Particle migration in viscoelastic microfluidics

Dan Yuan
*University of Wollongong*

Follow this and additional works at: [https://ro.uow.edu.au/theses1](https://ro.uow.edu.au/theses1)

---

**Copyright Warning**

You may print or download ONE copy of this document for the purpose of your own research or study. The University does not authorise you to copy, communicate or otherwise make available electronically to any other person any copyright material contained on this site.

You are reminded of the following: This work is copyright. Apart from any use permitted under the Copyright Act 1968, no part of this work may be reproduced by any process, nor may any other exclusive right be exercised, without the permission of the author. Copyright owners are entitled to take legal action against persons who infringe their copyright. A reproduction of material that is protected by copyright may be a copyright infringement. A court may impose penalties and award damages in relation to offences and infringements relating to copyright material. Higher penalties may apply, and higher damages may be awarded, for offences and infringements involving the conversion of material into digital or electronic form.

Unless otherwise indicated, the views expressed in this thesis are those of the author and do not necessarily represent the views of the University of Wollongong.

---

**Recommended Citation**


Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au
Particle migration in viscoelastic microfluidics

Dan YUAN

"This thesis is presented as part of the requirements for the award of the Degree of Doctor of Philosophy in Mechanical Engineering University of Wollongong"

June 2018
ABSTRACT

Particle migration is very important and essential for focusing, separating, counting, detecting or analysis in numerous biological and chemical applications. A variety of microfluidic devices have been designed to realize particle migration in Newtonian fluids. With the aid of external force fields, specially designed channel structures, or hydrodynamic forces, particles can migrate to one or several equilibrium positions in Newtonian fluids. While extensive research on fundamentals and application based on particle migration in Newtonian fluids has been conducted, there are fewer research on particle migration in non-Newtonian fluids. Actually, non-Newtonian fluids such as blood, cytoplasm, and many other body fluids, are very ubiquitous in our daily life and in real world issues. Therefore, it is important to invest our research focus on particle migration in non-Newtonian fluids to develop deep understanding of cell behaviours in these body fluids.

Recently, the research interests on particle migration based on non-Newtonian fluid have been increasing. The increasing attention on particle migration based on non-Newtonian fluids is a result of its interesting intrinsic fluid properties. Compared with particle focusing in a Newtonian fluid, 3D particle focusing in a non-Newtonian fluid can be easily realized in simple channels without the need of any external force fields; and particles with a much smaller size, such as from submicrometer to even nanometer particles can be manipulated. Particle migration in non-Newtonian fluids can break the limitations of requiring extra external components, lower throughput, complex fabrications in Newtonian fluids. Moreover, it can develop simpler, more flexible and versatile particle manipulation methods with lower costs. These advantages of non-Newtonian fluids allow fast growth of microfluidic applications based on viscoelasticity-induced particle migration, enabling the development of various micro-devices for biomedical and chemical analysis.

The main purpose of this research is to investigate particle migration in viscoelastic microfluidics, and develop microfluidic devices based on viscoelastic fluids to realize more efficient particle focusing, separation or solution exchange. These devices can be used as much easier particle manipulation methods in various biomedical or chemical
fields. Firstly, the concept of particle migration in viscoelastic fluid, and its applications were reviewed. Particle behaviours in viscoelastic fluid in a straight channel with asymmetrical expansion–contraction cavity arrays (ECCA channel) were investigated. Under the Dean-flow-coupled elasto-inertial effect, this device could offer a continuous, sheathless, and high throughput (>10000 s⁻¹) 3D particle focusing performance. Additionally, based on the same principle, continuous plasma extraction with high purity was achieved in this channel by simply adding polymer to blood. After two series of filtration with the same ECCA channel, the purity of 3 µm, 4.8 µm and 10 µm diameter particles reached 100%, and the plasma purity reached 99.99%.

Further, a novel microfluidic device for sheathless particle focusing and separation in viscoelastic fluid is proposed. The device consists of two stages: straight channel section with asymmetrical expansion–contraction cavity arrays (ECCA section) for sheathless Dean-flow-coupled elasto-inertial particle focusing (1st stage), and straight channel section for viscoelastic particle separation (2nd stage). This work investigates the on-chip washing process of microparticles and cells using co-flow configuration of viscoelastic fluid and Newtonian fluid in a straight microchannel.

Additionally, particle lateral migrations in sample-sheath flow with different properties were experimentally investigated. By using viscoelastic sample flow and Newtonian sheath flow, a selective particle lateral migration can be achieved in a simple straight channel, without any external force fields. Furthermore, the on-chip washing process of microparticles and cells using this co-flow configuration in a straight microchannel was studied. This technique may be a safer, simpler, cheaper, and more efficient alternative to the tedious conventional centrifugation methods, and may open up a wide range of biomedical applications.

Overall, viscoelastic fluids show a lot of advantages, therefore, allowing fast growth of microfluidic applications based on viscoelastisity-induced particle migration. The proposed devices can realize particle migration in an easier way, which show potential to be better alternatives in various biomedical and chemical detection and analysis.
ACKNOWLEDGEMENTS

First of all, I would like to express my greatest gratitude to my supervisor, Professor Weihua Li, for his great support and guidance on my way of exploring this novel and interesting research field. It’s his encouragements that kept me on when I came across difficulties. Moreover, he is not only an excellent supervisor in my research but also an approachable friend. I would like to express my deep gratitude to my supervisor, for his professional guidance, inspirational encouragement and patient support, and also for always being my inspiration.

Special thanks go to Dr. Jun Zhang, who is always warm at heart, selfless to share his working experience and give instructions. He helped me out of a lot of difficulties in my research work, and gave me many inspirations. I really feel grateful to him. He is not only a perfect colleague, but also a great friend who gave me a lot of help and advice in both life and work.

Then, I would like to thank Prof. Ronald Sluyter from School of Biological Sciences and Illawarra Health and Medical Research Institute, University of Wollongong, who kindly gave me a lot of technical training and support involving cell culture, staining, testing, and so on, which are very crucial in many of my research work.

Furthermore, I much appreciated the help and support given by Prof. Gursel Alici, Prof. Haiping Du and Prof. Enbang Li in this research, as well as Dr. Say Hwa Tan and Professor Nam-Trung Nguyen from Griffith University. I am also grateful to other colleagues who helped me in this or that way: Mr. Qianbin Zhao, Dr Shiyang Tang, Dr. Sheng Yan, Miss Xinxin Shao, Miss Weiping Hu, Dr. Jian Yang, Dr. Shuaishuai Sun, and etc.

I appreciate financial support from University of Wollongong-China Scholarship Council joint scholarships, which give me a chance to continue my higher degree study in this beautiful country, and broaden my visions and life experiences.

Last but not least, I’d like to give my deep gratitude to my family, especially my father Mr. Dezeng Yuan and my mother Mrs Xiuying Zhao, for their support and encouragement. They make this work more meaningful than it is.
TABLE OF CONTENTS

ABSTRACT ............................................................................................................................. I

ACKNOWLEDGEMENTS .................................................................................................... III

TABLE OF CONTENTS ........................................................................................................ IV

TABLE OF FIGURES ......................................................................................................... VIII

TABLE OF TABLES ............................................................................................................. I

1. INTRODUCTION ........................................................................................................ 1
   1.1. Background and motivation ................................................................................ 1
   1.2. Objectives .............................................................................................................. 2
   1.3. Outline of the thesis ............................................................................................... 2

2. LITERATURE REVIEW .............................................................................................. 6
   2.1. Hydrodynamic forces in viscoelastic fluids .......................................................... 8
       2.1.1. Inertial lift force ............................................................................................. 9
       2.1.2. Elastic force .................................................................................................. 9
       2.1.3. Drag force .................................................................................................... 10
   2.2. Basic particle migration in viscoelastic fluids in straight channels ...................... 11
       2.2.1. Particle migration in elasto-inertial flow ....................................................... 12
   2.3. Applications ......................................................................................................... 16
       2.3.1. Particle separation ....................................................................................... 16
       2.3.2. Cell deformability measurement and alignment ........................................... 21
       2.3.3. Particle Solution exchange ......................................................................... 24
       2.3.4. Rheometry-on-a-chip .................................................................................. 24
       2.3.5. Other applications (Mixing, heat transfer, droplet generation and sorting) .... 25
   2.4. Conclusion .............................................................................................................. 27

3. DEAN-FLOW-COUPLED ELASTO-INERTIAL THREE-DIMENSIONAL PARTICLE FOCUSING UNDER VISCOELASTIC FLOW IN A STRAIGHT CHANNEL WITH ASYMMETRICAL EXPANSION–CONTRACTION CAVITY ARRAYS .......................................................................................................................... 28
3.1. Introduction ..................................................................................................... 28
3.2. Principle of Dean-flow-coupled elasto-inertial particle focusing .............. 30
3.3. Methods ........................................................................................................... 33
   3.3.1. Design and fabrication of microfluidic device ........................................ 33
   3.3.2. Suspending fluids and particles ............................................................... 34
   3.3.3. Experimental setup .................................................................................. 34
3.4. Results ............................................................................................................. 35
   3.4.1. Effects of elasticity on particle migration ............................................... 35
   3.4.2. Effects of dean flow induced by expansion–contraction cavity arrays in ECCA channel ......................................................................................................... 37
   3.4.3. Effects of different flowing conditions ................................................... 38
   3.4.4. Side view of the channel ......................................................................... 40
   3.4.5. Effects of different distances from the inlet ............................................ 41
3.5. Conclusion ...................................................................................................... 42

4. CONTINUOUS PLASMA EXTRACTION UNDER VISCOELASTIC FLUID IN A STRAIGHT CHANNEL WITH ASYMMETRICAL EXPANSION–CONTRACTION CAVITY ARRAYS ........................................................................................................ 44

4.1. Introduction ..................................................................................................... 44
4.2. Schematic of plasma extraction based on dean-flow-coupled elasto-inertial effects ........................................................................................................................................................................ 46
4.3. Methods ........................................................................................................... 49
   4.3.1. Design and fabrication of microfluidic device ........................................ 49
   4.3.2. Preparation and rheology of the PEO solution ........................................ 49
   4.3.3. Particle preparation ............................................................................... 52
   4.3.4. Experimental setup ................................................................................ 52
4.4. Results ............................................................................................................. 53
   4.4.1. Effects of PEO concentrations and flow rates ........................................ 53
   4.4.2. Separation of particles with different sizes ............................................. 55
   4.4.3. Plasma extraction in diluted blood sample with different PEO concentrations ................................................................................................................................. 58
   4.4.4. Effects of distance from inlet ................................................................. 60
   4.4.5. Effects of blood hematocrit on focusing behavior .................................. 61
4.4.6. Purity of collected blood plasma ............................................................. 62
4.5. Conclusion ...................................................................................................... 63

5. SHEATHLESS DEAN-FLOW-COUPLED ELASTO-INERTIAL PARTICLE FOCUSING AND SEPARATION IN VISCOELASTIC FLUID ......................... 65

5.1. Introduction ..................................................................................................... 65
5.2. Schematic of sheathless particle focusing and separation ............................. 67
5.3. Methods ........................................................................................................... 68
5.3.1. Design and fabrication of microfluidic device ........................................ 68
5.3.2. Preparation of the PEO solution .............................................................. 68
5.3.3. Particle preparation ................................................................................. 69
5.3.4. Experimental setup .................................................................................. 70
5.4. Results ............................................................................................................. 70
5.4.1. Effects of flow rates ................................................................................ 70
5.4.2. Effects of distance from inlet .................................................................. 72
5.4.3. Separation of Jurkat cells and yeast cells ................................................ 75
5.5. Conclusion ...................................................................................................... 78

6. INVESTIGATION OF PARTICLE LATERAL MIGRATION IN SAMPLE-SHEATH FLOW OF VISCOELASTIC FLUID AND NEWTONIAN FLUID .......... 79

6.1. Introduction ..................................................................................................... 79
6.2. Schematic of lateral particle migration in sample-sheath flow of PEO solution-DI water, DI water-PEO solution, and DI water-DI water ...................... 80
6.3. Methods ........................................................................................................... 84
6.3.1. Design and fabrication of microfluidic device ........................................ 84
6.3.2. Preparation of the PEO solution .............................................................. 85
6.3.3. Particle preparation ................................................................................. 85
6.3.4. Experimental setup .................................................................................. 85
6.4. Results ............................................................................................................. 86
6.4.1. Effects of channel length ......................................................................... 86
6.4.2. Effects of flow rate .................................................................................. 87
6.4.3. Effects of PEO concentration .................................................................. 89
6.5. Conclusion ...................................................................................................... 91
7. ON-CHIP MICROPARTICLE AND CELL WASHING USING CO-FLOW OF VISCOELASTIC FLUID AND NEWTONIAN FLUID ......................................................... 92

7.1. Introduction ........................................................................................................ 92
7.2. Schematic diagram of particles’ on-chip washing using co-flow of viscoelastic fluid and Newtonian fluid ....................................................................... 94
7.3. The particle migration processes in sample viscoelastic fluid and sheath Newtonian fluid ................................................................................................. 96
7.4. Methods .............................................................................................................. 100
  7.4.1. Design and fabrication of microfluidic device .............................................. 100
  7.4.2. Preparation and rheology of the PEO solution ........................................... 100
  7.4.3. Particle and cell preparation ..................................................................... 101
  7.4.4. Experimental setup .................................................................................. 102
7.5. Results ............................................................................................................... 102
  7.5.1. On-chip particle washing ....................................................................... 102
  7.5.2. On-chip Jurkat cell washing .................................................................. 107
7.6. Conclusion ........................................................................................................ 111

8. CONCLUSION AND FUTURE WORK .................................................................. 112

8.1. Conclusion ....................................................................................................... 112
  8.1.1. Sheathless particle 3D focusing and separation ....................................... 112
  8.1.2. Particle solution exchange .................................................................. 113
8.2. Future work ...................................................................................................... 114
  8.2.1. More fundamental studies in viscoelastic fluids are needed .................. 114
  8.2.2. Particle interaction and the associated particle dynamics in viscoelastic fluid need further investigation ................................................................. 115
  8.2.3. Viscoelastic particle migration based on particle shape or deformability 116
  8.2.4. Realize real “label-free” bio-particle migration ...................................... 116

REFERENCES .......................................................................................................... 118

CONTRIBUTIONS TO THE THESIS ......................................................................... 135

OTHER PUBLICATIONS .......................................................................................... 137
TABLE OF FIGURES

Figure 2-1 Outline of particle migration in viscoelastic fluids. ........................................... 8

Figure 2-2 Schematic of the forces exerting on particles in viscoelastic fluids..................... 9

Figure 2-3 Separatrix in straight microchannels with (a) wide slit, W/H>>1; (b) circular cross section; (c) square cross section. Particles migrate towards different locations depending on their initial positions. They migrate toward centerline when they are inside the separatrix, and migrate towards the wall/corner when they are outside of the separatrix. (d) Secondary flows induced by the second normal stress difference (left) and the unstable separatrices (right) [52]. .................................................................................................. 12

Figure 2-4 (a) Particle equilibrium positions in inertial dominant focusing, elastic dominant focusing, and elasto-inertial particle focusing [47]. (b) Cross-sectional particle focusing in a shear-thinning PEO fluid in a microscale pipe flow at different flow rates [56]. (c) Cross-sectional particle focusing in a shear-thinning PEO fluid in a square microchannel at different flow rates [57]. (d) “Elasto-inertial” size-based particle separation in channel straight microchannel with a high width/height ratio in 1000 ppm PEO fluids [63]. (e) Multi-train particle elasto-inertial focusing in straight rectangular channels with high aspect ratios at different flow rates [59]. (f) “Elasto-inertial” single-line to multiple-line particle focusing in a high aspect ratio channel [60].......................... 15

Figure 2-5. Particle separation in viscoelastic fluids. (a) Elasto-inertial particle separation in PEO fluids in a square channel with high purity [66]. (b) Elasto-inertial exosomes separation from cell culture media or serum in a continuous, size-dependent, and label-free manner in a straight channel [67]. (c) “Elasto-inertial pinched flow fractionation” (eiPFF) size-based particle separation in PEO solutions in a T-shaped microchannel. {Lu, 2015 #201} (d) Sheathless particle focusing in a circular channel, followed by size-based particle separation using a symmetric bifurcation channel in elasticity dominant PVP fluids [69]. (e) Dean-flow-coupled elasto-inertial particle focusing and separation in spiral channel in PEO fluids [70]. (f) Sheathless, and high purity plasma extraction using dean-flow-coupled elasto-inertial effect in ECCA channel [71]. (g) Viscoelastic nonmagnetic particle 3D focusing, followed by size-based negative magnetophoresis particle separation in viscoelastic ferrofluid [72]................................................................. 17
Figure 2-6 Applications of viscoelasticity-induced particle migration in microfluidics. (a) Viscoelastic 3D particle pre-focusing, followed by cell deformability measurement based on the extensional field in rectangular channel in PVP solution [83]. (b) Sheath-free RBC vertical concentration and positioning induced by fluid viscoelasticity in a rectangular channel [84]. (c) On-chip particle washing using coflow of a PEO-contained viscoelastic fluid and a Newtonian fluid [85]. (d) Elastic effects on bubble generation processes in a microchannel [86].

Figure 3-1 Simulation results of the flow field in the cross section 10 μm away from the cavity along the x direction and the schematic illustration of the focusing mechanism in Newtonian (a) and non-Newtonian (b) fluid. (a) The red circles represent two equilibrium positions that resulted from a balance between the inertial lift force $F_L$ and secondary flow drag $F_D$, and (b) The red circle represents the single equilibrium position by the synergetic effect of inertia, secondary flow and viscoelasticity. The dashed circles are the unstable equilibrium positions. The arrows represent the magnitude and direction of the secondary flow field induced by the abrupt contraction of the channel.

Figure 3-2 Schematic diagrams of particle focusing in Newtonian and non-Newtonian fluids in ECCA channel. Particles are randomly injected to the inlet, and finally focused in a single line on the opposite cavity side at the outlet in non-Newtonian fluids in Dean-coupled elasto-inertial effects, while focused to two equilibrium positions on the cavity side at the outlet in Newtonian fluids in the combination of Dean and inertial effects.

Figure 3-3 The schematic geometry of the microfluidic channel with triangular expansion–contraction cavity arrays, $L_0 = 48$ mm, $L_1 = 900$ μm, $L_2 = 900$ μm.

Figure 3-4 The fluorescent images of 3.2-μm, 4.8-μm, 13-μm particles at the outlet under the flow rate of 60 μl/min$^{-1}$ in Newtonian and non-Newtonian fluids, respectively, and corresponding fluorescent intensity profiles for each particle type along the width of the channel.

Figure 3-5 The fluorescent images of 4.8-μm particles at the outlet in straight rectangular channel under the flow rate of 150 μl/min$^{-1}$ in DI water and in PEO under different flow rates, respectively. (b) The fluorescent intensity profile in DI water. Particles focus to four equilibrium positions centred at the faces of the channels. The middle fluorescent
intensity peak, which is higher than the other two peaks, represents two overlap equilibrium positions. (c) The fluorescent intensity profiles in PEO under different flow rates. Particles in 100 μm × 50 μm channel can only distribute in a wide central area along the width of the channel without the aid of secondary flow. ........................................37

Figure 3-6 The fluorescent images of 4.8μm particle at the outlet at the flow rate of 30 μl/min⁻¹, 60 μl/min⁻¹, 120 μl/min⁻¹, 240 μl/min⁻¹ in 500ppm PEO and DI water fluids, respectively, and corresponding fluorescent intensity profiles for different flow rates along the width of the channel. ..........................................................................................39

Figure 3-7 Fluorescent images of the side view of the channel when particle focusing in 500ppm PEO solution and DI water, respectively, and corresponding fluorescent intensity profiles ..............................................................................................................................41

Figure 3-8 4.8 μm particle distributions in ECCA channel at different distances from the inlet in 500ppm PEO. (a) At the inlet, no focusing is observed and the particles are still randomly distributed. (b) At the middle of the channel (approximately 2.5 cm from the inlet), the particles are confined within a narrow band along the channel wall on the opposite side of cavity. (c) At the outlet of the channel (approximately 4.5 cm from the inlet), 3D focusing on a line is achieved. (d) The fluorescence intensity profiles at the inlet, middle and outlet ........................................................................................................42

Figure 4-1 Schematic diagram of blood cells focusing in diluted blood sample with PEO in ECCA channel, and experimental demonstrations at outlet of ECCA channel in diluted blood sample with and without PEO. (a) Schematic diagram of blood cells focusing in diluted blood sample with PEO in ECCA channel. (b) Simulation results of velocity magnitude and shear rate in the cross section of ECCA channel. (c) Simulation results of the flow field 10 μm away from the cavity. (d) Experimental demonstrations at outlet of ECCA channel in diluted blood sample with and without PEO. ................................................................47

Figure 4-2 Viscosity of PEO solutions as a function of shear rate for polymer concentration of 500 ppm, 1000 ppm, 2000 ppm ........................................................................50

Figure 4-3 Effects of PEO concentrations and flow rates for focusing of 4.8 μm particles. The captured fluorescent picture for distribution of 4.8 μm particles at outlet section in
500 ppm, 1000 ppm and 2000 ppm PEO solution, respectively, with flow rates ranging from 20 µl/min to 80 µl/min, and corresponding fluorescent intensity profile. The green bar beside each PEO concentration shows the optimal flow rate range for 4.8 µm particle filtration.

