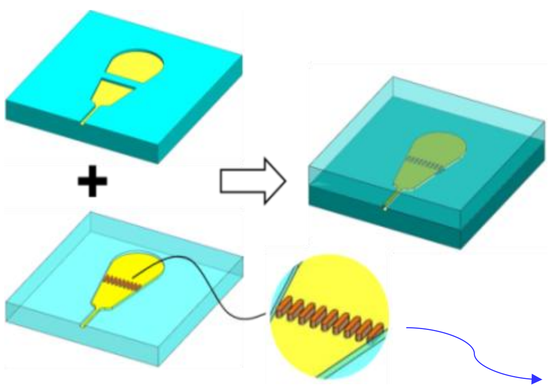
Cytokine IL6

Streptavidin-PE (fluorescent component)

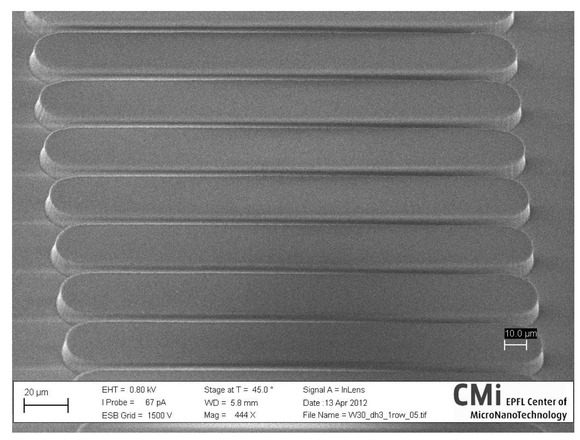
Fabrication

- Complete chip consisting of two PDMS parts, created by replica molding using the photoresist SU-8™ as a master mold material
- *Bottom part* comprising chambers for cell culturing and the magnetic immunoassay with a barrier in between
- *Top part* also containing the two chambers and the pillar-array filter arranged on top of the barrier after bonding the two parts

Bonding top and bottom PDMS part;
Section: pillar-array filter



- Providing a chip containing two parallel fluidic units
- Each unit comprising two chambers with a total depth of 60µm
- Chambers separated by a filter with a pore size of 10µm x 5µm

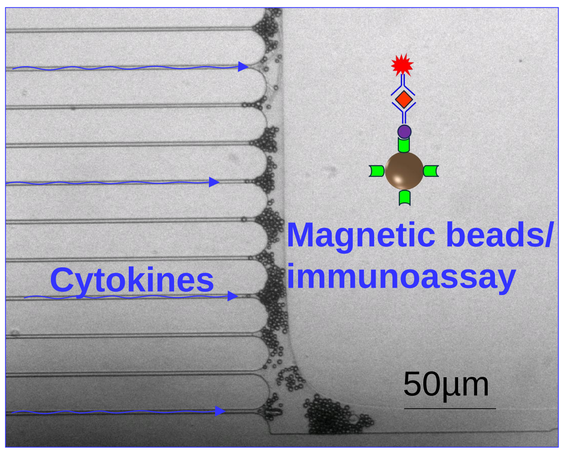


SEM image of the pillar-array filter (top PDMS part)

Results

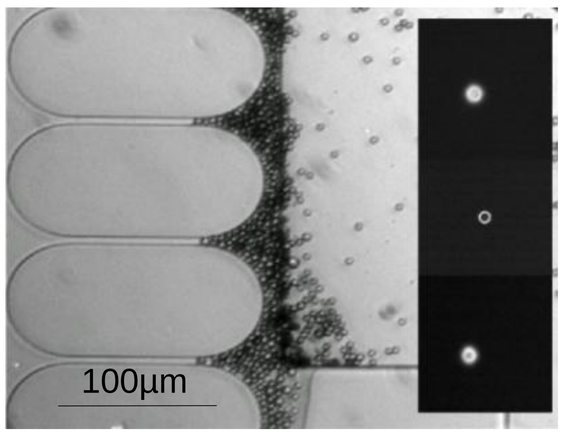
Microfluidic chip with cytokine/ magnetic bead-complexes in the immunoassay chamber (cell-culture chamber not shown)

Cell-culture chamber and stimulation



Magnetic beads retained by the pillar-array filter

Insert: off-chip detection of fluorescent anti-IL6 coated magnetic beads



Conclusions

The presented microfluidic system serves to investigate the response of human immune cells to the consumption of milk and dairy milk products. The variation of the pro-inflammatory cytokine IL-6 secreted by monocyte cells U937 as well as monocyte-derived macrophages after the stimulation with LPS and in-vitro digested milk will be detected using a magnetic immunoassay in combination with fluorescence microscopy.