Size-based chromosome separation in a microfluidic particle separation device using viscoelastic fluids

Therese R. Wassberg[1], *Mathilde L.* Witt[1], *Murat* Serhatlioglu[1], *Christian F.* Nielsen[2], *Ian D.* Hickson[2], and *Anders* Kristensen[1*]

[1]Department of Health Technology, Technical University of Denmark, Ørsteds Plads, Building 345C, 2800 Kgs. Lyngby, Denmark
[2]Center for Chromosome Stability, Department of Cellular and Molecular Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, 2200 København N., Denmark

Abstract. Viscoelastic flow-based particle manipulation techniques enable bio-particle focusing, separation, and enrichment by precisely tuning the rheological parameters, flow conditions, and microchannel geometry. In this study, we fabricated a PDMS-based single inlet/outlet microchannel to separate bio-particles by their size ranging from 1-10 µm. Flow conditions and rheological properties are optimized using 2 µm and 4 µm Polystyrene beads to reach the best particle separation condition. We demonstrated the size-based separation of human chromosomes by separating 1-2 µm size small chromosomes from 8-10 µm size large chromosomes. Thanks to its miniaturized size and simplicity, the isolation chip and unique viscoelastic separation method have great potential to be used as a future pioneering tool for genetic applications to study chromosome abnormalities such as fragile-X and trisomy.

1 Introduction

Miniaturized optofluidic platforms are essential for in-vitro bio-analysis and diagnostic applications [1]. Low sample requirements and rapid analysis capabilities are advantageous for such systems.

Optofluidic sensory tools such as optical tweezers directly manipulate bio-particles, such as chromosomes; however, current systems have limitations due to the low purification level of the prepared chromosome samples. Metaphase chromosome samples are prepared using cell lysates, with many impurities such as cell debris and nuclei with chromosomes [2]. Such impurities generate misinterpretation during device operation and data analysis.

This study aims to improve sample preparation for the chromosome analysis platforms by developing a new microfluidic device to separate chromosomes and extend the purity of chromosome sample solutions. The proposed device is a planar PDMS channel consisting of contraction/expansion cascades (Fig. 1). The device uses the viscoelastic particle separation technique to generate size-based multiple particle trajectories at the outlet using viscoelastic force and the pinched flow fractionation method [3, 4]. Metaphase cell lysates (a mixture of cell debris, chromosomes, and nuclei) have particles spanning the 1-10 µm size range. The elutes will be mixed with biocompatible viscoelastic buffer solutions and pumped through the microchannel inlet. It will be possible to reach size-based multiple particle trajectories at the channel outlet by carefully tuning the flow parameters (flow rate and viscosity). As the last step, multiple trajectories of different size chromosomes and cell debris will be collected (not demonstrated here) from multiple chip outlets.

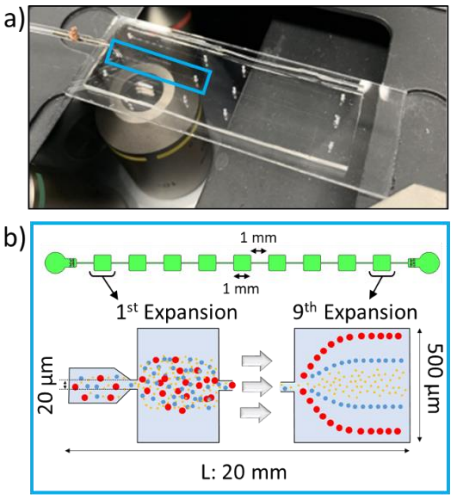

Fig. 1. a) The fabricated PDMS device and experimental setup, and b) schematic illustration of channel design and particle separation.

The demonstrated chromosome isolation chip and unique viscoelastic isolation method have the potential as a pioneering tool in chromosome research. It also has further potential applications for size-based isolation/enrichment of microorganisms in environmental science applications.

* Corresponding author: akri@dtu.dk
T. R. Wassberg, and M. L. Witt are contributed equally to this work as first authors.

2 Material and Methods

Two different molecular weights (600 kDa and 2 MDa) Polyethylene Oxide (PEO) viscoelastic polymers are used to prepare buffer solutions at various concentrations in 1X-PBS. PBS/PEO_{600kDa} buffer solutions were prepared at 2000, 5000, and 9000 ppm, and PBS/PEO_{2MDa} buffers were prepared at 1000, 2000, and 5000 ppm concentrations. Spherical polystyrene calibration beads of 2 µm and 4 µm diameter (Duke Scientific Inc.) were suspended in prepared viscoelastic buffer solutions at a concentration of 1×10^6 particles/ml. Flow experiments are demonstrated with calibration beads and optimized.