Figure 4-4 Filtration of particles with different size in 1000 ppm PEO solution with the flow rate range from 20 µl/min to 80 µl/min (captured fluorescent picture and corresponding fluorescent intensity profile), and the optimal flow rate range for filtration of 3 µm, 4.8 µm, and 10 µm particles respectively.

Figure 4-5 3 µm, 4.8 µm, and 10 µm particle concentrations measured from both inlet and filtrated PEO solution from outlets (ii) and (iii) and corresponding fluorescent images.

Figure 4-6 Plasma extraction in diluted blood sample with PEO concentration 500 ppm, 1000 ppm, and 2000 ppm from 20 µl/min flow rate to 80 µl/min flow rate (bright fields and corresponding intensity profile), and optimal filtration flow rate range for each PEO concentration.

Figure 4-7 Effects of distance from inlet.

Figure 4-8 Effects of blood hematocrit on the focusing behavior.

Figure 4-9 Purity of collected blood plasma. (a) Blood cells filtration in diluted blood sample with 1000 ppm PEO at flow rate of 50 µl/min. (b) Blood cell concentration before and after two series filtration. (c) Working device. (d) The original blood sample at the inlet, and blood cells from outlet (i), plasma from outlets (ii) and (iii) after first filtration, and plasma from outlets (ii) and (iii) after second filtration. (e) The events in blood sample at inlet detected by flow cytometry. (f) The events in the plasma collected at the outlets (ii) and (iii) after second filtration.

Figure 5-1 Schematic of the device for sheathless particle viscoelastic focusing and separation.

Figure 5-2 Distribution of particles at the expansion area at outlet: (a) The captured fluorescent images and corresponding normalized fluorescent intensity profiles at
different flow rates; (b) Normalized lateral position and width of particles at different flow rates..................................................72

Figure 5-3 Distribution of particles at different channel positions: (a) The captured fluorescent images at $Q = 5 \mu l/min$ and corresponding normalized fluorescent intensity profiles at different positions; (b) Normalized lateral position and focusing width of particles at different distances from inlet.................................................................73

Figure 5-4 (a) Comparison of particle separation in a viscoelastic fluid (PEO solution) and a Newtonian fluid (DI water) in the same integrated ECCA straight channel. (b) Comparison of particle separation in the integrated ECCA straight channel and pure straight channel in viscoelastic fluids.................................................................75

Figure 5-5 Cells’ distributions at different channel positions.............................................76

Figure 5-6 Separation of Jurkat cells and yeast cells in the viscoelastic fluids. (a) The distribution of Jurkat and yeast cells at the expansion region under different flow rates (captured images in bright field and corresponding normalized cell count). (b) The recovery rate and purity of cells at each outlet at $Q = 20 \mu l/min$.................................77

Figure 6-1 (a) Schematic diagram of particle lateral migration in sample-sheath flow of 1000 ppm PEO solution and DI water in a straight channel. (b) Simulation results of velocity profile. (c) Shear rate and forces experienced by particles. (d) Experimental demonstration of lateral particle migration in sample-sheath flow of 1000 ppm PEO solution-DI water in straight channel at inlet and outlet (e) with a flow rate of 5 $\mu l/min$ sample flow-20 $\mu l/min$ sheath flow and corresponding fluorescent intensity profiles. The red area indicates the distribution and intensity of red-dyed PEO solution, and the green area indicates the distribution of the green-dyed particles. 82

Figure 6-2 Fluorescent images of particle lateral migration in sample-sheath flow of (a) DI water -DI water, and (b) DI water-1000 ppm PEO solution at 10 mm from the inlet and corresponding fluorescent profiles .................................................................84
Figure 6-3 Particle lateral migration phenomenon at (a) the inlet; (b) 5 mm downstream; (c) 10 mm downstream from the inlet; (d) the corresponding normalised fluorescent intensity profiles.

Figure 6-4 The fluorescent images of 10 mm downstream from inlet, and (b) The corresponding fluorescent intensity profiles along the width of the channel. (c) The difference between the lateral positions corresponding to 0.2 normalised fluorescent intensity of red-dyed PEO solution and the peak fluorescent intensity of green-dyed particles.

Figure 6-5 The effects of different PEO concentrations (a) 500 ppm; (b) 1000 ppm; (c) 5000 ppm on particle lateral migration and the corresponding fluorescent intensity profiles.

Figure 6-6 Rheological properties of 500 ppm, 1000 ppm, 5000 ppm PEO solutions. (a) the amplitude dependence of the viscoelastic modulus (G' and G") of each viscoelastic fluid, where G' and G" represent storage modulus and loss modulus, respectively; (b) the damping ratio (the ratio of loss modulus and storage modulus, G"/G') of each PEO solution.

Figure 7-1 Schematic diagram of on-chip particle washing using co-flow of a viscoelastic fluid and a Newtonian fluid.

Figure 7-2 Particle migration processes.

Figure 7-3 Viscosity of Newtonian fluid and 1000 ppm PEO solution as a function of shear rate.

Figure 7-4 On-chip particle washing. (a) Fluorescent images of particle migration processes for mixture of 0.8-µm + 2-µm, 0.8-µm + 3-µm, 0.8-µm + 5-µm, 0.8-µm + 10-µm particles at inlet, 1.5mm from inlet, 3mm from inlet, and outlet. (b) Corresponding normalised fluorescent intensity profiles.

Figure 7-5 Particle lateral positions along the channel length at the inlet (0 mm), 1.5 mm from the inlet, 3 mm from the inlet, and the outlet (5 mm).
Figure 7-6 Fluorescent images of mediums from the inlet (left panels), and outlets 1 and 2 (as indicated) for particle mixtures of (a) 0.8-µm + 2-µm, (b) 0.8-µm + 3-µm, (c) 0.8-µm + 5-µm, and (d) 0.8-µm + 10-µm on a hemocytometer. ........................................ 106

Figure 7-7 On-chip Jurkat cells washing. (a) Jurkat cell migration processes at inlet, 1.5 mm from the inlet, 3mm from the inlet, and the outlet. (b) The original Jurkat cell culture medium and the mediums exit from outlet 1 and outlet 2 after cell washing process. (c) Hemocytometer images of the inlet and the two outlets after cell washing. (d) Flow cytometer results of the two outlets. ............................................................. 110

Figure 7-8 The absorbance spectra of the mediums from the two inlets and the two outlets tested by plate reader. ............................................................................................................ 110
TABLE OF TABLES

Table 1 $R_c$ and $W_i$ for 500 ppm, 1000 ppm, 2000 ppm PEO solutions .......................... 51

Table 2 $R_c$ and $W_i$ for 1000 ppm PEO solution............................................................. 69
1. Introduction

1.1. Background and motivation

Microfluidics, which can precisely manipulate and control fluids and particles at micron and submicron dimensions, has many advantages compared with the traditional macroscale platform, including reduced sample volumes, low cost, fast processing and analysing, high efficiency, high sensitivity, and high accuracy [1, 2]. Currently, it has been widely used in medical, chemical, biological and environmental fields [3-5]. Particle manipulating technologies such as focusing [6, 7], separation [8], trapping [9], and stretching [10, 11], are indispensable abilities in microfluidics. A variety of particle manipulating technologies have been developed based on either active approaches using external forces induced by electric [12-15], magnetic [16, 17], acoustic [18-20] and optical [21] fields; or passive intrinsic hydrodynamic forces [6, 22-28]. However, most of these manipulating methods are performed and studied in Newtonian fluids. Besides, active microfluidic methods often require extra external components, have lower throughput, and passive methods always need complex fabrication processes and lack flexibility. Therefore, it’s of great importance to break these limitations and develop simpler and easier particle manipulation methods with lower costs.

Recently, the research interests on particle migration based on viscoelastic fluids have been increasing. Viscoelastic fluids such as blood, cytoplasm, and many other body fluids, are very ubiquitous in our daily life and in real world issues. Therefore, it is important to invest our research focus on particle migration in non-Newtonian fluids to develop deep understanding of cell behaviours in these body fluids.

A non-Newtonian fluid is a fluid that does not follow Newton's Law of Viscosity. Newton’s law of viscosity defines the relationship between the shear stress and shear rate of a fluid subjected to a mechanical stress. The non-Newtonian fluids either has a shear-dependent or shear-independent viscosity. The non-Newtonian fluids can be formed by dissolving long chain molecules (PEO (poly (ethylene oxide)), PVP (poly (vinyl pyrrolidone)), PAA (polyacrylamide), PAM (polyacrylamide), DNA, HA (hyaluronic acid)) in Newtonian base medium. The polymers can induce viscoelastic effect in their dissolved solutions.

The increasing attention on particle migration based on viscoelastic fluids is a result of
its interesting intrinsic fluids properties. Compared with particle focusing in a Newtonian fluid, in a viscoelastic fluid 3D particle focusing can be easily realized in simple channels without the need of any external force fields, and particles with a much smaller size, such as from submicrometer to even nanometer particles can be manipulated. These advantages of viscoelastic fluids allow fast growth of microfluidic applications based on viscoelastisity-induced particle migration, enabling the development of various micro-devices for biomedical and chemical analysis.

1.2. Objectives

Viscoelastic fluids exhibit a lot of advantages due to its intrinsic properties. The purpose of this work is to investigate particle migration in viscoelastic fluids, aiming to find simpler particle manipulation methods, and better alternatives in various biomedical and chemical detection and analysis. The major objectives of the work include:

1) To investigate particle behaviours in viscoelastic fluids based on an ECCA channel, and develop a continuous, sheathless, and high throughput (>10000 s⁻¹) 3D particle focusing method.
2) To continuously isolate plasma with a high purity from blood under viscoelastic fluids in an ECCA channel by exploiting the dean-flow-coupled elasto-inertial effects.
3) To design and fabricate a novel integrated microfluidic device for sheathless particle focusing and separation in viscoelastic fluids.
4) To develop a new particle lateral migration technique by using sample-sheath flow of viscoelastic fluid and Newtonian fluid.
5) To achieve an on-chip washing process of microparticles and cells using co-flow configuration of viscoelastic fluid and Newtonian fluid in a straight microchannel.

1.3. Outline of the thesis

Chapter 1 provides the background and motivation of this work. A brief introduction of the latest techniques of particle manipulation in microfluidics, including active and passive methods, is presented. The current importance and advantages of particle migration in viscoelastic fluids are described. The objectives of this work are also discussed.
Chapter 2 summarizes recent progress of particle migration in viscoelastic fluids. Firstly, the fundamental hydrodynamic forces in viscoelastic fluids are introduced. Next, the basic particle migration in viscoelasticity-dominant fluid and elasto-inertial fluids in straight channels are elaborated. After that, a comprehensive review of the applications of viscoelasticity-induced particle migration in microfluidics is prescribed; this includes particle separation, cell deformability measurement and alignment, particle solution exchange, and many other discovered applications in microfluidics.

Chapter 3 investigates particle behaviours in viscoelastic fluids in a straight channel with asymmetrical expansion–contraction cavity arrays (ECCA channel). Under the Dean-flow-coupled elasto-inertial effect, this device can offer a continuous, sheathless, and high throughput (>10000 s⁻¹) 3D particle focusing performance. Particle focusing was demonstrated experimentally in this channel with Newtonian and non-Newtonian fluids using three different sized particles (3.2µm, 4.8 µm, 13 µm), respectively. Also, the effects of dean flow (or secondary flow) induced by expansion–contraction cavity arrays were highlighted by comparing the particle distributions in a single straight rectangular channel with that of in an ECCA channel. Finally, the influences of flow rates and distances from the inlet on focusing performance in the ECCA channel were studied. The results show that in the ECCA channel particles were focused on the cavity side in Newtonian fluids due to the synthesis effects of inertial and dean-drag force, whereas on the opposite cavity side in non-Newtonian fluids due to the addition of viscoelastic force. Compared with the focusing performance in Newtonian fluids, the particles are more easily and better focused in non-Newtonian fluids. Besides, the Dean flow in visco-elastic fluids in an ECCA channel improves the particle focusing performance compared with that in a straight channel. A further advantage is three-dimensional (3D) particle focusing that in non-Newtonian fluids is realized according to the lateral side view of the channel while only two-dimensional (2D) particle focusing can be achieved in Newtonian fluids. Conclusively, this novel Dean-flow-coupled elasto-inertial focusing technique may be valuable in various applications from high speed flow cytometry to cell counting, sorting, and analysis.

Chapter 4 realized continuous plasma extraction under viscoelastic fluids in the ECCA channel by exploiting the dean-flow-coupled elasto-inertial effects. Firstly, 4.8 µm
diameter particles, to mimic the behaviour of red blood cells (RBCs), were used to study the effects of poly(ethylene oxide) (PEO) concentrations and flow rates on particle viscoelastic focusing. Then, 3 µm, 4.8 µm and 10 µm diameter particles, which are comparable in size to platelets, RBCs, and white blood cells (WBCs), were used to study the effect of particle sizes on particle viscoelastic focusing. Finally, plasma extraction from diluted blood sample under viscoelastic conditions was conducted, and purity of collected blood plasma was measured. After two series of filtration with the same ECCA channel, the purity of 3 µm, 4.8 µm and 10 µm diameter particles reached 100%, and the plasma purity reached 99.99%, measured by a hemocytometer. In addition, flow cytometry data further validated the filtration performance of blood plasma. By exploiting the dean-flow-coupled elasto-inertial effects, this ECCA channel offers a continuous, sheathless, and high purity plasma extraction.

Chapter 5 proposes a novel microfluidic device for sheathless particle focusing and separation in viscoelastic fluids. The device consists of two stages: a straight channel section with asymmetrical expansion–contraction cavity arrays (ECCA section) for sheathless Dean-flow-coupled elasto-inertial particle focusing (1st stage), and a straight channel section for viscoelastic particle separation (2nd stage). At stage 1, particles with diameters of 4.8 µm and 13 µm were both focused at the opposite side of the cavities. Then, the particles were subsequently separated at 2nd stage based on the differential focusing dependency on size. The effects of flow rates and channel length on particle separation were investigated. Particle separation in both viscoelastic fluids and Newtonian fluids was also compared to elucidate the differences. In addition, particle separation in the straight channel and the integrated ECCA straight channel was also studied. The proposed device was used to separate human Jurkat cells (an immortalized T cell line) and yeast cells. Experimental results show that this technique offers an efficient, continuous, and sheathless particle separation in viscoelastic fluids.

Chapter 6 develops a new particle lateral migration technique by using sample-sheath flow of viscoelastic fluids and Newtonian fluids. The 4.8 µm micro-particles were dispersed in a PEO (polyethylene oxide) viscoelastic solution, then the solution was injected into a straight rectangular channel with a DI (deionised) water Newtonian sheath flow. Micro-particles suspended in PEO solution migrated laterally to a DI water stream,
but migration in the opposite direction from a DI water stream to a PEO solution stream or from one DI water stream to another DI water stream could not be achieved. The lateral migration of particles depends on the viscoelastic properties of the sample fluids. Furthermore, the effects of channel length, flow rate, and PEO concentration were studied. By using viscoelastic sample flow and Newtonian sheath flow, a selective particle lateral migration was achieved in a simple straight channel, without any external force fields. This particle lateral migration technique could be potentially used in solution exchange fields such as automated cell staining and washing in microfluidic platforms, and holds numerous biomedical applications.

Chapter 7 achieved an on-chip washing process of microparticles and cells using co-flow configuration of viscoelastic fluids and Newtonian fluids in a straight microchannel. By adding a small amount of biocompatible polymers into the particle medium or cell culture medium, the induced viscoelasticity can push particles and cells laterally from their original medium to the co-flow Newtonian medium. This behavior can be used for particle or cell washing. First, we demonstrated on-chip particle washing by the size-dependent migration speed using a co-flow of viscoelastic fluids and Newtonian fluids. The critical particle size for efficient particle washing was determined. Second, we demonstrated continuous on-chip washing of Jurkat cells using co-flow of viscoelastic fluids and Newtonian fluids. The lateral migration process of Jurkat cells along the channel length was investigated. In addition, the cell washing quality was verified by hemocytometry and flow cytometry with a recovery rate as high as 92.8%. Scanning spectrophotometric measurements of the media from the two inlets and the two outlets demonstrated that diffusion of the co-flow was negligible, indicating efficient cell washing from culture medium to phosphate-buffered saline medium. This technique may be a safer, simpler, cheaper, and more efficient alternative to the tedious conventional centrifugation methods, and may open up a wide range of biomedical applications.

Chapter 8 highlights the major findings and contributions of this thesis, as well as several directions for future work.
2. Literature Review*

Initially, particle migration in a viscoelastic non-Newtonian fluid was investigated in a channel with a macroscale dimension. These studies were generally conducted using two typical flows: Poiseuille flow in a circular pipe and Couette flow in two parallel plates. In 1966, Karnis and Mason first studied the migration of a sphere in a viscoelastic liquid in a pipe flow (pipe diameter ~ cm) when inertia is negligible, and observed the sphere approaches the centre of the pipe regardless of its initial position [29]. After that, the motion of particles in Couette flows (the laminar flow of a viscous fluid in the space between two parallel plates, of which one is moving relative to the other) was studied experimentally [30, 31]. Tehrani [32] investigated experimentally on particle migration in viscoelastic fluids used in hydraulic fracturing. He found that the migration occurs toward the region of lower shear rate in the suspensions with a moderate particle volume fraction. Thus in Boger fluid [33], an elastic fluid with constant viscosity which is independent of the shear rate, particles are pushed towards the centreline in case of Poiseuille flow (steady viscous fluid flow driven by an effective pressure gradient established between the two ends of a long straight pipe with a uniform circular cross-section) while aggregate close to the outer wall in a Couette flow. Ho and Leal [34] theoretically studied the lateral migration of a neutrally buoyant rigid sphere suspended in a second order fluid and demonstrated that the particle migration is induced by the non-uniform normal stress distribution and particles move towards the low normal stress difference regions. Later, Huang et al. [35] conducted a direct simulation of the motion of solid particles in Couette and Poiseuille flows of viscoelastic fluids, and found inertia, elasticity, shear thinning and the blockage ratio of the channel affect the stable equilibrium positions of neutrally buoyant particles. Moreover, through the same method, Huang and Joseph [36] specifically studied the effects of shear thinning on migration of neutrally buoyant particles in a pressure driven flow. As opposite to the fact that the inertia tends to push the particles away from both the walls and the centerline in Newtonian fluids, in viscoelastic fluids with shear-thinning effects, an annular particle-
free zone at an intermediate radii can be created due to the fact that the elasticity causes them to migrate toward the centreline, and shear thinning moves the particles away from the centerline but toward the channel walls. The annular particle-free zone expands with the increment of shear thinning.

Recently, due to the advancement of micro- and nano-fabrication technology and blooming of microfluidics, researchers started to investigate particle behaviours in viscoelastic fluids in a confined channel with a micrometer scale. Based on 3D numerical simulations and experiments, D’ Avino [37] demonstrated the presence of a bistability scenario for transversal migration of particles suspended in a viscoelastic fluid in a micro-pipe. They observed a neutral cylindrical surface, which acts as a separatrix of particles’ initial positions and divides particles to migrate either to the channel wall or the centreline.

Recent review articles from D’Avino et al. [38] and Liu et al. [39] describe the mechanism of viscoelasticity-driven particle cross-flow migration from both experiments and theoretical analysis, and various passive particle manipulation techniques, respectively. Given that the fundamentals involving viscoelastic fluids have been extensively studied, and studying and applying viscoelastic fluids in a wide range of applications within the context of microfluidics has recently gaining momentum and is evolving very quickly, in this chapter, we discuss the up-to-date opportunities provided by conducting particle migration in viscoelastic fluids mainly from the aspect of applications, laying out a comprehensive perspective on their potentials in future lab-on-a-chip platforms. The outline of particle migration in viscoelastic fluids is shown in Figure 2-1.
2.1. **Hydrodynamic forces in viscoelastic fluids**

In viscoelastic fluids, both the inertial effect and viscoelastic effect may contribute to particle migration. Three hydrodynamic forces may exert on particles flowing in viscoelastic fluids: elastic force $F_E$, inertial lift force $F_L$, and drag force $F_D$. Consequently, the particle trajectories and equilibrium positions are tuned by competition of these forces. A schematic of the forces exerting on particles in viscoelastic fluids is shown in Figure 2-2.
2.1.1. Inertial lift force

When particles are flowing in a fluid with un-negligible inertia, they are affected by an inertial lift force. The inertial effect has been used for various microfluidic applications such as focusing [40, 41], separation [42, 43], and filtration [44, 45]. The inertial lift force $F_L$, which is comprised of the shear gradient lift force and wall lift force, induces the lateral migration of particles toward equilibrium positions between channel centerline and channel walls. The inertial lift force can be expressed as [6, 46]:

$$F_L = \frac{\rho_f V_m^2 a^4}{D_h^2} f_L(R_c, x_c)$$

(1)

$$R_c = \frac{\rho V_m D_h}{\mu_f}$$

(2)

where $\rho_f$ is the fluid density, $\mu_f$ is the dynamic viscosity, $D_h$ is the hydraulic diameter for a channel, $V_m$ is the average velocity of the channel flow, $a$ is the particle diameter, $f_L(R_c, x_c)$ is the lift coefficient of net inertial lift force [6], $R_c$ is the Reynolds number.

2.1.2. Elastic force

When particles are flowing in a viscoelastic fluid, they are affected by an elastic force. The Weissenberg number $W_i$ (or Deborah number $D_e$), which is the ratio of the relaxation time $\lambda$ of the fluid to the characteristic time $t_f$ of the channel flow, is a dimensionless number to characterise the elastic effects [47]:

Figure 2-2 Schematic of the forces exerting on particles in viscoelastic fluids.

$F_L$: Inertial Lift Force
$F_D$: Drag Force
$F_E$: Elastic Force
\[ W_i = \frac{\lambda}{t_f} = \dot{\gamma} = \lambda \frac{2V_w}{D_h} \]  \hspace{1cm} (3)

where \( \dot{\gamma} \) is the average (characteristic) shear rate, and can be expressed as \( 2V_w / D_h \). In viscoelastic fluids, both the first \( (N_1 = \tau_{xx} - \tau_{yy}) \) and second normal stresses \( (N_2 = \tau_{yy} - \tau_{zz}) \) contribute to particle migration. \( \tau_{xx}, \tau_{yy}, \) and \( \tau_{zz} \) are normal stresses that direct toward the flow, the velocity gradient and vorticity direction, respectively. Normally, the effects of \( N_2 \) can be neglected in the diluted viscoelastic solutions, because \( N_1 \) is much larger than \( N_2 \) [48, 49]. When the \( N_2 \) is very small, the elastic force \( F_E \) originates from an imbalance in the distribution of \( N_1 \) over the size of the particle [39] can be expressed as:

\[ F_E = C_{el} a^3 \nabla N_1 = C_{el} a^3 (\nabla \tau_{xx} - \nabla \tau_{yy}) = -2C_{el} a^3 \eta_p \lambda \nabla \dot{\gamma}^2 \]  \hspace{1cm} (4)

where \( C_{el} \) is the non-dimensional elastic lift coefficient, \( \eta_p \) is the polymeric contribution to the solution viscosity.

The relative importance of elastic to inertial effects can be defined as \( El \), which results in different particle migration behaviours in viscoelastic fluids. \( El \) is expressed as the ratio of Weissenberg number \( W_i \) and Reynolds number \( R_c \):

\[ El = \frac{W_i}{R_c} = \frac{2\lambda \mu_f}{\rho_f D_h^2} \]  \hspace{1cm} (5)

2.1.3. Drag force

The viscous drag force, which is induced due to the velocity difference of fluid element and particles, can also affect particle migration. Assuming a spherical particle travelling in a uniform Stokes flow, the drag force can be expressed as [6, 28]:

\[ F_D = 3\pi \mu_f a (\nu_f - \nu_p) \]  \hspace{1cm} (6)

where \( \nu_f \) and \( \nu_p \) represent the velocities of fluid elements and particles, respectively.
2.2. Basic particle migration in viscoelastic fluids in straight channels

In viscoelastic fluids, the relative importance of elastic effect to inertial effect leads to different particle migration phenomenon. In the following sections, we’ll describe the basic particle migration in (i) viscoelasticity-dominant flow, and (ii) in elasto-inertial flow.

If particles migrate in viscoelastic fluids flow with negligible inertia, it’s called the viscoelasticity-dominant particle migration.

Numerical simulations and experiments were employed to study viscoelasticity-induced focusing of particles in pressure-driven wide slit [50], circular [51] and square-shaped microchannel [52-54] under inertialess conditions.

In general, in the elasticity dominant flow with inertia neglected, a separatrix divided the particles migration direction according to particles initial positions, Figure 2-3(a-c). Particles migrate toward centerline if they are inside the separatrix, and migrate towards the wall/ corner if they are outside of the separatrix. The separatrix can be regarded as the locus of particle positions where migration velocity of particles is zero. However, these positions are unstable positions, because the net forces on particles along both sides are directing outward, and a slight disturbance may push particles toward the close walls or the centerline. Besides, the position of the separatrix depends on rheological properties of the suspending fluid. The separatrix moves towards the wall as the shear-thinning become less pronounced. A weaker shear-thinning can also slow down the lateral migration of particles. Meanwhile, by increasing De (or Wi), the separatrix moves towards the channel centerline, thus increasing the corner-attractive region [52].

In Giesekus fluids (viscoelastic fluids based on Giesekus model), due to the existence of a secondary normal stress difference, the secondary flow which is orthogonal to the flow direction appears, Figure 2-3(d). The existence of secondary flows strongly affects the particle migration dynamics, giving rise to complex behaviors depending on the blockage ratio and the Deborah number. For a sufficiently small particle size and relatively large Deborah number, the velocity components of the secondary flows overcome the migration velocity, and new attractor appears to drive particles to follow vortex trajectories. On the other hand, as the Deborah number decreases and particle diameter increases, the attractor will disappear [52].
Figure 2-3 Separatrix in straight microchannels with (a) wide slit, W/H>>1; (b) circular cross section; (c) square cross section. Particles migrate towards different locations depending on their initial positions. They migrate toward centerline when they are inside the separatrix, and migrate towards the wall/corner when they are outside of the separatrix. (d) Secondary flows induced by the second normal stress difference (left) and the unstable separatrices (right) [52].

2.2.1. Particle migration in elasto-inertial flow

When particles migrate in viscoelastic fluids flow with a finite fluid inertia, the inertial effect becomes apparent. With a proper inertial effect, the particle focusing phenomenon becomes intriguing, and the throughput for particle focusing may be increased. The wall-lift force induced by the finite inertia can impair the corner attraction effect of elasticity,
and equilibrium positions at corners become unstable, and particles focus only at the channel centerline, which is termed as elasto-inertial focusing [55].

The concept of “elasto-inertial particle focusing” was first proposed to illustrate the 3D sheathless focusing in a straight square channel by Yang et al. [47]. They proposed three different focusing regions according to the importance of elasticity and inertia, as shown in Figure 2-4 (a). In inertia dominant flow, the particles are gathered at four equilibrium positions facing the center of each wall due to the inertial lift force. In elastic dominant flow, particles migrate to the lower first normal stress regions corresponding to the centerline and four corners under the pure elastic effects. The multiple equilibrium positions are not favorable in practical applications. Then, the equilibrium positions can be reduced to one at the center by increasing the flow rate and regulating the relative importance of inertial and elastic effects.

Meanwhile, the elasto-inertial particle migration with an increasing inertia was investigated in straight microchannels with circular [56] and square [57] cross sections, respectively, using a holographic technique. In a circular pipe flow in a PEO fluid with shear-thinning property, particles can only be confined within the channel central area at lower flow rates, but disperse again as the flow rate increases [56], as shown in Figure 2-4(b). Similarly, in a square microchannel in PEO fluids, under a balanced condition of inertia and elasticity, the particles can be focused tightly, but the increased flow rate and small blockage ratio are not in favor of particle focusing [57], as shown in Figure 2-4(c). The re-disperse phenomenon is believed to be due to the increased inertial effects (the shear-gradient lift force directs to the channel wall) as well as the substantially decreased viscosity for shear-thinning fluids. However, in elasticity-dominant PVP fluids in a circular pipe flow [56] or a square microchannel [57], the particles migrate toward the tube center as flow rate, blockage ratio and channel length increase.

Besides the circular and square cross sections, elasto-inertial particle migrations in rectangular channels with different aspect ratios were studied. The continuous-flow sheath-free size-based particle separation in viscoelastic fluids in high width/height rectangular microchannels were realized [58], Figure 2-4 (d). Multiple particle focusing lines were observed in rectangular channels with a high aspect ratio [59] [60], Figure 2-4(e and f). The reason is that as the inertial effect increases, the shear-gradient lift begins
to take effect. As its direction points towards the channel wall, it may defocus the particles from the centerline.

Besides the widely used PEO and PVP viscoelastic aqueous solution some other solutions are used as viscoelastic fluids. Since relatively high flow rates are needed to turn on inertial effects in elasto-inertial focusing, the throughput is higher than that in viscoelasticity-dominant focusing. Moreover, if the solution is with a low viscosity but high elasticity, such as DNA or hyaluronic acid (HA) solutions, the throughput can be enhanced significantly.

Based on the larger size and longer relaxation time of DNA, particle focusing in DNA solution in good quality over a wide range of flow rates (0.08-30µl/min) was achieved [61]. Furthermore, Lim et al. [62] reported successful “elasto-inertial” particle focusing at an extremely high flow rate (50 ml/min) in HA solution, which is the highest reported flow rate for particle viscoelastic focusing up till now.
Figure 2-4 (a) Particle equilibrium positions in inertial dominant focusing, elastic dominant focusing, and elasto-inertial particle focusing [47]. (b) Cross-sectional particle focusing in a shear-thinning PEO fluid in a microscale pipe flow at different flow rates [56]. (c) Cross-sectional particle focusing in a shear-thinning PEO fluid in a square microchannel at different flow rates [57]. (d) “Elasto-inertial” size-based particle separation in channel straight microchannel with a high width/height ratio in 1000 ppm PEO fluids [63]. (e) Multi-train particle elasto-inertial focusing in straight rectangular channels with high aspect ratios at different flow rates [59]. (f) “Elasto-inertial” single-line to multiple-line particle focusing in a high aspect ratio channel [60].
2.3. Applications

The basic particle migrations in viscoelasticity-dominant flow and elasto-inertial flow in straight channels have been discussed above. The intrinsic viscoelasticity of fluid brings many intriguing and unique phenomenon on particle migration. In addition, viscoelasticity-induced particle migration in microfluidics can be effectively used in a wide range of applications, which will be elaborated in the following sections.

2.3.1. Particle separation

Separation of biological particles (DNA, cells, virus, bacteria, exosomes etc.) is highly important in various biomedical and biotechnological applications [64, 65], because they are promising biomarkers in the early detection and diagnosis of many diseases. Many researchers have developed the bioparticles separation techniques in viscoelastic fluids.

2.3.1.1. Aid of sheath flow

In viscoelastic fluids, particles are prone to migrate to the center of the channel under a proper elasto-inertial effect, and the migration speed is proportional to particle size. By pre-aligning all the particles with different sizes at the same initial position, the following size-dependent lateral migration speed will create a lateral gap between different sized particles. Therefore, particle separation based on lateral position difference can be achieved.

With the aid of sheath flow, elasto-inertial particle separation in a PEO fluid in a square channel with high purity was realized [66], as shown in Figure 2-5 (a). Viscoelastic sample suspension was injected from two side inlets, while the viscoelastic sheath flow was injected from the middle inlet, squeezing the particles to the sidewalls of the channel. The four inertial equilibrium positions are broken by the elastic forces, whose direction is away from the wall, leaving one equilibrium position in the centerline of the channel. As the inertial and elastic forces are both proportional to the particle sizes, larger particles migrate more quickly than smaller ones, resulting in their separation. Through this separation principle, platelets were separated from diluted whole blood with high purity (nearly 99.9%). RBCs and WBCs with larger sizes migrated to the center of the channel, while platelets with smaller sizes remained focused along the side walls with a minimum
lateral displacement.

Figure 2-5. Particle separation in viscoelastic fluids. (a) Elasto-inertial particle separation in PEO fluids in a square channel with high purity [66]. (b) Elasto-inertial exosomes separation from cell culture media or serum in a continuous, size-dependent, and label-free manner in a straight channel [67]. (c) “Elasto-inertial pinched flow fractionation” (ePFF) size-based particle separation in PEO solutions in a T-shaped microchannel. [68] (d) Sheathless particle focusing in a circular channel, followed by size-based particle separation using a symmetric bifurcation channel in elasticity dominant PVP fluids [69]. (e) Dean-flow-coupled elasto-inertial particle focusing and separation in spiral channel in PEO fluids [70]. (f) Sheathless, and high purity plasma extraction using dean-flow-coupled elasto-inertial effect in ECCA channel [71]. (g) Viscoelastic nonmagnetic particle 3D focusing, followed by size-based negative magnetophoresis particle separation in viscoelastic ferrofluid [72].
Based on the size difference of blood cells and bacteria, bacteria were separated successfully from whole blood using elasto-inertial effect in straight channel with the aid of sheath flow in viscoelastic fluids [73]. Similarly, Liu et al. [67] successfully separated exosomes from cell culture media or serum in a continuous, size-dependent, and label-free manner with a high separation purity (> 90%) and a high recovery (> 80%) in a straight channel, as shown in Figure 2-5 (b). The proposed technique may serve as a versatile platform to facilitate exosome analyses in diverse biochemical applications.

Based on the same principle, Lu and Xuan [68] proposed the “elasto-inertial pinched flow fractionation” (eiPFF) to separate particles by size in PEO solutions in a T-shaped microchannel, as shown in Figure 2-5 (c). They investigated in detail the factors influencing the separation performance in eiPFF method, including the PEO concentration, the flow rate and flow rate ratio of sample flow and sheath flow, the particle size, the aspect ratio of the channel. Furthermore, shape-based particle separation in the eiPFF was demonstrated [74]. Particles with spherical and peanut-shaped rigid particles of equal volume were separated in 1000ppm PEO solution, whereas separation cannot be realized in Newtonian fluids.

Apart from the normally used PEO solution, separation of particles with four different sizes in DNA solution was also achieved using the same mechanism [61]. Compared with the particle separation in Newtonian fluids, the separation resolution has been proven significantly enhanced in viscoelastic DNA fluid.

2.3.1.2. Symmetric bifurcation channel

Besides sheath flow, symmetric bifurcation channel were proposed to initially align particles along the sidewall to optimize the particle differential migration in viscoelastic fluids. Nam et al. [69] realized particle focusing in a circular channel, followed by size-based particle separation using a symmetric bifurcation channel in elasticity dominant PVP fluids, as shown in Figure 2-5 (d). The difference in lateral displacements of particles was magnified by a sudden expansion region. Besides, they also realized the size-based elasto-inertial separation of RBCs, platelets, and 15µm polystyrene particles. Although the flow rate was limited, it was the first report to realize sheathless particle initialization
and continuous label-free particle separation in viscoelastic flow. Moreover, they found that microchannel with a high-aspect ratio in the first focusing stage and a low-viscosity polymer (HA solution) can improve the separation throughput [75]. Additionally, they successfully separated malaria parasite from WBCs with high recovery rate (94%) and purity (99%) [75]. They further modified the device using a commercially available circular capillary tube for particle pre-focusing [76], and realized separation of MCF-7 cells from leukocytes with a high recovery rate and a high purity based on the same principle.

2.3.1.3. Aid of Dean flow

In a curved or a straight channel with contraction and expansion cavity arrays, the Dean flow appears and can be adjusted to enhance particle separation.

In a spiral channel, when the inertia effect is not negligible, particles are affected by the “dean-flow-coupled elasto-inertial” effect. Particles can be focused in a 3D manner or separated by the combined effects of three forces: inertial lift force $F_L$, including the shear-gradient lift force ($F_{LS}$), wall-repulsion force ($F_{LW}$), the Dean drag force $F_D$ resulting from the curved channel geometry, and elastic force $F_E$ induced by the nature of the viscoelastic medium. Based on the effect, different sized particles are focused differentially at the outlet, and particle separation was realized [70], as shown in Figure 2-5 (e). The differential migration and separation of particles was also achieved in a novel integrated 2-spiral microdevice in viscoelastic fluids [77]. The throughput and separation efficiency have been improved significantly.

Precise manipulation of sub-micrometer to nanometer-scale particles in passive microfluidics is technically challenging because the Brownian motion may become dominant once the particles size is reduced to the nanometer-scale. However, recently it has been proven that it may be achievable by employing fluid viscoelasticity [78, 79]. The submicron polystyrene (PS) beads were successfully focused in the center and corners in a rectangular channel in PEO solution by the pure elastic effect, and flexible DNA molecules were also focused in the centerline of the channel by the integration of elastic force and wall lift force originating from the flexibility of DNA molecules [78].
Flow focusing of sub-micrometer particles in cylindrical channels has been reported by De Santo et al. [79]. In addition, they proposed a simple theoretical model that supports the experimental evidence, and links the trapping force to a dimensionless parameter comparing viscoelastic normal forces and Brownian forces. Using a double spiral channel, \(\lambda\)-DNA molecules/blood platelets were focused and separated successfully in a low molecular weight PEO solution [80]. The superior manipulation capacity by viscoelastic fluids on submicro to nano particles is a significant advantage of viscoelastic microfluidics compared with other passive microfluidic manipulation technologies.

2.3.1.4. **Aid of magnetophoresis**

Viscoelastic particle migration is one of the passive methods, when integrated with other active methods, the device can be more versatile, and provide more precise control of target bio-particles. By integrating positive magnetophoresis with viscoelastic particle focusing, for the first time, Del Giudice and his coworkers [81] realized the high efficiency magnetic particle separation in a H-shaped channel in PAM solution. The magnetic particles were firstly pre-focused at the centerline of rectangular channel by viscoelastic force, and then attracted laterally to the sheath fluid by a permanent magnet. The pre-focusing in viscoelastic fluids prevents particles from getting stuck on the wall, and flowing out to the undesired outlet in the magnetic deflection section. Using this method, the magnetic and non-magnetic particles are separated with a high efficiency. Later, Zhang et al. [72] and Kim et al. [82] proposed similar methods combining viscoelastic focusing and negative magnetophoresis to separate non-magnetic particles in the ferrofluid with viscoelastic base medium. The viscoelastic force was used to align particles at the channel centreline before the magnetic region, and size-dependent magnetophoretic force was applied on particles to separate particles by size, as shown in Figure 2-5 (f). The integration of viscoelastic particle migration and active methods makes the viscoelastic microfluidics more flexible and versatile, with potential more functionality.
2.3.2. Cell deformability measurement and alignment

Figure 2-6 Applications of viscoelasticity-induced particle migration in microfluidics. (a) Viscoelastic 3D particle pre-focusing, followed by cell deformability measurement based on the extensional field in a rectangular channel in PVP solution [83]. (b) Sheath-free RBC vertical concentration and positioning induced by fluid viscoelasticity in a rectangular channel [84]. (c) On-chip particle washing using coflow of a PEO-contained viscoelastic fluid and a Newtonian fluid [85]. (d) Elastic effects on bubble generation processes in a microchannel [86].