Human chromosomes are isolated from their cell lysates and suspended in viscoelastic buffer solution to a concentration of 2×10^6 chromosomes/ml. Chromosome separation experiments are performed at the best-optimized flow conditions.

The microfluidic PDMS device is fabricated using conventional soft lithography techniques in a cleanroom. A 10 µm thick SU-8 mold was prepared on a silicon substrate. Then 3-mm thick PDMS layer was cast and cured on Si mold. Later cured PDMS peeled and bonded to a glass slide to form the microchannel. The PDMS device is placed on an inverted microscope, and high-speed camera videos are recorded at 5000 fps. Recorded videos are image-stacked to show the formation of particle separation before the outlet, the 9th expansion.

3 Experimental Results

Initially, the performance of the microfluidic device was characterized by using various-sized model polystyrene microparticles. Later, in-vitro analysis of metaphase chromosomes was performed.

Calibration experiments were performed using 2 µm and 4 µm PS beads with mixture suspensions to determine the best particle separation condition. Results were analyzed for flow rates varying from 480 µl/h to 720 µl/h using six different viscoelastic buffer solutions. Multiple particle trajectories with clear size bands for the2 µm, 4 µm, and larger aggregates (>4 um) were achieved at 720 µl/h using 9000 ppm PBS/PEO_{600kDa} (Fig. 2).

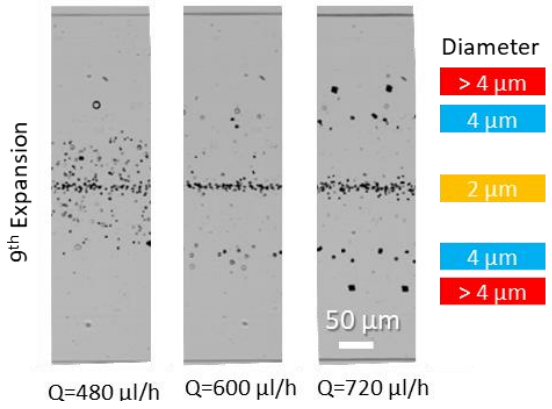

Fig. 2. Image stacks of 2 and 4µm PS bead mixture suspended in 9000 ppm PBS/PEO_{600kDa} at 480, 600, and 720 µL/h flow rates.

Size-based chromosome separation experiments were conducted at a 720 µl/h flow rate using 9000 ppm PBS/PEO_{600kDa} buffer solution (Fig. 3). Particle trajectories of individual chromosomes are generated for different size chromosomes. Single frame snapshots were inserted in Fig. 3 to show the size-based particle trajectories for chromosomes observed.

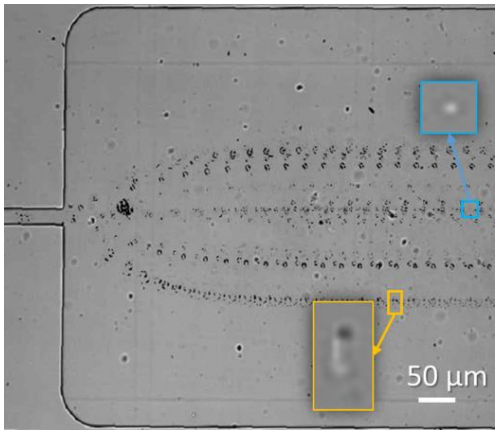

Fig. 3. Image stacked picture of chromosomes, suspended in 9000 ppm PBS/PEO_{600kDa} at 720 µL/h flow rate. The inserted images are the single-frame snapshots (7 times enlarged) from high-speed camera video recordings showing the location of 2.5 µm (blue outline) and 8.1 µm (yellow outline) size chromosomes.

4 Conclusion

The presented study investigated size-based human chromosome separation using PEO-based viscoelastic buffer solutions and viscoelastic pinched flow fractionation method in a cascaded configuration microchannel. Viscoelastic particle separation performance was optimized by tuning the fluid rheology and flow conditions using PS particles with diameters of 2 and 4 µm. Efficient size separation of chromosomes based on their size was achieved. The proposed device configuration is promising for the future of clinical studies in genetics, specifically aiming for the analysis of size-related chromosomal abnormalities.

This work was funded by Novo Nordisk Foundation Interdisciplinary Synergy Programme, under grant agreement No. 34948 (ChromoCapture).

References

1. M. Asghari, M Serhatlioglu, B Ortaç, M E Solmaz, C Elbuken, *Sci Rep* **7**, (2017)
2. H. Feng, M. Hockin, M. Capecchi, B. Gale, H. Sant, *Biomicrofluidics* **14** (2020)
3. D. Li, X. Lu, X. Xuan, *Anal Chem* **88**, (2016)
4. B. Ismail, *J Chromatogr A*, **30**, **(**2021)