Cell deformability is a promising label-free biomarker for the diagnosis of diseases [87, 88]. The alteration in the deformability of cells compared with healthy cells can be utilized to detect blood diseases [89], cancers [87, 88, 90, 91] and so on. For example, the alteration in the deformability in RBCs can help diagnose the malaria, sickle cell anemia, and diabetes diseases. The reduction in stiffness of the cells in abnormal tissue is an indication of cancer. Compared with the conventional molecular-based biomarkers, which require pre-processing steps, costly antibodies or dyes, the deformability as a biomarker is label-free, simpler, cheaper and has the potential for automated measurement [92, 93].
Villone et al. has studied the lateral migration of deformable particle in tube flow of Newtonian and viscoelastic media through 3D finite element method numerical simulations [94]. They found that if the particle is initially not at the channel axis, it attains an asymmetric shape. In a Newtonian liquid, the migration is always directed towards the tube axis. In a viscoelastic liquid, the migration direction and velocity depend on the competition among particle deformability, fluid elasticity, or fluid viscosity shear thinning. In a certain range of parameters, an unstable radial position appears, which separates the region where the migration is directed towards the axis from the region where it is directed towards the wall.

The migration phenomenon in rectangular channels is different. As we know that the rigid spherical particles in viscoelastic fluids without inertia in rectangular channels migrate towards the centerline and corners, while the deformability effect results in additional wall repulsion forces to exert on deformable particles or cells and selectively entrains them at the centerline. Based on this principle, Yang et al. [95] successfully extracted WBCs (collected along the channel corners) from RBCs (concentrated at the centerline of the channel) with a purity of 35% from a dilute input solution with WBC/RBC ratio of 0.17%.

Recently, based on the viscoelastic 3D particle pre-focusing, cell deformability measurement with a high detection efficiency using the extensional flow field in rectangular channel in PVP solution was realized [83], as shown in Figure 2-6 (a). After initially homogenization of cell trajectories, the RBC will be delivered to a stagnation point for stretch measurement. The detection efficiency was improved compared with that in Newtonian fluids. They also found that after heat treatment, RBC deformability can be changed; after nutrient starvation in human mesenchymal stem cells, their deformability will be decreased. Using the similar method, Kim et al. performed shape measurement of ellipsoidal particles in the cross-slot microchannel utilizing viscoelastic particle focusing [96]. The shapes of ellipsoidal particles with various aspect ratios were successfully measured. This method can break the limitation of particle aggregation or uncertainty out-of-plane arrangement of particles in conventional methods such as optical microscopy, and can be useful in a wide range of applications such as shape measurement of nonspherical cells.
RBCs are normally arbitrarily oriented in Newtonian fluids, which is not favorable for accurate cell monitoring and counting in optical imaging system. Sheath-free RBC vertical concentration and positioning induced by fluid viscoelasticity in a rectangular channel has been demonstrated [84], as shown in Figure 2-6 (b). Monitored by a digital holographic microscopy, RBCs can be in high uniformity under the effect of the elastic force, and the cell overlapping can be reduced. Therefore, the out-of-focus blurring can be eliminated, and the detection sensitivity can be increased.

Moreover, the abnormal erythrocyte deformability which is potential for label-free diagnosis of hematological diseases was studied [97]. By employing digital in-line holographic microscopy (DIHM), they investigated the lateral migration of human erythrocytes induced by viscoelastic fluids flow in a rectangular microchannel. Different from rigid spheres and hardened erythrocytes, deformable normal erythrocytes become more dispersed in the channel center area as the flow rate increases. Additionally, normal erythrocytes have a higher angle of inclination than hardened erythrocytes in the region near the side-walls of the channel.

Most recently, a striking collective swimming of bovine sperm in dynamic clusters, enabled by the viscoelasticity of the fluid was reported [98]. Sperm oriented in the same direction within each cluster, and cluster size and cell-cell alignment strength increased with viscoelasticity of the fluid. The collective swimming induced by elasticity may facilitate sperm migration and contribute to successful fertilization. This work implies that the fluid elasticity may be of great importance in biological function, since almost all biological fluids are viscoelastic in nature.

For the first time, Holzner et al. investigated the elasto-inertial focusing of mammalian cells and bacteria using low molecular and low viscosity PEO solutions [99]. This kind of PEO solution exhibits negligible shear thinning, and particles can be focused over a wide range of elasticity numbers and a large range of Reynolds numbers. In addition, the influence of blockage ratio, volumetric flow rate, cell concentration, and polymer chain length was assessed. The current method can be used to focus cells without inducing bodily rotation, which is especially useful in morphology-based analysis of disease-infected cells and suggests the realization of an optofluidic platform for imaging and cytometric analysis in the short term.
2.3.3. Particle Solution exchange

Particle focusing and separation using sample-sheath flow with the same fluid property (either Newtonian fluids or non-Newtonian fluids) has been explored extensively, while sample-sheath flow with different fluid properties has rarely been studied. Jayaprakash et al. [100] investigated the dynamics of aqueous droplets of different size and viscosity at the interface of a coflowing stream of immiscible oils (silicone oil as primary and mineral oil as secondary continuous phases). They elaborated that the competing noninertial lift and interfacial tension forces govern the interfacial migration of the droplets. Sorting of droplets based on size contrast is demonstrated as well. Later, they reported the dynamical migration behavior of rigid polystyrene microparticles at an interface of coflowing streams of primary (aqueous) and secondary (oils) immiscible phases at low Reynolds numbers in a microchannel [101]. They found that the migration criterion depends on the sign of the spreading parameter and the presence of surfactant at the interface, and the interfacial perturbation can cause detachment of microparticles from the interface. Moreover, size based sorting of microparticles was demonstrated. Recently, Ha et al. [102] have studied the lateral migration behaviors of particles using the co-flow configuration of viscoelastic and Newtonian fluids. Ha et al. [102] implemented particle separation using $\lambda-DNA$ viscoelastic and Newtonian fluids. Instead of using $\lambda-DNA$ to form viscoelastic fluids, as shown in Figure 2-6 (c). Recently, the viscoelastic/Newtonian interfacial effect has been investigated, and a size-based separation of microparticles in co-flow of Newtonian (water or PBS) and viscoelastic fluids (PEO) were illustrated with the aid of interfacial effect [103]. Small particles cannot traverse the interface between Newtonian and viscoelastic media due to the dominance of wall-directed interfacial elastic lift forces, whereas large particles cross the interface due to the dominated inertial lift forces. Additionally, they realized the separation of Staphylococcus aureus (1 μm) from platelets (2-3 μm) with high efficiency and purity.

2.3.4. Rheometry-on-a-chip

Microfluidic viscometers for the shear rheology and extensional rheology of complex fluids and biofluids have been investigated extensively recently [104-106]. These miniaturized devices are helpful especially in the case of low viscosity, weakly elastic fluids, low sample volume and so on. Rheometry-on-a-chip has been proposed by...
exploiting the characteristics of viscoelastic fluids in microfluidic channels. By testing creep recovery in a microfluidic device, the measurement of relaxation time of viscoelastic fluids at low strain has been realized [107]. Microfluidic rheometric devices that are capable of measuring fluid relaxation times down to 1 ms have also been proposed by Zilz et al. [108] based on the scaling behaviour of the onset of a purely-elastic flow instability in a microfluidic serpentine channel. This microfluidic rheometric device can assess lower molecular weight materials, solvent viscosities or concentrations than that using the state-of-the-art commercial rheometers. Besides, Del Giudice et al. [109] found an effective way to measure relaxation times of viscoelastic fluids based on the particle migration phenomenon occurring when the suspending viscoelastic fluids flow in straight microfluidic channels, which does not need a calibration curve, and more easily detectable compared with conventional techniques. Two glycerol-water solutions on PEO and PAM solutions at various concentrations were tested. Later, they used a homemade µ-rheometer based on the viscoelasticity-induced cross-flow migration to measure a characteristic relaxation time of polyelectrolytes solutions [110]. Relaxation time as small as 60 µs was detected, which is the highest resolution compared with the conventional and other µ-rheometrical techniques. Results from two different microrheometrical techniques: relaxation times in shear flow measured through the µ-rheometer based on the viscoelastic alignment of particles in a straight microchannel, and in extensional flow measured in a microfluidic optimized cross-slot configuration based on the onset of the flow-induced birefringence were compared [111]. It is found that microfluidic techniques can capture very small relaxation times of dilute polymer solutions, and there is a good agreement from the two platforms. The advances in microfluidic rheology will help us better understand these complex fluids, and uncover their properties, as well as mechanisms in relation to the behaviors of living biofluids.

2.3.5. Other applications (Mixing, heat transfer, droplet generation and sorting)

Efficient mixing is required in a wide range of microfluidic applications, such as chemical synthesis and micro or nanoparticle production. Microfluidic flows are typically laminar because of the low Reynolds number $R_c$. The viscoelasticity of polymer solutions can generate unstable flows even when $R_c$ is very small and can significantly enhance mixing efficiency. Based on the chaotic vortex dynamics of a viscoelastic flow, an efficient
microfluidic mixer was proposed by Hong et al. [112]. In viscoelastic fluids, as the Reynolds number $R_c$ and Weissenberg number $W_i$ both increase to a certain value, the instability of elasto-inertial flow will appear, leading to chaotic vortices in the side wells. This kind of instability can enhance the mixing of adjacent fluid streams. In order to improve mixing, a channel that had sudden expansion and contraction along with teeth patterns along the channel walls was employed by Julius et al. to perform viscoelastic mixing [113]. They also completed on-chip erythrocytes lysis based on the efficient mixing effect. The erythrocytes are gradually lysed along the channel, leaving WBCs at the outlet.

By employing the instability of a viscoelastic fluid with a lower viscosity, fluid mixing at subcritical Reynolds numbers was induced, thus the heat transfer in a serpentine channel was improved [114]. Due to the low pumping cost of the fluid, it would be an attractive cooling method, which can be used in industrial applications such as next generation photonic devices for enhanced local hot-spot cooling. Heat transfer in both Newtonian and non-Newtonian viscoelastic fluids was investigated by embedding Ti–Pt films on the bottom of channel wall as temperature sensors [115]. They concluded that due to the instability of the viscoelastic fluids, which can induce fluid irregular motion, it has a better heat transfer performance compared with that of Newtonian fluids.

Viscoelastic fluids have also been used for droplet generation and sorting. Droplet separation was achieved based on the intrinsic viscoelastic fluid properties [116]. Droplets with different intrinsic viscous and viscoelastic properties relative to the continuous oil phase would migrate to high or low shear rate regions. This passive droplet sorting method is simpler and in lower cost, and can be potentially used in the chemical or biological fields where droplets with different intrinsic viscoelastic properties need to be separated. The effect of elasticity on bubble generation processes in a microchannel was also investigated [86], as shown in Figure 2-6 (d). They found that even a small amount of polymer can have significant effect on bubble generation. Due to the instability of the flow, the generated bubble sizes are fluctuated. Compared with the bubble generated in Newtonian fluids, the bubble were thinner along the minor axis in the viscoelastic fluids due to the viscoelastic effect.
2.4. Conclusion

In this chapter, particle migration in viscoelastic fluids were discussed mainly from the aspect of applications. The hydrodynamic forces and the basic particle migration in viscoelasticity-dominant and elasto-inertial flow in straight channels were elaborated. After that, the applications of viscoelasticity-induced particle migration in microfluidics, including particle separation, cell deformability measurement and alignment, particle solution exchange, rheometry-on-a-chip and many other applications in microfluidics were comprehensively reviewed.
3. Dean-flow-coupled elasto-inertial three-dimensional particle focusing under viscoelastic flow in a straight channel with asymmetrical expansion–contraction cavity arrays*

3.1. Introduction

Particle focusing is very important and essential for separating [8], sorting, counting [117], detecting and analysis in numerous biological and chemical applications. Three-dimensional (3D) particle focusing, where particles can eventually form one single equilibrium position in flow field, is the most desirable focusing condition in various microfluidic applications such as the flow cytometers used for the detection and enumeration of bio-particles. Various microfluidic devices were designed to realize the particle migration and focusing to one or several equilibrium positions in a Newtonian fluid. The migration of particles is based on their intrinsic physical characteristics such as particle size, shape, density, polarizability and magnetic susceptibility. Focusing techniques can be classified to two categories: active methods and passive methods. Active methods are based on the application of external force fields. Various active methods have been proposed, such as dielectrophoresis (DEP) [12-15], magnetophoresis [16, 17], acoustophoresis [118], and optical tweezers [21]. These active methods can provide precise control of target bio-particles. However, they have a low throughput and require extra, expensive device components for the external forces. Passive methods are based on the microchannel geometrical effects and hydrodynamic forces [22], such as pinched flow fractionation (PFF) [26], hydrodynamic filtration [27], Dean-flow coupled inertial effects[6, 119], deterministic lateral displacement (DLD) [120], surface acoustic wave (SAW)-induced streaming [19]. These passive methods are simple, effective and have a high throughput.

Recently, particle manipulation including focusing and separation in non-Newtonian fluids have gained significant attention because the positive first normal stress difference

* Results of this chapter are published in: Dan Yuan, Jun Zhang, Sheng Yan, Chao Pan, Gursel Alici, Nam-Trung Nguyen, Weihua Li. Dean-flow-coupled elasto-inertial three-dimensional particle focusing under viscoelastic flow in a straight channel with asymmetrical expansion-contraction cavity arrays. Biomicrofluidics 07/2015; 9(4):044108.
($N_f$) arising in pressure driven flows of dilute polymer solutions can lead suspended particles or cells to migrate to the mid–plane of the channel [35, 121, 122]. Leshansky et al. observed that the particles migrate toward the centreline due to the imbalance in the first normal stress difference between the centreline and the walls in a slit channel. These particles are two-dimensionally focused under the conditions of negligible inertia and dominant elasticity. However, in planar rectangular channels, the elasticity results in particle migration towards the centreline and the corners of the channel which corresponds to the low first normal stress regions, so this approach cannot be directly applied to 3D focusing. Yang, et al [47], reported that the number of multiple equilibrium positions can be reduced to one equilibrium at the centreline by properly adjusting the flow rate due to the synergetic effect of inertia and viscoelasticity. By balancing the elastic and inertial forces, he demonstrated particle focusing on a flow centreline of a square microchannel by using a synergetic combination of elasticity and inertia of viscoelastic fluid flow. D’Avino et al. [37] performed 3D numerical simulations and experiments to demonstrate particle focusing through viscoelasticity-induced migration in pressure-driven flows in simple cylindrical micropipes. Seo, et al [56], investigated the elasto-migration of microparticles in a microscale pipe flow of viscoelastic fluids using a holographic technique, and evaluated the effects of blockage ratio, flow rate, and entry length on particle migration. Lu and Xuan presented an experimental study of continuous particle separation in viscoelastic solutions via a combined action of elastic and inertial lift forces, which is termed as elasto-inertial pinched flow fractionation (eiPFF).

3D particle focusing and separation is achieved in Newtonian fluids in channels with expansion–contraction cavity arrays by Je-Kyun Park’s group [123]. He exploits the dean flow effects and inertial effects and also uses the aid of sheath flow to realize 3D focusing. In this work, 3D sheathless particle focusing in an ECCA channel which also used the geometry of expansion-contraction cavity arrays was demonstrated by exploiting the Dean-flow-coupled elasto-inertial effects. The differences are that in an ECCA channel, the viscoelastic fluids is used instead of the Newtonian fluids, and thus 3D particle focusing can be sheathless and easily realized. In this work, we demonstrated particle focusing experimentally in this channel under Newtonian and non-Newtonian fluids using three different sized particles (3.2µm, 4.8 µm, 13 µm), respectively. The influences of flow rate and distance from the inlet on focusing performance were also studied. The
particles are focused on the cavity side in a Newtonian fluid by the synthesis of inertial and Dean-drag force, whereas on the opposite cavity side in a non-Newtonian fluid due to the addition of viscoelastic force. Besides, the Dean effects in the ECCA channel improves the particle focusing performance compared with that of in straight channel. To our best knowledge, the Dean-flow-coupled elasto-inertial focusing in such a device has not been explored yet. This Dean-flow-coupled elasto-inertial microfluidic device reported here could offer a continuous, sheathless, and high throughput (>10000 s⁻¹) 3D focusing performance, which may be valuable in various applications from high speed flow cytometry to cell counting, sorting, and analysis.

3.2. Principle of Dean-flow-coupled elasto-inertial particle focusing

The Dean-flow-coupled elasto-inertial particle focusing is to harmonize three kinds of forces: Lift force $F_L$, including the shear-gradient lift force ($F_{LS}$), wall-repulsion force ($F_{LW}$); the Dean drag force $F_D$ resulting from the curved channel geometry; and elastic force $F_E$ induced by the nature of the viscoelastic medium. Figure 3-1 shows the simulation results of the flow field in the cross section 10 μm away from the cavity along the x direction and the schematic illustration of the focusing mechanism in Newtonian (a) and non-Newtonian (b) fluids at the outlet in the ECCA channel. COMSOL Multi-physics 5.2 (COMSOL, Burlington, MA) finite element software was used to calculate the flow field. In order to save computational time and space, only 2 of 26 expansion–contraction cavities in a straight channel was analysed using this software. The calculated flow field was used to help analyse the mechanism of particles focusing in this microchannel. The laminar steady incompressible flow model was used. The inlet condition was set with a uniform average velocity calculated from the flow rate, and the non-slip boundary condition was applied onto the surfaces of the channel. The magnitude of the secondary flow field in this simulation is represented by $\sqrt{\nu_x^2 + \nu_y^2}$. When particles flowing in straight channels in Newtonian fluids, the shear gradient lift force pushes particles away from the centreline of the channel where corresponds to the low shear rate region, while the wall lift force drives particles away from the channel wall. The four equilibrium positions are formed as shows in Figure 3-1 (a) (the four black dashed circles). When the particles are not in straight channels but in the ECCA channel with a Newtonian fluid, the effects of inertial migration and secondary flow act in superposition on the particles, thus
the four equilibrium positions induced by Lift force $F_L$ in rectangular channels are destroyed by the dean drag force $F_D$ and particles become stable in two modified equilibrium positions, Figure 3-1 (a) (the red circles). The arrows in Figure 3-1 represent the magnitude and direction of the secondary flow field induced by the abrupt contraction of the channel. When particles flowing in the ECCA channels with a viscoelastic non-Newtonian fluid, the elastic force $F_E$ should be considered, which is directed away from the wall and decays with increasing distance from the wall. In a straight rectangular channel with non-Newtonian fluids, the particles tend to migrate to the centreline of the channel by the synergetic effect of inertia and viscoelasticity. In the ECCA channel, the particles become unstable when an additional secondary flow drag is exerted on the particles. Subsequently, a new equilibrium position can be achieved near the channel wall by the combined effects of lift force $F_L$, elastic force $F_E$ and the Dean drag force $F_D$, as shown in Figure 3-1 (b). Finally, 3D particle focusing is realized in a ECCA channel with the combined effect of Dean-flow-coupled elasto-inertial forces.

Figure 3-1 Simulation results of the flow field in the cross section 10 µm away from the cavity along the x direction and the schematic illustration of the focusing mechanism in Newtonian (a) and non-Newtonian (b) fluid. (a) The red circles represent two equilibrium positions that resulted from a balance between the inertial lift force $F_L$ and secondary flow drag $F_D$, and (b) The red circle represents the single equilibrium position by the synergetic effect of inertia, secondary flow and viscoelasticity. The dashed circles are the unstable equilibrium positions. The arrows represent the magnitude and direction of the secondary flow field induced by the abrupt contraction of the channel.
After a comprehensive review of the working principle of particle focusing in Newtonian and non-Newtonian fluids, particle focusing experiments were performed using both Newtonian and non-Newtonian fluids in the ECCA channel. Figure 3-2 shows the schematic diagram of particle focusing in Newtonian and non-Newtonian fluids in the ECCA channel. There are 26 repeated expansion–contraction triangular cavities in this channel, while only the inlet and outlet section of the channel is illustrated here. Particles are randomly injected to the inlet, so from the top view, cross section view and side view in the amplified inlet section the particles are dispersed. As the particle suspension flows, particles are driven into a single line along the channel wall on the opposite cavity side in non-Newtonian fluids with the Dean-flow-coupled elasto-inertial effects described above. However, in a Newtonian fluid, because there is no elastic effect induced by the polymers, particles are focused to two equilibrium positions on the cavity side at the outlet in a Newtonian fluid as result of Dean and inertial effects.

Figure 3-2 Schematic diagrams of particle focusing in Newtonian and non-Newtonian fluids in ECCA channel. Particles are randomly injected to the inlet, and finally focused in a single line on the opposite
cavity side at the outlet in non-Newtonian fluids in Dean-coupled elasto-inertial effects, while focused to two equilibrium positions on the cavity side at the outlet in Newtonian fluids in the combination of Dean and inertial effects.

3.3. Methods

3.3.1. Design and fabrication of microfluidic device

![Figure 3-3](image)

Figure 3-3 The schematic geometry of the microfluidic channel with triangular expansion–contraction cavity arrays, $L_0 = 48$ mm, $L_1 = 900$ μm, $L_2 = 900$ μm.

The right angled isosceles triangular cavities are patterned on one side of a straight channel. The channel has a cross section of 100 μm x 40 μm (width x height). The longest edge of the triangle is $L_1 = 900$ μm, and the space between two adjacent cavities is uniform at $L_2 = 900$ μm. The total length of the straight channel is $L_0 = 48$ mm, including 26 repeated expansion–contraction triangular cavities. Its schematic geometry is shown in Figure 3-3.

The device was fabricated by standard photolithography and soft lithography techniques. The fabrication included rapid prototyping on a silicon master, polydimethylsiloxane (PDMS) replica moulding, and sealing through plasma oxidation. Briefly, photoresist (SU-8 2025, MicroChem Co., Newton, MA) was spun on a silicon wafer at 2000 rpm to a thickness of 40 μm, and then exposed to UV light through a designed mask using a mask aligner system (ABM, San Jose, CA). After that the photoresist on the silicon wafer was developed in an SU-8 developer solution and rinsed by isopropylalcohol (IPA) to create a positive replica of channel geometry. A PDMS mixture with a 10:1 ratio of base to agent (Dow Corning, Midland, MI) was poured over the silicon master, degassed to remove bubbles in a vacuum oven, and cured at 100°C for 45 min. After the PDMS was cured and taken out of silicon master, the inlet and outlet holes were punched with a custom needle tip. Finally, the PDMS slide was bonded with another PDMS slide after exposure to oxygen plasma (PDC-002, Harrick Plasma, Ossining, NY) for 3 minutes.
3.3.2. Suspending fluids and particles

In this work, two kinds of fluids were prepared: Newtonian fluids (deionized water), and Non-Newtonian fluids (moderate elasticity fluid). For the moderate elasticity fluid, PEO (poly (ethylene oxide), $M_w = 2\times10^6$, Sigma-Aldrich) was added to deionized water in 500 ppm. The density of the fluid matches with the polystyrene (PS) particles (1.05 g cm$^{-3}$). The PEO solution is considered to have a constant shear viscosity of $3.12\times10^{-3}$ Pa s under the present experimental conditions and its relaxation time is $9.1\times10^{-3}$ s. The viscosities for deionized water is $1.0\times10^{-3}$ Pa s. Tween 20 (0.01 wt%, Sigma-Aldrich) was added to all the solutions to prevent particle-particle adhesion.

Internally dye d fluorescent polystyrene microspheres were purchased from Thermo Fisher Scientific, USA. A particle suspension was prepared by diluting 3.2 μm (product no.G0300, CV<5%), 4.8 μm (product no.G0500, CV < 5%), and 13 μm (product no.G1000, CV < 5%) particles to the concentration of ~$10^7$ particles ml$^{-1}$ by deionized water and PEO solution respectively. This concentration was considered to be low enough to neglect any interaction between particles in the micro-channel. Before the experiment, the particles are shaken by a vortex device to guarantee a good suspension.

3.3.3. Experimental setup

The particle suspensions were transferred to a 1 ml syringe, and then introduced into the microfluidic chip through a silicon tube by a syringe pump (Legato 100, Kd Scientific). The outflow of the particle suspension was collected in a glass bottle. The microfluidic chip was placed on an inverted microscope (CKX41, Olympus, Japan), and illuminated by a mercury arc lamp. The images of the fluorescent particles were observed and captured by a CCD camera (Rolera Bolt, Q-imaging, Australia) which had a maximum capturing speed of 50 frames per second. The fluorescent images were then post-processed and analysed with the software Q-Capture Pro 7 (Q-imaging, Australia). The flow rate in the experiment was increased from $10$ μl/min$^{-1}$ to $300$ μl/min$^{-1}$, which corresponds to an average fluid velocity from $0.04$ m s$^{-1}$ to $1.2$ m s$^{-1}$. A profile of the fluorescent intensity was taken from the outlet of the last cavity to examine the focusing performance of this microfluidic device in both Newtonian and non-Newtonian fluids.
3.4. Results

3.4.1. Effects of elasticity on particle migration

To investigate the effects of elasticity on particle migration, experiments were carried out in both Newtonian and non-Newtonian fluids in the ECCA channel using three different particle sizes (3.2 μm, 4.8 μm, 13 μm) with the flow rate increasing from 10 μl/min$^{-1}$ to 300 μl/min$^{-1}$. The results show that particles are more easily and better focused in non-Newtonian fluids. Figure 3-4 shows the fluorescent images of 3.2-μm, 4.8-μm, 13-μm particles at the outlet under the flow rate of 60 μl/min$^{-1}$ in Newtonian and non-Newtonian fluids, respectively, and corresponding intensity profiles for each particle type along the width of the channel. In the 500-ppm PEO solution, the three particle types were focused very well at the flow rate of 60 μl/min$^{-1}$ ($R_c = 4.62$, $W_t = 45.48$, $El = 9.84$) and in a specific range of flow rate around 60 μl/min$^{-1}$ as well. The particles experience the lift force $F_L$, the Dean drag force $F_D$ and the elastic force $F_E$ at the same time, and an equilibrium position was observed on the opposite side of cavity side due to the combined effects of the three forces. In a Newtonian fluid, however, no obvious particle focusing is observed at the outlet under 60 μl/min$^{-1}$ flow rate with the 3.2-μm, 4.8-μm particle size. The 13-μm particles begin to focus from 60-μl/min$^{-1}$ flow rate under the effects of the lift force $F_L$, the Dean drag force $F_D$. In this experiment, the hydrodynamic diameter $D_h = 2wh/(w+h) \approx 60$, and $a/D_h$ is approximately 0.05, 0.08, 0.22 for 3.2-μm, 4.8-μm, 13-μm particles, respectively. This phenomenon is consistent with the theory that particle focusing can only be achieved when $a/D_h$ is larger than 0.07. $^2,28,33$ It can also be seen from the fluorescent intensity profile (Figure 3-4 d,e,f) that the particles in DI water are mostly dispersed, while in PEO solution the particles are tightly focused. The width of the focusing line (derived from the difference of lateral position in the fluorescence intensity profile at 70% intensity peak) in PEO solution is similar to the diameter of the particles. We can conclude that single-particle focusing is achieved in the $x$-$y$ plane. Compared with particle focusing in a Newtonian fluid, the elasticity in a non-Newtonian fluid accelerates the particle focusing, improves the focusing performance, and finally focuses particles on the opposite side of cavity. Besides, particle focusing in non-Newtonian fluids requires less external energy and less pressure is exerted on the microfluidic device.
Figure 3-4 The fluorescent images of 3.2-µm, 4.8-µm, 13-µm particles at the outlet under the flow rate of 60 µl/min$^{-1}$ in Newtonian and non-Newtonian fluids, respectively, and corresponding fluorescent intensity profiles for each particle type along the width of the channel.
3.4.2. Effects of dean flow induced by expansion–contraction cavity arrays in ECCA channel

Figure 3-5 The fluorescent images of 4.8-μm particles at the outlet in straight rectangular channel under the flow rate of 150 µl/min⁻¹ in DI water and in PEO under different flow rates, respectively. (b) The fluorescent intensity profile in DI water. Particles focus to four equilibrium positions centred at the faces of the channels. The middle fluorescent intensity peak, which is higher than the other two peaks, represents two overlap equilibrium positions. (c) The fluorescent intensity profiles in PEO under different flow rates. Particles in 100 µm × 50 µm channel can only distribute in a wide central area along the width of the channel without the aid of secondary flow.

In order to investigate the effects of Dean flow induced by expansion–contraction cavity arrays in an ECCA channel, the particle distribution in a straight rectangular channel with cross section 100 µm × 50 µm (width × height) (Figure 3-5) was compared with that of in ECCA channel. As is known that particles focus in rectangular channels to four or two equilibrium positions centred at the faces of the channels at moderate flow rates of a Newtonian fluid as shown in Figure 3-5 (a). In a non-Newtonian fluid, Yang et al.
demonstrated that multiple particle equilibrium positions can be reduced to a single particle stream along the channel centreline when elastic and inertial forces are synergistically balanced\textsuperscript{22}, which was termed as elasto-inertial particle focusing. The dimension of the microchannel cross section used in Yang’s experiments was $50 \mu m \times 50 \mu m$, and particle size was $5.9 \mu m$. Finally, particles formed a single line at the centre of the channel at only several microlitres per minute. Whereas in the $100 \mu m \times 50 \mu m$ rectangular channel, single-particle focusing was not achieved due to a wider width, Figure 3-5 (a). This can be explained by the shape of velocity profile along the channel width. Velocity distribution along the width becomes increasingly flat as the channel width becomes wider. The fluid shear rate is very small along a large central area. As the particles tend to migrate towards the low shear rate region in a viscoelastic fluid, the particles in $100 \mu m \times 50 \mu m$ rectangular channel can only distribute in a wide central area along the width of the channel, Figure 3-5.

With the aid of the Dean flow induced by expansion–contraction cavity arrays, the particles can be tightly focused to a single line on the opposite cavity side in moderate flow rates although the channel dimension is still $100 \mu m \times 50 \mu m$ in rectangular section as shown in Figure 3-4. A further advantage is that in an ECCA channel the flow rate that allows particle focusing cold be dozens of or even hundreds of microlitre per minute, which is much higher than that in a straight channel, resulting in a higher throughput ($>10000 \text{ s}^{-1}$). So this Dean-flow-coupled elasto-inertial microfluidic device could offer a continuous, sheathless, and high-throughput focusing performance.

3.4.3. Effects of different flowing conditions

As the inertial lift force, secondary force and elastic force are all related to the flow rate. Under different flowing conditions, the particles will experience different dominant forces, which will lead to different particle distributions. Take 4.8-\(\mu\)m particle as an example, the 4.8-\(\mu\)m particles distribution was observed under different flowing conditions in 500ppm PEO fluid and DI water, as is shown in Figure 3-6. The flow rate \(Q\) varies from 10 \(\mu\)l/min\(^{-1}\) to 300 \(\mu\)l/min\(^{-1}\), which corresponds to the levels of \(Re\) ranging from 0.77 to 23.10, \(Wi\) ranging from 7.58 to 227.4, and \(El\) 9.84 which is independent of the flow rate. In DI water, \(Wi\) and \(El\) are both zero since Newtonian fluids does not exhibit elastic properties. When the flow rates are relatively low (<30 \(\mu\)l/min\(^{-1}\), \(Re = 2.31\), \(Wi\)
=22.74, $El =9.84$) with 500ppm PEO solution, the inertial effect and the Dean effect are negligible, while elastic force is dominant, and the particles are randomly distributed. As the flow rate increases, the inertial and Dean drag forces being exerted on a particle competes with elastic force, and the particles gradually migrate to the opposite side of the cavity in the channel and focused tightly (as shown in Figure 3-6 at 60-µl/min flow rate in PEO ($R_c = 4.62, W_l =45.48, El =9.84$)) due to the balance of the lift force $F_L$, Dean drag force $F_D$ and elastic force $F_E$. The focusing range is approximately from 30 µl/min$^{-1}$ to 120 µl/min$^{-1}$. When the flow rate reaches 120 µl/min$^{-1}$ ($R_c = 9.24, W_l =90.96, El =9.84$), another focusing line occurs on the cavity side. This is partly because inertia and secondary flow effects begin to dominant the particle migration. Consequently, the elastic force was eventually overwhelmed by the inertial force all the particles migrate and focus on the cavity side when the flow rate reaches 240 µl/min$^{-1}$ ($R_c = 18.48, W_l =181.92, El$}

Figure 3-6 The fluorescent images of 4.8µm particle at the outlet at the flow rate of 30 µl/min$^{-1}$, 60 µl/min$^{-1}$, 120 µl/min$^{-1}$, 240 µl/min$^{-1}$ in 500ppm PEO and DI water fluids, respectively, and corresponding fluorescent intensity profiles for different flow rates along the width of the channel.
=9.84). In DI water, the particles are dispersed until the flow rate reaches 120 μl/min⁻¹, then the particles are focused on the cavity side of the channel under the combined effect of the lift force \( F_L \) and the Dean drag force \( F_D \). The minimum focusing flow rate in a Newtonian fluid is higher than that in a non-Newtonian fluid and the focusing performance in a Newtonian fluid is not as good as in a PEO solution. That is to say, the elastic effect accelerates and improves the particle focusing performance. It can also be seen from the fluorescent intensity profiles of particles flowing with different flow rates in 500ppm PEO and DI water.

3.4.4. Side view of the channel

We checked if the focusing is three-dimensional focusing in Newtonian and non-Newtonian fluids in this ECCA channel by imaging the side view of the channel, Figure 3-7. Figure 3-7 (a) is the side view of the channel when particles flowing in 500ppm PEO solution at a flow rate of 60 μl/min⁻¹. It is verified that the particles flowing in non-Newtonian fluids is not only focused in the \( x-y \) plane, but also forms a single line in the centreline of the channel in the \( x-z \) plane. That means, the 3D particle focusing in non-Newtonian fluids in this ECCA channel is realized by properly balancing the effects of inertia, secondary flow and elasticity. This phenomenon was not observed for the case of a Newtonian fluid when particles focused in DI water in \( x-y \) plane as shown in Figure 3-7 (b) in 240 μl/min⁻¹. Instead, the particles in DI water are focused in two lines along the two channel walls in the \( x-z \) plane due to the synergetic effect of inertia and secondary flow, which is not suitable for practical applications such as one-by-one particle counting or sorting. It is confirmed that the equilibrium positions are reduced to single one by the additional elasticity effect in PEO solution in the ECCA channel. It’s also obvious from the corresponding fluorescent intensity profiles in PEO and DI water, respectively. In a PEO solution, only one fluorescence intensity peak exists in the middle of the lateral side view, while there are two peaks in DI water.
Figure 3-7 Fluorescent images of the side view of the channel when particle focusing in 500ppm PEO solution and DI water, respectively, and corresponding fluorescent intensity profiles.

3.4.5. Effects of different distances from the inlet

The particle distribution also strongly depends on the distance from the inlet. Figure 3-8 shows the fluorescent images of 4.8-μm particle distributions at three different distances from the inlet in the 500-ppm PEO solution. Figure 3-8 (a) shows that particles at the inlet are randomly distributed along the radial direction. However, at the middle of the channel, approximately 2.5 mm from the inlet, that almost all the particles have already migrated and been confined within a narrow band along the channel wall on the opposite side of cavity. Finally, at the outlet of the channel, strict particle focusing is observed. It can also be seen from the fluorescent intensity profiles at three different distances from the inlet that particle focusing strongly depends on the distance from the inlet. The focusing width at the outlet of the channel is approximately 5 μm, which is the same size of particles, indicating single-line particle focusing.
In this work, 3D particle focusing was demonstrated in a straight channel with asymmetrical expansion–contraction cavity arrays (ECCA channel) by exploiting the Dean-flow-coupled elasto-inertial effects. By properly controlling the flow rates to harmonize the inertial force, viscoelastic force and Dean-drag force, 3D particle focusing in non-Newtonian fluids along the opposite side of cavities was achieved. Particle focusing under Newtonian and non-Newtonian fluids using three different sized particles (3.2µm, 4.8 µm, 13 µm) were demonstrated. The particles were focused on the cavity side in a Newtonian fluid by the synthesis of inertial and Dean-drag force, whereas on the opposite cavity side in a non-Newtonian fluid due to the addition of the viscoelastic force. Compared with particle focusing in Newtonian fluids in an ECCA channel and in non-Newtonian fluids in straight channel, it can be concluded that particles in non-Newtonian fluid in an ECCA channel are more easily and better focused. Moreover, the effect of
Dean-flow-coupled elasto-inertial focusing can be fully developed as the flowing distances from the inlet becomes longer. This Dean-flow-coupled elasto-inertial microfluidic device could offer a continuous, sheathless, and high-throughput (>10000 s⁻¹) 3D focusing performance, which may be valuable in various applications from high speed flow cytometry to cell counting, sorting, and analysis.
4. Continuous plasma extraction under viscoelastic fluid in a straight channel with asymmetrical expansion–contraction cavity arrays *

4.1. Introduction

Blood is an important biological fluid that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. By volume, ~40% cells and ~60% plasma constitute the two main blood components [124]. Blood cells consist of three main classes: 

(i) erythrocytes or red blood cells (RBCs), which account for 98% of all blood cells, delivering oxygen to the body tissues, have a discoid shape and are about 8 µm in diameter and 2 µm in thick; 

(ii) leukocytes or white blood cells (WBCs), which account for 1% of all blood cells, play a critical role in the immune system and range from 5 to 20 µm in diameter; and 

(iii) platelets are responsible for clotting reaction and 1 to 3 µm in size [125, 126]. Blood plasma is clear, straw-yellow in color, and is host to a myriad of analytes including but not limited to proteins, metabolites, or circulating nucleic acids (CNAs), and these components are useful in diseases diagnostics and prognostics [124]. Plasma extraction to eliminate the blood cells is of great importance, because the accurate detection of these analytes and following analysis requires the plasma to be totally free of cells’ interference [126].

Centrifugation and filtration are two conventional plasma separation methods, although simple and remain ubiquitous, they have many limitations. For example, the centrifugation method is time-consuming, labour-intensive and may impair the analytes of interest due to the mechanical stress induced by high speed rotation; in filtration method, clogging is the most severe problem [126].

The microfluidic platforms, which can manipulate and control fluids and particles at micron and submicron dimensions [8], present alternative methods for blood plasma

* Results of this chapter are published in: Dan Yuan, Jun Zhang, Ronald Sluyter, Qianbin Zhao, Sheng Yan, Gursel Alici, Weihua Li. Continuous plasma extraction under viscoelastic fluid in a straight channel with asymmetrical expansion–contraction cavity arrays. Lab on a Chip, 2016, 16(20): 3919-3928. Cover page paper.
separation. Microfluidic methods offer many advantages, including (i) reduced sample volumes, (ii) lower time and cost, (iii) higher sensitivity and accuracy, (iv) increased portability [2]. According to the operating principle, the microfluidic techniques are classified to active and passive methods. Active methods are based on the application of external force fields such as acoustic [127], dielectrophoretic (DEP) [14, 128], and magnetic [129] forces. The active methods face the challenges of low throughput, complexity and auxiliary expensive equipment, although they can provide more precise manipulation of particles or cells. Passive methods are based on the microchannel geometrical effects and intrinsic hydrodynamic forces, such as sedimentation [130, 131], microfiltration [132], deterministic lateral displacement (DLD) [133], hydrophoresis [14, 134] and inertial microfluidics [44, 135]. They are generally simpler, cheaper and more robust. Both active and passive techniques have been extensively explored for blood plasma separation. Kersaudy-Kerhoas and Sollier [124], Sajeesh et al.[4], Gossett et al. [5], and Hou et al. [136] have made a comprehensive review on microfluidic blood plasma separation. In order to improve the separation efficiency or throughput, so far researchers are still striving to develop novel mechanisms for plasma extraction in microfluidic platform. For example, Rafeie et al. [137] developed a multiplexing spiral microchannels with a trapezoidal cross-section for ultra-fast plasma separation. Through parallelization of 16 spiral channels along planar and vertical direction, the total flow rate for blood plasma separation can be as high as 24 ml/min. Tripathi et al. [138] presented a microfluidic device for plasma separation from pure blood using biophysical and geometrical effects. And this microdevice can achieve almost 100% separation efficiency on undiluted blood.

Recently, particle manipulation including focusing and separation in non-Newtonian fluids and its application on blood cells have gained significant attention [37, 50, 52, 53, 56, 58, 78, 83, 95, 122, 139-141]. Yang et al. [47] reported that the number of multiple equilibrium positions can be reduced to one equilibrium position at the centreline by properly adjusting the synergetic effect of inertia and viscoelasticity. Isolation of WBCs from diluted whole blood was obtained based on cell deformability in a rectangular channel using medium viscoelasticity [95]. Seo et al. [84] observed that the viscoelasticity induced the high uniformity in depth position and reduction in cell overlap, and they demonstrated counting and monitoring of red blood cells flowing in a microchannel using
phase holographic microscopy for quantitative phase imaging. Liu et al. [58] separated MCF-7 cells and *Escherichia coli* (*E. coli*) bacteria from RBCs in straight microchannels with different cross-sections. Nam et al. [66] successfully separated platelets from diluted whole blood using the elasto-inertial characteristic of non-Newtonian fluids in a square channel with the aid of sheath flow, and separated malaria parasites from white blood cells at high throughput using viscoelastic fluids for ultrasensitive PCR detection [75].

In this work, based on our previous study on Dean-flow-coupled elasto-inertial three-dimensional particle focusing under viscoelastic flow in an ECCA channel [140], we investigated continuous plasma extraction in the ECCA channel using blood sample diluted by PEO PBS solution. By exploiting the dean-flow-coupled elasto-inertial effects, this ECCA channel offers a continuous, sheathless, and high purity (99.99%) plasma extraction; Moreover, this method can simultaneously focus and filtrate platelets and white blood cells (WBCs), in addition to red blood cells (RBCs), as verified by flow cytometric data. To the best of authors’ knowledge, this is the first report to realize blood plasma extraction using dean-flow-coupled elasto-inertial effects in microfluidics.

4.2. Schematic of plasma extraction based on dean-flow-coupled elasto-inertial effects

Figure 4-1 shows the schematic diagram of blood cells focusing in diluted blood sample with PEO in the ECCA channel, and experimental demonstrations at outlet of ECCA channel in diluted blood sample with and without PEO. Figure 4-1 (a) shows the overview of the ECCA channel, which includes 26 consecutive expansion–contraction triangular cavities, one inlet and a trifurcating outlet with three branches. Insets A, B and C represent amplified sections at inlet, middle and outlet of the channel, respectively. At inlet (A), all the blood cells (platelets, WBCs, and RBCs) are randomly distributed; as the blood sample flows, blood cells are pushed towards the channel wall on the opposite cavity side, as can be seen at amplified middle section (B); at outlet (C), platelets, WBCs, and RBCs are focused to a tight stream and flow out at outlet (i), and plasma can be collected at outlets (ii) and (iii).
Figure 4-1 Schematic diagram of blood cells focusing in diluted blood sample with PEO in ECCA channel, and experimental demonstrations at outlet of ECCA channel in diluted blood sample with and without PEO. (a) Schematic diagram of blood cells focusing in diluted blood sample with PEO in ECCA channel. (b) Simulation results of velocity magnitude and shear rate in the cross section of ECCA channel. (c) Simulation results of the flow field 10 \( \mu \text{m} \) away from the cavity. (d) Experimental demonstrations at outlet of ECCA channel in diluted blood sample with and without PEO.

In the ECCA channel containing a diluted blood sample with PEO, the blood cells are driven by three kinds of forces: Lift force \( F_L \), including the shear-gradient lift force \( F_{LS} \), wall-repulsion force \( F_{LW} \); the Dean drag force \( F_D \) resulting from the channel geometry; and elastic force \( F_E \) induced by the nature of the viscoelastic medium. Figure 4-1 (b) is
the simulation results of velocity magnitude (left) and shear rate (right). The maximum velocity occurs in the central part of the rectangular cross section (dark red area), and decreases gradually from the centreline to the channel wall (dark blue area). The shear rate is the differential of the flow velocity, which is expressed as 
\[ \dot{\gamma} = \left[ \left( \frac{\partial u}{\partial y} \right)^2 + \left( \frac{\partial u}{\partial z} \right)^2 \right]^{1/2}. \]
In the shear rate map, there are five zero shear rate regions in the cross section, corresponding to the five blue areas. Some researchers reported that the particles migrate towards the five zero shear rate regions when the second normal stress difference in viscoelastic fluids is negligible [52]. Yang et al. [47] thought that the number of multiple equilibrium positions can be reduced to central area only by adjusting the flow rate to cope with the synergetic effect of inertia and viscoelasticity. Figure 4-1 (c) is the simulation results of the cross sectional flow field 10 μm away from the cavity.

The arrows represent the magnitude and direction of the secondary flow field induced by the abrupt contraction of the channel. When particles or cells flowing in ECCA channels in fluids with viscoelasticity, apart from the inertial lift force and secondary drag force, the elastic force \(F_E\) should be considered, which is directed away from the wall and decays with increasing distance from the wall. In a straight rectangular channel with viscoelastic fluids, the particles tend to migrate to the centreline of the channel by the synergetic effect of inertia and viscoelasticity, as the dashed circle in the center of the cross section in Figure 4-1 (c). In the ECCA channel, the particles become unstable when an additional secondary flow drag is exerted on the particles. Subsequently, a new equilibrium position can be achieved near the channel wall by the combined effects of lift force \(F_L\), elastic force \(F_E\) and the dean drag force \(F_D\), as shown in Figure 4-1(c). Finally, particles or cells’ focusing is realized in an ECCA channel with the combined effect of Dean-flow-coupled elasto-inertial forces. In plasma extraction experiment, platelets, WBCs, and RBCs are expected to focus along the channel wall on the opposite cavity side in the diluted blood sample with PEO by the combined effect of Dean-flow-coupled elasto-inertial forces; while for the blood sample diluted by pure PBS without PEO, there is no elastic force, and the blood cells cannot be focused decently by the effect of inertial and secondary forces solely.

Figure 4-1 (d) shows the experimental demonstrations at outlet of ECCA channel in diluted blood sample with and without PEO. In diluted blood sample with PEO, the blood cells (the black stream) are focused as a tight stream and flow out at outlet (i) at the flow
rate of 50 µl/min (left figure); while at the same flow rate, the blood cells (the dark area) are dispersed across the whole channel at the outlet when the blood sample is diluted with pure PBS without PEO (right figure). It can be seen that the elastic effect in diluted blood sample with PEO improves the cell focusing performance.

4.3. Methods

4.3.1. Design and fabrication of microfluidic device

The schematic geometry of the microfluidic channel has been presented in previous work [140]. The right angled isosceles triangular cavities are patterned on one side of a straight channel. The channel has a cross section of 100 µm × 40 µm (width × height). The longest edge of the triangle is 900 µm, and the space between two adjacent cavities is uniform at 900 µm. The total length of the straight channel is 48 mm, including 26 consecutive expansion-contraction triangular cavities.

The device was fabricated using standard photolithography and soft lithographic techniques [142, 143]. This fabrication included rapid prototyping on a silicon master, and polydimethylsiloxane (PDMS) replica molding and sealing through plasma oxidation.

4.3.2. Preparation and rheology of the PEO solution

Poly(ethylene oxide) (PEO; 2,000,000 Da; Sigma-Aldrich) was diluted in distilled water (DI water) and phosphate-buffered saline (PBS) to yield concentrations of 500 ppm, 1000 ppm, and 2000 ppm. Tween 20 (0.01% (v/v), Sigma-Aldrich) was added to all the solutions to prevent particle from aggregation. The rheological properties of the fluids were measured by a rotational rheometer (Antonpaar MCR 301) that has a parallel plate configuration and a diameter of 20 mm. These experiments were performed at room temperature (24 ± 1°C). Figure 4-2 shows the viscosity of the PEO solutions as a function of the shear rate for PEO concentrations of 500 ppm, 1000 ppm, 2000 ppm. The shear viscosity of the viscoelastic fluids was measured in shear rates ranging from 200 s⁻¹ to 10³ s⁻¹. The PEO solutions slightly show shear thinning behaviors. The shear viscosity of the PEO solution increased rapidly with increments of PEO concentration because the higher the PEO concentration, the more couplings and entanglements are formed by the internal polymer chains; this action caused the viscosity to increase. According to Figure
The estimated viscosity for 500 ppm, 1000 ppm, and 2000 ppm PEO solutions are $2 \times 10^{-3}$, $3 \times 10^{-3}$, and $5 \times 10^{-3}$ Pa·s, respectively.

The relaxation times for PEO solutions were estimated from the previous empirical relaxation times ($\lambda$) measured with CaBER. The relaxation time depends on the polymer conformation, and can be defined by the polymer concentration $c$ as:

$$\lambda = 18\lambda_z(c / c^*)^{0.65}$$  \hspace{1cm} (7)

where $\lambda_z$ is the Zimm relaxation time and $c^*$ is the polymer overlap concentration (858 ppm [144]),

$\lambda_z$ was calculated as $7.1 \times 10^{-4}$ s from equation [144, 145]:

$$\lambda_z = f[\eta](M_w)\eta_s / RT$$  \hspace{1cm} (8)

where $f$ (=0.463) is the prefactor dependent upon solvent quality, $[\eta]$ (=0.916 m$^3$ kg$^{-1}$) is the intrinsic viscosity, $\eta_s$ (=2.05×10$^{-3}$ Pa·s) is the solvent viscosity, $R$ (=8.314 J mol$^{-1}$ K) is the gas constant, and $T$ (=293K) is the absolute temperature.
However, the equation can only be applied when $c/c^* <= 1$, so only 500 ppm PEO solution was calculated using the above equations. The empirical relaxation time for 500 ppm PEO solution is estimated to be 9.1 ms [47]. The relaxation time for 1000 ppm and 2000 ppm PEO solutions are referred from Xuan’s work [68], which is 12.4 ms and 19.5 ms respectively.

According to equation (2) (3), $R_c$ and $W_i$ for 500 ppm, 1000 ppm, and 2000 ppm PEO solutions from flow rate $Q = 10 \mu l/min$ to $Q = 80 \mu l/min$ in this ECCA channel were calculated in Table 1.

Table 1 $R_c$ and $W_i$ for 500 ppm, 1000 ppm, 2000 ppm PEO solutions

<table>
<thead>
<tr>
<th></th>
<th>500 ppm</th>
<th>1000 ppm</th>
<th>2000 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q= 10 \mu l/min$</td>
<td>$R_c =0.3$</td>
<td>$R_c =0.2$</td>
<td>$R_c =0.12$</td>
</tr>
<tr>
<td></td>
<td>$W_i =7.64$</td>
<td>$W_i =10.41$</td>
<td>$W_i =16.22$</td>
</tr>
<tr>
<td>$Q= 20 \mu l/min$</td>
<td>$R_c =0.6$</td>
<td>$R_c =0.4$</td>
<td>$R_c =0.24$</td>
</tr>
<tr>
<td></td>
<td>$W_i =15.28$</td>
<td>$W_i =20.82$</td>
<td>$W_i =32.44$</td>
</tr>
<tr>
<td>$Q= 30 \mu l/min$</td>
<td>$R_c =0.9$</td>
<td>$R_c =0.6$</td>
<td>$R_c =0.36$</td>
</tr>
<tr>
<td></td>
<td>$W_i =22.92$</td>
<td>$W_i =31.23$</td>
<td>$W_i =48.66$</td>
</tr>
<tr>
<td>$Q= 40 \mu l/min$</td>
<td>$R_c =1.2$</td>
<td>$R_c =0.8$</td>
<td>$R_c =0.48$</td>
</tr>
<tr>
<td></td>
<td>$W_i =30.56$</td>
<td>$W_i =41.64$</td>
<td>$W_i =64.88$</td>
</tr>
<tr>
<td>$Q= 50 \mu l/min$</td>
<td>$R_c =1.5$</td>
<td>$R_c =1$</td>
<td>$R_c =0.6$</td>
</tr>
<tr>
<td></td>
<td>$W_i =38.2$</td>
<td>$W_i =52.05$</td>
<td>$W_i =81.8$</td>
</tr>
<tr>
<td>$Q= 60 \mu l/min$</td>
<td>$R_c =1.8$</td>
<td>$R_c =1.2$</td>
<td>$R_c =0.72$</td>
</tr>
<tr>
<td></td>
<td>$W_i =45.84$</td>
<td>$W_i =62.46$</td>
<td>$W_i =97.35$</td>
</tr>
<tr>
<td>$Q= 80 \mu l/min$</td>
<td>$R_c =2.4$</td>
<td>$R_c =1.6$</td>
<td>$R_c =0.96$</td>
</tr>
<tr>
<td></td>
<td>$W_i =61.12$</td>
<td>$W_i =83.28$</td>
<td>$W_i =129.76$</td>
</tr>
</tbody>
</table>
4.3.3. Particle preparation

Particle suspensions were prepared by diluting 3 μm and 10 μm internally red dyed fluorescent polystyrene microspheres (ThermoFisher Scientific, CV5%), and 4.8 μm internally green dyed fluorescent polystyrene microspheres (Thermo Fisher Scientific, CV5%) using 500ppm, 1000ppm, and 2000ppm PEO solution. The concentration for 3 μm, 4.8 μm, and 10 μm particle solution is ~ 10^7 particles ml\(^{-1}\), ~ 3 × 10^6 particles ml\(^{-1}\), and ~ 10^6 particles ml\(^{-1}\) respectively. As these microspheres have similar dimensions with blood cells, they were used to mimic platelets, RBCs and WBCs, respectively.

Peripheral blood was taken from healthy adult male volunteers using VACUETTE lithium heparin tubes (Greiner Bio-One,) in accordance with and approval from the Human Ethics Committee (University of Wollongong, Wollongong, Australia). Blood was then diluted 20 times in PBS containing PEO at concentrations of 500 ppm, 1000 ppm or 2000 ppm. For comparison, blood was also diluted with PBS without PEO. Before commencing each experiment the particles were resuspended by vortex and blood samples were manually stirred to provide uniform suspensions.

4.3.4. Experimental setup

The particle and blood suspension were transferred to 1 ml syringes and then introduced into the microfluidic chip through silicon tubes by syringe pumps (Legato 100, KD Scientific). The microfluidic chip was then placed onto an inverted microscope (CKX41, Olympus), and images were observed and captured by a CCD camera (Optimos, Q-imaging), and then were post-processed and analysed with Q-Capture Pro 7 (Q-imaging) software. The particle and cell concentration were measured by a haemocytometer (Qiu Jing Biochemical Reagents Instrument co.) according to the manufacturer’s instructions. The plasma extraction performance was further tested using a LSR II flow cytometry (BD Biosciences). Flow cytometric data was analysed using FlowJo software (Tree Star, Inc.).
4.4. Results

4.4.1. Effects of PEO concentrations and flow rates

Figure 4-3 Effects of PEO concentrations and flow rates for focusing of 4.8 µm particles. The captured fluorescent picture for distribution of 4.8 µm particles at outlet section in 500 ppm, 1000 ppm and 2000 ppm PEO solution, respectively, with flow rates ranging from 20 µl/min to 80 µl/min, and corresponding fluorescent intensity profile. The green bar beside each PEO concentration shows the optimal flow rate range for 4.8 µm particle filtration.

RBCs account for 98% of all cells in blood (with $5 \times 10^9$ RBCs per ml, 2–5×10⁸ platelets per ml, and 5–10×10⁶ WBCs per ml) [146]. Therefore, the separation of blood cells mainly focuses on RBCs. Although RBCs have a discoid shape and about 8 µm × 2 µm,
they behave similarly to ~4 µm spherical particles [147]. In the current study, 4.8 µm internally green dyed fluorescent polystyrene microspheres were used to mimic the behavior of RBCs.

The effects of PEO concentrations and flow rates on the focusing of 4.8 µm particles were studied and are shown in Figure 4-3. Figure 4-3 shows the captured fluorescent picture for distribution of 4.8 µm particles at the outlet section in 500 ppm, 1000 ppm and 2000 ppm PEO solution, respectively, with flow rates ranging from 20 µl/min to 80 µl/min, and corresponding fluorescent intensity profile. The x and y axis for each fluorescent intensity profile figure is normalized lateral channel distance y/w, and normalized fluorescent intensity respectively. The vertical red dashed line at outlet section in each figure represents the position of fluorescent intensity profile. The horizontal red dashed line in each fluorescent intensity profile represents the background fluorescent intensity. The value of background fluorescent intensity for each PEO concentration is slightly different. The curve above this red dashed line implies the existence of fluorescent particles. The green bar beside each PEO concentration shows the optimal flow rate range for 4.8 µm particle filtration in 500 ppm, 1000 ppm and 2000 ppm PEO solution, respectively. In 500 ppm PEO solution, at 20 µl/min (R_c=0.6, W_i=15.28) flow rate, the inertial effect and the dean effect are negligible, while elastic force is dominant, so the particles are dispersed, as can be seen from the captured fluorescent picture and corresponding intensity profile that the particles flow out from all three outlets; as the flow rate increases, the inertial and dean drag forces exerted on a particle compete with elastic force, and most of the particles gradually migrate to the opposite side of the cavity in the channel, as can be seen at 30 µl/min (R_c=0.9, W_i=22.92) flow rate; as flow rate increases to 40 µl/min (R_c=1.2, W_i=30.56), all the particles are focused tightly and flow out from outlet (i) due to the balance of the lift force F_L, dean drag force F_D and elastic force F_E, and the focusing lasts until 60 µl/min (R_c=1.8, W_i=45.84); when the flow rate reaches 80 µl/min (R_c=2.4, W_i=61.12), the particles become dispersed again because the inertia and secondary flow effects begin to dominate the particle migration, as can be seen from the fluorescent profile that the curve in outlet (ii) section exceeds above the background intensity line. A similar phenomenon is observed for 1000 ppm PEO solution, but particles are focused more tightly in 1000 ppm PEO solution. In 2000 ppm PEO
solution, a large proportion of particles tended to be dispersed and flow out from all outlets, despite an obvious focusing streak observed at the opposite cavity side of the channel, as can been seen from the corresponding fluorescent profiles that the curves in outlet \( (ii) \) and \( (iii) \) section exceed above the background intensity line. It’s probably due to stronger elastic turbulence effect [148, 149] in solution with high PEO concentration that this instability makes particles disperse again. The optimal flow rate range for 4.8 \( \mu \)m particles is 40 \( \mu \)l/min - 60 \( \mu \)l/min in both 500 ppm \( (1.2 \leq R_c \leq 1.8, 30.56 \leq W_i \leq 45.84) \) and 1000 ppm \( (0.8 \leq R_c \leq 1.2, 41.64 \leq W_i \leq 62.46) \) PEO solutions; while particles could not be focused tightly in 2000 ppm PEO solution.

4.4.2. Separation of particles with different sizes

Before the filtration of blood cells, 3 \( \mu \)m, 4.8 \( \mu \)m, and 10 \( \mu \)m diameter particles, which are comparable to the sizes of platelets, RBCs, and WBCs, respectively, were first tested (platelets: 1 to 3 \( \mu \)m in diameter; RBCs: 8 \( \mu \)m in diameter \( \times \) 2 \( \mu \)m in thick; WBCs: 5 to 20 \( \mu \)m in diameter). Figure 4-4 shows the filtration of particles with different size in 1000 ppm PEO solution with the flow rate range from 20 \( \mu \)l/min \( (R_c =0.4, W_i=20.82) \) to 80 \( \mu \)l/min \( (R_c=1.6, W_i=83.28) \) (captured fluorescent picture and corresponding fluorescent intensity profile), and optimal flow rate range for filtration of 3 \( \mu \)m, 4.8 \( \mu \)m, and 10 \( \mu \)m particles respectively. It can be seen that the 3 \( \mu \)m particles begin to focus well and be filtrated out of outlet \( (i) \) at the flow rate of 40 \( \mu \)l/min \( (R_c =0.8, W_i =41.64) \) (as can been seen from the fluorescent profile that the curve in outlet \( (ii) \) and \( (iii) \) section overlap with the background intensity line), and begin to disperse at the flow rate of 60 \( \mu \)l/min \( (R_c =1.2, W_i =62.46) \) (as can been seen from the fluorescent profile that the curve in outlet \( (ii) \) begins to exceed the background intensity line); the 4.8 \( \mu \)m particles are focused and filtrated out of outlet \( (i) \) from flow rate 40 \( \mu \)l/min \( (R_c =0.8, W_i =41.64) \) to 60 \( \mu \)l/min \( (R_c =1.2, W_i =62.46) \); while 10 \( \mu \)m particles are more easily and better focused, which are focused well from 30 \( \mu \)l/min \( (R_c =0.6, W_i =31.23) \) to 80 \( \mu \)l/min \( (R_c =1.6, W_i =83.28) \). The optimal flow rate range for 3 \( \mu \)m, 4.8 \( \mu \)m, and 10 \( \mu \)m particles filtration are 40 \( \mu \)l/min \( \sim \) 50 \( \mu \)l/min \( (0.8 \leq R_c \leq 1, 41.64 \leq W_i \leq 52.05) \), 40 \( \mu \)l/min \( \sim \) 60 \( \mu \)l/min \( (0.8 \leq R_c \leq 1.2, 41.64 \leq W_i \leq 62.64) \), and 30 \( \mu \)l/min \( \sim \) 80 \( \mu \)l/min \( (0.6 \leq R_c \leq 1.6, 31.23 \leq W_i \leq 83.28) \).


\( \leq W_i \leq 93.28 \) respectively. After filtration, there are few particles in PEO solution collected from outlet (ii) and (iii).

The focusing performance improved as the particle size increases. The inertial lift force, elastic force and dean drag force scale differently with particle diameter \( (F_L \propto a^4, F_E \propto a^3, F_D \propto a^1) \). Assuming particles are in a straight rectangular channel, the particles are affected by inertial lift force and elastic force. For inertial lift force, the particles are affected by a significant wall repulsion force between the particle and the wall, and this force will induce cross-lateral focusing toward the channel centre. As this force is strongly dependent on particle size, the cross-lateral focusing is not fully developed as larger particles [57]. Furthermore, the elastic force is proportional to the third power of the particle size, therefore the larger particle will experience a stronger elastic force. Thus, larger particles are more tightly focused at the channel centreline by this elasto-inertial effect at appropriate flow rate. In our ECCA channel, the additional dean drag force will adjust centreline focusing position to one side of the channel, and the particle focusing status will remain during the process. Therefore, particles with larger sizes are more easily affected and better focused by the balance of these three forces.
Figure 4-4 Filtration of particles with different size in 1000 ppm PEO solution with the flow rate range from 20 µl/min to 80 µl/min (captured fluorescent picture and corresponding fluorescent intensity profile), and the optimal flow rate range for filtration of 3 µm, 4.8 µm, and 10 µm particles respectively.

The performance evaluation of this dean-flow coupled elasto-inertial particle filtration was tested by comparing the original particle concentration with that after filtration. Figure 4-5 shows the 3 µm, 4.8 µm, and 10 µm particle concentrations measured from both the inlet and filtrated PEO solution from outlets (ii) and (iii) (left figure), and corresponding fluorescent images (right figure). After first filtration, approximately 99.2% of the 3 µm particles, and 100% of the 4.8 µm and 10 µm particles were removed from the original PEO solution, measured by hemocytometer. The purity for 3 µm particles could reach 100% after a second round of filtration.
4.4.3. **Plasma extraction in diluted blood sample with different PEO concentrations**

After performing the filtration of different-sized particles, the ECCA channel was used to filtrate blood cells. The blood sample was diluted 20 times by PEO PBS solution to reduce the cell-to-cell interactions, prior to be transferred to the ECCA channel. Figure 4-6 shows plasma extraction in the diluted blood sample with PEO concentration 500 ppm, 1000 ppm, and 2000 ppm from 20 µl/min flow rate to 80 µl/min flow rate (bright fields and corresponding intensity profile), and optimal filtration flow rate range for each concentration. The black streaks indicate the streaks of RBCs, while the platelets and WBCs are too rare to be seen here. It can be seen that the filtration of RBCs is similar to that of 4.8 µm particles except the optimal flow rates are slightly different (Figure 4-6 (b)). The optimal flow rate range for blood cells is 50 µl/min - 60 µl/min ($1.5 \leq R_c \leq 1.8, 38.2 \leq W_i \leq 45.84$), 30 µl/min - 50 µl/min ($0.6 \leq R_c \leq 1, 31.23 \leq W_i \leq 52.05$), 30 µl/min - 40 µl/min ($0.36 \leq R_c \leq 0.48, 48.66 \leq W_i \leq 64.88$) in 500 ppm, 1000 ppm, and 2000 ppm PEO PBS solutions, respectively. Although similar, the shape, actual sizes and deformability of RBCs differ to rigid sphere 4.8 µm particles, providing a possible explanation for differences in optimal filtration flow rate ranges.

Figure 4-5 3 µm, 4.8 µm, and 10 µm particle concentrations measured from both inlet and filtrated PEO solution from outlets (ii) and (iii) and corresponding fluorescent images.
Figure 4-6 Plasma extraction in diluted blood sample with PEO concentration 500 ppm, 1000 ppm, and 2000 ppm from 20 µl/min flow rate to 80 µl/min flow rate (bright fields and corresponding intensity profile), and optimal filtration flow rate range for each PEO concentration.
4.4.4. Effects of distance from inlet

![Figure 4-7 Effects of distance from inlet.](image)

The red blood cells’ focusing behaviour after every expansion–contraction cavity was investigated. Figure 4-7 shows the captured figure of focusing behaviour after certain number of the cavities. The number on the axis means the cavity order counted from inlet. It can be seen that at inlet, the red blood cells are randomly distributed, from the 3rd to the 6th cavity, the cells are gradually confined after the 10th cavity, most cells are focused, but not very well; until after the 16th cavity, all the cells are focused into a tight stream. The focusing after 16th cavity is as good as that of the cavity at further distance from inlet, as can be seen from the captured picture after the 19th, 22th, and the last cavity. Therefore, the length of our ECCA channel can be shortened to approximately 40mm while maintaining the good focusing performance.
4.4.5. Effects of blood hematocrit on focusing behavior

We’ve investigated the effects of blood hematocrit on the focusing behavior by diluting whole blood 100 times (0.45% HCT), 50 times (0.9% HCT), 20 times (2.25% HCT), 10 times (4.5% HCT), and 5 times (9% HCT) respectively. It can be seen that as the blood hematocrit increases, the focusing width of blood cells in our ECCA channel becomes wider (Figure 4-8). This is because the higher the blood hematocrit, the more significant the interaction between blood cells. And strong interaction of blood cells deteriorates the focusing quality. Therefore, the blood hematocrit has an obvious effect on focusing behavior. The focusing width becomes tighter as the whole blood dilute more times.
4.4.6. Purity of collected blood plasma

Figure 4-9 Purity of collected blood plasma. (a) Blood cells filtration in diluted blood sample with 1000 ppm PEO at flow rate of 50 µl/min. (b) Blood cell concentration before and after two series filtration. (c) Working device. (d) The original blood sample at the inlet, and blood cells from outlet (i), plasma from outlets (ii) and (iii) after first filtration, and plasma from outlets (ii) and (iii) after second filtration. (e) The events in blood sample at inlet detected by flow cytometry. (f) The events in the plasma collected at the outlets (ii) and (iii) after second filtration.

In Figure 4-9 (a), blood cells were filtrated out from outlet (i), and plasma was extracted from outlets (ii) and (iii) in diluted blood sample with 1000 ppm PEO at flow rate of 50 µl/min. Two series of filtration were conducted in the same ECCA channel, and the blood
cell concentration before and after two series filtration were plotted in Figure 4-9 (b). The purity after the first filtration was 99.93%, and was improved to 99.99% after the second filtration, measured by hemocytometer. Figure 4-9 (c) shows the working device. Figure 4-9 (d) demonstrates the original blood sample at the inlet, and blood cells from outlet (i), plasma from outlets (ii) and (iii) after first filtration, and plasma from outlets (ii) and (iii) after second filtration. The twice filtration takes less than 2 hours. The filtration results demonstrate the good performance of this ECCA channel using dean-flow-coupled elasto-inertial filtration. The original blood sample at the inlet, and plasma from outlets (ii) and (iii) after second filtration were further tested using flow cytometry. Before testing, both samples were diluted another 25 times. Flow cytometric data, displayed as forward scatter (FSC-A: relative event size) and side scatter (SSC-A: relative cell surface and intracellular complexity) further supports the high separation performance of our device. Figure 4-9 (e) shows the events (total number: 382917) in the blood sample at inlet. Events of low forward scatter (<200 arbitrary units) represent platelets, events of mid forward scatter represent RBCs (200-500 arbitrary units) and events of high forward scatter represent WBCs (>500 arbitrary units). Figure 4-9 (f) shows the events (total number: 2) in the plasma collected at the outlets (ii) and (iii) after second filtration after same testing volume with sample from inlet. It can be seen that there are few events remain in the plasma. The flow cytometry data verified that the ECCA channel can simultaneously focus platelets, RBCs and WBCs, and can extract cell-free plasma with a high purity.

4.5. Conclusion

Continuous plasma extraction under viscoelastic fluids was achieved by exploiting the dean-flow-coupled elasto-inertial effects in an ECCA channel. The effects of PEO concentrations and flow rates on particle viscoelastic focusing were studied. Filtration of particles with different sizes and blood plasma extraction were also demonstrated using this device. The focusing of 3 μm, 4.8 μm and 10 μm particles under the same flow rate and the flow cytometry test results verified that this method can simultaneously focus platelets, RBCs and WBCs. Following the first filtration, approximately 99.2% of the 3 μm particles, and 100% of the 4.8 μm particles and 10 μm particles were removed from the original PEO solution, and 100 % purity for 3 μm particles as well after second
filtration; the plasma purity was improved to 99.99% from 99.93% after second filtration using the same ECCA channel. By exploiting the dean-flow-coupled elasto-inertial effects, this ECCA channel offers a continuous, sheathless, and high purity plasma extraction, which could potentially aid in the realization of lab-on-a-chip bioanalysis devices.
5. Sheathless Dean-flow-coupled elasto-inertial particle focusing and separation in viscoelastic fluid *

5.1. Introduction

Particle or cell separation in a continuous and label-free manner is essential in a wide range of applications such as disease diagnostics, chemical and biological analysis, and environmental assessment [3-5]. Centrifugation and filtration are two conventional separation methods. However, the centrifugation method is time-consuming, labour-intensive and may impair the analytes of interest due to the mechanical stress induced by high speed rotation. Filter clogging is an inherent problem which is often difficult to avoid [126].

In the last two decades, microfluidic devices have been proven as a promising platform for particle/cell manipulation due to their advantages of lower cost, reduced sample volume needed, higher efficiency and accuracy [2]. Recently, particle manipulation in viscoelastic fluids has gained increasing attention. Particle or cell manipulation in viscoelastic fluids can be more flexible due to the fact that Many studies regarding 3D particle focusing in viscoelastic fluids have been performed [37, 47, 53, 61, 62, 78, 139, 140]. However, few studies have achieved particle separation in viscoelastic fluids. Yang et al. [47] demonstrated sheathless elasto-inertial particle focusing and continuous separation of different sized particles from the size dependence of the elastic force in a straight rectangular microchannel. Ahn et al. [150] achieved “elasto-inertial” particle separation by size in poly(ethylene oxide) (PEO) solution in a square channel. Because of the random initial distribution of particles, their approaches are limited by the low separation efficiency. Researchers also found that the effect of elasto-inertia on lateral migration can be further optimized when particles are injected along the channel wall at the entrance. Therefore, sheath flow or specific channel geometry to induce particles to one side of the wall can be applied initially, then separation can be realized downstream.

* Results of this chapter are published in: Dan Yuan, Say Hwa Tan, Qianbin Zhao, Sheng Yan, Ronald Sluyter, Nam-Trung Nguyen, Jun Zhang, Weihua Li. Sheathless Dean-flow-coupled elasto-inertial particle focusing and separation in viscoelastic fluid. RSC Advances, 2017, 7(6): 3461-3469.
This has been demonstrated in several studies [61, 66, 68, 81]. However, sheath flow is not beneficial for channel parallelization design and high-throughput processing. Instead of using sheath flow, Nam et al. [69] used specific channel geometry to align particles before separation. They used a circular channel followed by a symmetric bifurcation channel and a sudden expansion region to realize the initialization of the particle position and continuous particle separation in elasticity dominant polyvinyl pyrrolidone (PVP) fluids. However, the length of the circular channel section for pre-focusing and straight channel has to be carefully calculated and designed according to particle sizes.

In all the above papers demonstrating particle manipulation in viscoelastic fluids, the blockage ratio $\beta = a/D_h$, where $D_h = 2wh/(w+h)$ is the hydraulic diameter for a rectangular channel with $w$ and $h$ representing the width and height of the channel cross section are below the value of 0.25. Particle separation is based on the mechanism that the particles are prone to be focused at the centerline of the channel, but the lateral migration speed is highly dependent on particle size because the driving elastic force acting on the particles is proportional to particle sizes, which result in different lateral positions at outlet. However, when the value of blockage ratio $\beta$ is above 0.25, the effect of normal stresses is strengthened by the effect of the blockage. Unlike the traditional centerline focusing position, the centerline of the channel is no longer a stable equilibrium position, and the particles will be pushed towards the sidewalls [35, 151]. In this way, particles with blockage ratio below $\beta = 0.25$ will migrate to centerline of the channel regardless of their initial positions, while particles with blockage ratio above $\beta = 0.25$ will be attracted to the sidewalls.

Based on the new separation principle, this work reports a device consisting of two stages: a straight channel section with asymmetrical expansion–contraction cavity arrays (ECCA section) for sheathless particle initialization (1st stage), and a straight channel section for label free viscoelastic particle separation (2nd stage) is proposed. Particles with diameter of 4.8 µm ($\beta = 0.13$) and 13 µm ($\beta = 0.35$) are separated effectively in this channel. Separation of human Jurkat cells with diameter of 15 µm ($\beta = 0.4$) and yeast cells with diameter of 5 µm ($\beta = 0.13$) are achieved as well. To the best of our knowledge, only Liu et al. [58] realized the size-based separation of particles and cells suspended in viscoelastic fluids in straight microchannels using this new principle. However, the
approach was limited as the viscoelastic fluids containing PEO had to be denaturalized through storage at room temperature without exposure in sunlight for 3 months. In the current study, the PEO solution does not need to be denatured. Moreover, with the aid of an expansion–contraction cavity array (ECCA) section, all the particles are focused to a single line along one sidewall due to the Dean-flow-coupled elasto-inertial effects before the separation process. The ECCA pre-focusing section enhanced the separation performance of the straight section, and the newly prepared PEO solution in this channel provided good separation performance.

5.2. Schematic of sheathless particle focusing and separation

Figure 5-1 shows a schematic of the device for sheathless particle focusing and viscoelastic separation. The microfluidic device consists of two stages: an ECCA section for sheathless particle initialization (1st stage) and a straight channel section for label free particle separation (2nd stage). At the entrance region of the 1st stage, all particles are randomly distributed. Inertial lift, elastic and Dean drag forces affect the particles flowing through the ECCA section. Therefore, 3D particle pre-focusing at the opposite cavity side is realized by the synthesis effect of the three forces. This can be seen from the top and cross-sectional view at “B end of ECCA channel” section. Then, the particles enter the straight channel section. Since the blockage ratio for larger 13 µm particles (β = 0.35) exceed 0.25, the particles were displaced from the centerline and the fluid tends to flow through the larger gap between the particles and the wall. Therefore, the enhanced compressive normal stress at the near center side of the particles induced by the intensified shear rates will drive particles towards the sidewalls [35]. For the smaller 4.8-µm particles (β = 0.13), the effect of the blockage ratio is small, and particles will migrate to centerline of the channel regardless of their initial positions. This can be seen from the top and the cross-sectional view at “C outlet” section. From the fluorescence intensity profile at the end of first stage (B), the peak of the two curves are overlapped, indicating small and large particles are focused at the same position; at the end of second stage (C), the two curves are split up, indicating the pre-focused small and large particles are separated along the lateral direction.
5.3. Methods

5.3.1. Design and fabrication of microfluidic device

The microfluidic device has one inlet and two outlets, and comprised a 1st ECCA stage for sheathless particle initialization along the opposite cavity side, and a 2nd straight channel stage for viscoelastic particle separation. In the ECCA section, the right angled isosceles triangular cavities are patterned on one side of a straight channel. The longest edge of the triangle is 900 μm, and the space between two adjacent cavities is uniform at 900 μm. Two stages have a uniform cross section of 50 μm × 30 μm (width × height). The channel lengths in the 1st and 2nd stages are 3 cm and 2 cm respectively.

The device was fabricated using standard photolithography and soft lithography techniques [142, 143]. This fabrication included rapid prototyping on a silicon master, and polydimethylsiloxane (PDMS) replica molding and sealing through plasma oxidation.

5.3.2. Preparation of the PEO solution

PEO (2,000 kDa; Sigma-Aldrich) was diluted to 1000 ppm in distilled water (DI water) containing 0.01% (v/v) Tween 20 (Sigma-Aldrich) (PEO solution). Tween 20 was included in the PEO solution to prevent particle aggregation. For Jurkat cell and yeast
separation, PEO was added to a phosphate-buffered saline (PBS) (Sigma-Aldrich) at 1000 ppm.

The rheological property of the fluid was measured by a rotational rheometer (Antonpaar MCR 301) that has a parallel plate configuration and a diameter of 20 mm. The experiment was performed at room temperature (24 ± 1°C). The estimated viscosity of 1000 ppm PEO solution is $3 \times 10^{-3}$ Pa·s. The relaxation time was 12.4 ms [68, 152]. $R_c$ and $W_i$ for 1000 ppm PEO solution from flow rate $Q = 5 \mu$l/min to $Q = 20 \mu$l/min were in Table 2.

Table 2 $R_c$ and $W_i$ for 1000 ppm PEO solution

<table>
<thead>
<tr>
<th>$Q$ (μl/min)</th>
<th>$R_c$</th>
<th>$W_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.7</td>
<td>28</td>
</tr>
<tr>
<td>10</td>
<td>1.4</td>
<td>56</td>
</tr>
<tr>
<td>15</td>
<td>2.1</td>
<td>84</td>
</tr>
<tr>
<td>20</td>
<td>2.8</td>
<td>112</td>
</tr>
</tbody>
</table>

5.3.3. Particle preparation

Particle suspension was prepared by diluting 4.8-μm internally green dyed fluorescent polystyrene microspheres (ThermoFisher Scientific, CV 5%), and 13-μm internally red dyed fluorescent polystyrene microspheres (Thermo Fisher Scientific, CV 5%) in the 1000 ppm PEO solution. For comparison, the 4.8-μm and 13-μm particles are diluted in DI water as well. The blockage ratio for 4.8-μm and 13-μm particles is 0.13 and 0.35, respectively.

For cell experiments, Jurkat cells (ATCC), an immortalized human T cell line (average diameter around 15 μm), were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (ThermoFisher Scientific) containing 10% fetal bovine serum (Bovogen Biologicals) and 2 mM L-glutamine (ThermoFisher Scientific) in a humidified incubator (Thermo Scientific) at 37°C and 95% air/5% CO₂. Before the cell experiments, Jurkat
cells were centrifuged (300 x g) and re-suspended in PBS containing 1000 ppm PEO. Yeast from Saccharomyces cerevisiae (Sigma-Aldrich) was diluted by PBS containing 1000 ppm PEO. Jurkat and yeast cells were mixed to yield final concentrations of 1.6 x 10^6 cells/ml and 6 x 10^7 cells/ml, respectively. Before commencing each experiment, the particle mixture solutions were re-suspended by vortex and cell samples were manually stirred to provide uniform suspensions.

5.3.4. Experimental setup

The particle and cell suspensions were transferred to 1-ml syringes and then introduced into the microfluidic chip through silicon tubes by Legato 100 syringe pumps (KD Scientific). The microfluidic chip was then placed onto a CKX41 inverted microscope (Olympus), and images were observed and captured using a CCD camera (Optimos, Q-imaging). Images were post-processed and analysed with Q-Capture Pro 7 (Q-imaging) software. The cell distributions were analyzed using a customized MATLAB program.

5.4. Results

5.4.1. Effects of flow rates

The mixture of both 4.8-µm and 13-µm particles are focused to a tight stream at the 1st stage; after the initialized particles enter the 2nd stage, 4.8 µm particles (β = 0.13) migrate to the center of the channel, while 13 µm particles (β = 0.35) are attracted near the channel walls. As mentioned above, the fluid tends to flow through the larger gap between the particle and the wall when the particle migrates from the centerline, and the enhanced compressive normal stress at the near center side of the particle induced by the intensified shear rates will drive particles towards the side walls. In this channel, the existence of an asymmetrical expansion–contraction cavity arrays in the 1st stage, results in the secondary flow which pushes the particles to the opposite side of cavities. Therefore, only one equilibrium position exists along the channel wall for the 13-µm particles in straight channel section at the 2nd stage.

Figure 5-2 shows the distribution of particles at the expansion area in the outlet. The captured fluorescent images and corresponding normalized fluorescent intensity profiles at different flow rates are shown in Figure 5-2 (a). At \( Q = 5 \, \mu\text{l/min} \) (\( Re = 0.7, \, Wi = 28 \)), \( Q = 10 \, \mu\text{l/min} \) (\( Re = 1.7, \, Wi = 28 \)), \( Q = 20 \, \mu\text{l/min} \) (\( Re = 3.4, \, Wi = 28 \)), \( Q = 30 \, \mu\text{l/min} \) (\( Re = 5.2, \, Wi = 28 \)), \( Q = 50 \, \mu\text{l/min} \) (\( Re = 8.7, \, Wi = 28 \)) and \( Q = 70 \, \mu\text{l/min} \) (\( Re = 12.2, \, Wi = 28 \)).
13-µm particles (red fluorescence line) and 4.8-µm particles (green fluorescence line) are focused well in the Dean-flow-coupled elasto-inertial effect at 1st stage, then they are separated accordingly (13-µm particles are attracted to the upper channel wall, 4.8-µm particles are focused tightly at the centerline). As the flow rate increases to \( Q = 10 \mu\text{l/min} \) \((c_R = 1.4, W_i = 56)\), there is insufficient time for the 4.8-µm particles to migrate to the centerline of the channel by the elastic force. With the flow rate increasing further \((15 \mu\text{l/min} \ (R_c = 2.1, W_i = 84) \text{ and } 20 \mu\text{l/min} \ (R_c = 2.8, W_i = 112))\), the time for particle lateral migration is shorter, therefore the distance between the two-sized particles reduces. Moreover, as the flow rate increases, the inertial effect becomes more dominant, thus particles gradually become less focused. As seen from the normalized lateral position and the width of particles stream at different flow rates (Figure 5-2 (b)), the distance between the normalized lateral positions of two particles becomes smaller as the flow rate increases, and the particles become more dispersed at the same time (the symbols represent the peak fluorescent intensity position of 13 µm particles and 4.8 µm particles respectively at each flow rate; the error bars represent the focusing width at 50% peak fluorescent intensity). The optimal flow rate for particle separation at this integrated ECCA straight channel is \( Q = 5 \mu\text{l/min} \) \((R_c = 0.7, W_i = 28)\). The optimal flow rate could be enhanced by extending the straight channel section at the 2nd stage.
Figure 5-2 Distribution of particles at the expansion area at outlet: (a) The captured fluorescent images and corresponding normalized fluorescent intensity profiles at different flow rates; (b) Normalized lateral position and width of particles at different flow rates.

5.4.2. Effects of distance from inlet

Particles have different distributions at varying channel positions, Figure 5-3. Figure 5-3(a) shows the captured fluorescent images along channel length from inlet at $Q = 5 \mu l/min$ ($R_e = 0.7$, $W_t = 28$) and their corresponding normalized fluorescent intensity profiles. At the inlet, the mixture of 4.8-µm and 13-µm particles are randomly distributed. As the particles flow, they are confined within a narrow band at opposite cavity side (10
mm from inlet). Then, the focusing band becomes narrower at 20 mm distance from the inlet. At the end of the 1st stage (30 mm from inlet), all particles are focused to a tight

![Figure 5-3 Distribution of particles at different channel positions](image)

Figure 5-3 Distribution of particles at different channel positions: (a) The captured fluorescent images at $Q = 5 \mu l/min$ and corresponding normalized fluorescent intensity profiles at different positions; (b) Normalized lateral position and focusing width of particles at different distances from inlet.

stream by the Dean-flow-coupled elasto-inertial effects. This can be seen from the overlapping lines in the fluorescent images and the intensity profiles. After the particles enter the straight channel section, 4.8-µm particles migrate to the center of the channel by the elastic force, while 13-µm particles shift to the channel wall (40 mm from inlet) due to the blockage ratio effect. At a position of 50 mm from the inlet, two different equilibrium positions are formed at outlet and particles with different sizes are separated consequently. It can also be proven from the normalized lateral positions and focusing width of particles at different distances from inlet (Figure 5-3 (b)), 4.8-µm and 13-µm particles came together to a single line at the 1st stage, then migrated to adverse directions and are separated gradually (the symbols represent the peak fluorescent intensity position.
of 13 µm particles and 4.8 µm particles respectively at each position; the error bars represent the focusing width at 50% peak fluorescent intensity). Comparison of particle separation in viscoelastic fluids and Newtonian fluids, in pure straight channel and integrated ECCA straight channel.

The particle distribution in a viscoelastic fluid (PEO solution) and a Newtonian fluid (DI water) were compared in the same integrated ECCA straight channel, Figure 5-4 (a). No particle focusing is observed at the outlet in a Newtonian fluid. However, single-line focusing is achieved by the combination of inertial force, elastic force and Dean drag force in the viscoelastic fluids. Furthermore, particles with different blockage ratios are successfully separated at the outlet in viscoelastic fluids.

The particle distribution in the integrated ECCA straight channel and the single straight channel in viscoelastic fluids are compared as well, Figure 5-4(b). In the pure straight channel, larger particles are focused at the center of narrower channel walls and form two equilibrium positions along the two sides of wider channel walls, but the particles cannot be focused very well. However, in the integrated ECCA straight channel, the secondary flow reduces two equilibrium positions to one for larger particles. Also, the pre-focusing of particles by the Dean-flow-coupled elasto-inertial effect at the 1st stage facilitates particles’ distinct lateral migration at the 2nd stage and improves the separation performance significantly.
5.4.3. Separation of Jurkat cells and yeast cells

The previous section demonstrates the separation of 13-µm (β = 0.35) particles and 4.8-µm (β = 0.13) particles. In order to utilise the potential of our device, separation of Jurkat cells from yeast cells was performed. The mean size of Jurkat and yeast cells is about 15 µm (β = 0.4) and 5 µm (β = 0.13), respectively.

Cells have different distributions at different channel positions (Figure 5-5). The cells within the red circles are Jurkat cells, and the yeast cells are pointed by blue arrows. At the inlet, a mixture of Jurkat and yeast cells are randomly distributed; as the cells flow, they are confined within a narrow band (10 mm from inlet); then the focusing band becomes narrower at 20 mm distance from inlet; at the end of the 1st stage (30 mm from inlet), all cells are focused to a tight stream by the dean-flow-coupled elasto-inertial effects; after cells enter straight channel section, yeast cells migrate to the center of the channel by the elastic force, while Jurkat cells shift to the channel wall (40 mm from inlet) under the blockage ratio effect; at 50 mm from inlet, two different equilibrium positions are formed at outlet and cells with different sizes are separated consequently.
Figure 5-6 (a) shows the distribution of Jurkat and yeast cells at expansion region at different flow rates (captured images in bright field and corresponding normalized cell frequency). The red circles and blue arrows indicate the Jurkat cells and yeast cells, respectively. The number of cells for each cell type within different segments in the expansion region was quantified using a customized MATLAB program. The channel width was divided into twenty virtual segments, and the number of cells in each segment was normalized by the total number of cells traversing the entire region. Out of expectation, at $Q = 5 \mu$l/min ($R_e = 0.7$, $W_i = 28$), larger cells distributed at both sides of the channel walls, and most smaller cells are in the middle; as the flow rate increases (10 $\mu$l/min ($R_e = 1.4$, $W_i = 56$)), the number of larger cells decreased at the lower channel wall side; with the flow rate increasing to 20 $\mu$l/min ($R_e = 2.8$, $W_i = 112$), the separation performance is improved, as well as the focusing performance for both cells. At $Q = 20$ $\mu$l/min ($R_e = 2.8$, $W_i = 112$), the Dean-flow-coupled elasto-inertial focusing and separation based on differential focusing for cells with different sizes are achieved in this integrated ECCA straight channel. The optimal separation flow rate for the cells is different from that for particles. This is most likely due to differences in the compressibility of cells and particles, as cell focusing requires a higher flow rate under the Dean-flow-coupled elasto-inertial focusing effect at the 1st stage.
Figure 5-6 Separation of Jurkat cells and yeast cells in the viscoelastic fluids. (a) The distribution of Jurkat and yeast cells at the expansion region under different flow rates (captured images in bright field and corresponding normalized cell count). (b) The recovery rate and purity of cells at each outlet at $Q = 20 \, \mu l/min$.

To evaluate the separation performance of the device, the recovery rate (the ratio of the number of target cells collected in the target outlet to the total number of target cells collected from both outlets), purity (the ratio of the number of target cells to the total number of cells collected at the target outlet), and enrichment ratio (the ratio of the number of target cells to the number of untargeted cells at the inlet divided by the ratio of the number of target cells to the number of untargeted cells collected at the target outlet) were calculated, respectively. The recovery rate, purity and enrichment ratio of Jurkat cells collected at the outlet 1 were 99.6%, 24% and 11.9, respectively. Figure 5-6 (b) shows the recovery rate and purity at each outlet at $Q = 20 \, \mu l/min$ ($R_e = 2.8$, $W_i = 112$). Since the initial ratio of Jurkat cells in the total cells is only 2.6%, even a small portion of yeast cells mixed with Jurkat cells at the outlet will significantly reduce the purity.
However, this device can still have a high recovery rate and enrichment ratio. These results demonstrated that our device can enrich sample concentration significantly and potentially improve the sensitivity of downstream detection.

5.5. Conclusion

In summary, an innovative microfluidic device for sheathless particle focusing and separation in viscoelastic fluids is described in this work. Particles with different blockage ratios are firstly focused on the opposite cavity side by Dean-flow-coupled elasto-inertial effects at the 1st stage, then separated at the 2nd stage based on the differential focusing of particles with different sizes in viscoelastic fluids. The effects of flow rates and distance from the inlet on particle separation were investigated. The comparison of particle separation in a viscoelastic fluid and a Newtonian fluid; in single straight channel and integrated ECCA straight channel were also studied. This device was further applied for separation of Jurkat cells and yeast cells. The recovery rate, purity, and enrichment ratio for Jurkat cells can reach 99.6%, 24% and 11.9, respectively. The described device offers an efficient, continuous, and sheathless particle separation method in viscoelastic fluids. This device could be potentially used for clinical and biological applications, where biological particles with various sizes need to be separated, or for bio-sample enrichment to improve the sensitivity of the downstream detection unit.
6. Investigation of particle lateral migration in sample-sheath flow of viscoelastic fluid and Newtonian fluid *

6.1. Introduction

Microfluidic platforms can manipulate, control fluids and particles in micron and submicron domains [8]; microfluidics has the potential to be used in a variety range of applications [153], such as single cell or molecule analysis and manipulation [154], drug screening and delivery [155], point of care diagnostics and therapeutics [156], and so on.

Particle or cell manipulation in microfluidics such as focusing, separation and counting is very important and essential in numerous biological and chemical applications. The manipulation methods are based on the particles’ intrinsic physical characteristics such as particle size, shape, density, compressibility, polarizability and magnetic susceptibility. The manipulation methods can then be classified into two categories according to the source of dominant forces: active methods and passive methods. Active methods are based on the application of external force fields such as dielectrophoresis (DEP) [12, 14, 157], magnetophoresis [16], acoustophoresis [18] and optical tweezers [21]. Passive methods are based on the microchannel geometrical effects and hydrodynamic forces [22], such as pinched flow fractionation (PFF) [26], hydrodynamic filtration [27], inertial microfluidics [6, 158], deterministic lateral displacement (DLD) [120].

Among various manipulation methods, sheath flow is often used in microfluidics to pre-focus particles or pinch fluid to one side of the channel. For example, in flow cytometry [159], sheath flow focusing is often used to align particles or cells one by one for downstream counting, detecting, and sorting. Recently, sheath flow configuration was used to focus particles in 3D by dean flow [160] and vertical confinement [161]. Moreover, sheath flow was integrated with DEP [162], magnetic [163], optical methods [164], or iPFF (inertial-enhanced pinched flow fractionation) [165] to realise particle separation. Besides particle focusing and separation, sheath flow is essentially important

in solution exchange when integrated with other active [21, 166, 167] or passive methods [168-171].

However, in these works, the fluids of sample-sheath flow are always with the same property, and most of them are in co-flow of Newtonian and Newtonian fluids. Particle migration in viscoelastic fluids, recently, has attracted more interest [37, 50, 52, 53, 56, 58, 78, 83, 95, 122, 139, 140], and sample-sheath flow with the same viscoelasticity was applied to achieve particle separation [61, 66, 68, 81]. Currently, none of publications has reported about particle manipulation in sample-sheath flow with different fluids properties. In this work, we experimentally investigated particle migration in sample-sheath flow with different fluid properties, one is viscoelastic fluids and the other is Newtonian fluids. The particles can easily migrate from viscoelastic flow (1000 ppm PEO solution) to Newtonian sheath flow (DI water). In normal laminar flow, the migration of particles or cells across streamlines is difficult to achieve without assistance of external force fields or specific channel structures [169], while by using viscoelastic sample flow and Newtonian sheath flow, particle lateral migration can be easily achieved in a simple straight channel, without any external force fields.

6.2. Schematic of lateral particle migration in sample-sheath flow of PEO solution-DI water, DI water-PEO solution, and DI water-DI water

Figure 6-1 (a) shows the schematic diagram of particle lateral migration in sample-sheath flow of 1000 ppm PEO solution and DI water in a straight channel. At the inlet section of the channel the particles are randomly distributed in the original sample PEO solution stream; as the fluid flows, the particles are transported laterally and focused into a tight streak, after which they progressively move across the boundary of the PEO solution and migrate to DI water solution. Figure 6-1 (b) and (c) show the simulation results of velocity profile and shear rate. COMSOL Multi-physics 5.2 (COMSOL, Burlington, MA) finite element software was used to calculate the velocity profile and shear rate in the cross section of the rectangular microchannel. The laminar steady incompressible flow model was used. The inlet condition was set with a uniform average velocity calculated from the flow rate, and the non-slip boundary condition was applied onto the surfaces of the channel. (As the shear viscosity in non-Newtonian fluid is considered constant, there is no difference between Newtonian and viscoelastic flows in
the velocity distribution within a cross-section at the same flow rate. The color bar represents the magnitude of the velocity. The magnitude of velocity increases from the bottom of the bar (dark blue) to the top of the bar (dark red). It can be seen from the simulation results that the maximum velocity occurs in the central part of the rectangular channel, and decreases gradually from the centre line to the channel wall, which validates that the velocity profile is a parabolic curve. The simulation results of the shear rate, which is the differential of the flow velocity. There are five zero shear rate regions in the cross section, corresponding to the blue areas in Figure 6-1 (c). Particles focusing position in viscoelastic fluids is controversial. Some researchers reported that the particles migrate towards the five zero shear rate regions when the second normal stress difference in viscoelastic fluids is negligible [52]; Yang et al. [47] believed that the number of multiple equilibrium positions can be reduced to central area only by adjusting the flow rate to
cope with the synergetic effect of inertia and viscoelasticity; while Del Giudice et al. [53] observed that no matter the inertia is negligible or not, the particles migrate to the channel centerline for all the experimental conditions, and they believed the elastic effects are much stronger than the inertial effects and the PEO solution exhibits purely elastic effects. In our experiments, no particle migration along four corners was observed, and the particle Reynolds number \( R_P \approx 0.03 \) (Q<30µl/min) is much smaller than 1. Although we are not sure whether the negligible inertial effect has eliminated the equilibrium positions in four corners, it’s verified in the following experiments that it’s the dominant elastic force rather than the inertial effect that induces the lateral migration of particles. Figure 6-1 (d) and (e) show the experimental results of lateral particle migration in sample-sheath flow of 1000 ppm PEO solution-DI water in straight channel at inlet and outlet respectively, with a flow rate of 5 µl/min sample flow-20 µl/min sheath flow, as well as the corresponding fluorescent intensity profiles.
In this work, the direction of particle migration is from a non-Newtonian viscoelastic PEO solution to a Newtonian DI water solution, so it was presumed that the lateral transport of particles is induced by the elastic force $F_E$. At the same time, these particles also experience an opposite drag force as they move laterally across the streamlines. As the particles migrate across the PEO boundary, the elastic force disappears when particles completely escape from PEO solution, however, particles can still migrate a certain lateral distance within the DI water solution. We suspect that this may be due to the inertia of particle lateral migration when particles migrate across the PEO boundary. In the sample-sheath flow of PEO solution and DI water, diffusion is inevitable and the lateral particle migration across the boundary of viscoelastic fluids and Newtonian fluids occurs when the velocity of the migrating particles is higher than the speed of diffusion for the PEO solution.

To further verify the fact that a viscoelastic solution provides the lateral driving forces needed for particle lateral migration, comparative experiments were carried out using sample-sheath flow of DI water sample solution-DI water sheath flow and DI water sample solution-PEO sheath flow solution, as shown in Figure 6-2. In Figure 6-2 (a), instead of suspending particles in a PEO solution, internally dyed 4.8 µm particles were suspended in DI water and the sheath flow was also DI water. The result was that the green-dyed particles and red-dyed DI water solution still overlapped 10 mm away from the inlet, and the particles cannot migrate from DI water to the other DI water stream. In Figure 6-2 (b), the particles were suspended in DI water, although a 1000 ppm PEO solution was used as the sheath flow; the particles still could not be transferred from the DI water to the PEO solution which means that the particles can be transferred laterally, but only when they are in a viscoelastic PEO solution.

These two comparative experiments proved that the lateral migration of particles is selective and the direction of migration can be controlled by adding PEO to the original sample solution.
6.3. Methods

6.3.1. Design and fabrication of microfluidic device

A simple straight rectangular channel with a 30 × 50 μm (width × height) cross section and length of 10 mm was used. The sample flow and sheath flow were injected from two inlets. The device was fabricated using standard photolithography and soft lithographic techniques [142, 143]. This fabrication included rapid prototyping on a silicon master, and polydimethylsiloxane (PDMS) replica molding and sealing through plasma oxidation. Fabrication also included spinning a photoresist (SU-8 2025, MicroChem Co., Newton, MA) on a silicon wafer at 2000 rpm until it became 50 μm thick. This was then exposed to UV light through a mask designed using a mask aligner system (ABM, San Jose, CA); the photoresist on the silicon wafer was then developed in an SU-8 developer solution and rinsed with isopropanol (IPA) to create a positive replica of channel geometry. A PDMS mixture at a 10:1 ratio of base to curing agent (Dow Corning, Midland, MI) was poured over the silicon master, which was degassed in a vacuum oven to remove bubbles, and then cured at 100°C for 45 min. After curing, the PDMS was peeled off the silicon master and then a custom needle tip was used to punch the inlet and outlet holes. The PDMS slide was then bonded to a glass slide after being exposed to oxygen plasma (PDC-002, Harrick Plasma, Ossining, NY) for 3 minutes.
6.3.2. Preparation of the PEO solution

A PEO (poly (ethylene oxide), Mw = 2, 000, 000, Sigma-Aldrich) aqueous solution with three different levels of concentration were prepared (500 ppm, 1000 ppm, and 5000 ppm in DI water) as the viscoelastic fluids. DI water was used as the Newtonian fluids. Tween 20 (0.01 wt%, Sigma-Aldrich) was added to all the solutions to prevent particle-to-particle aggregation. The estimated viscosity for 500 ppm, 1000 ppm, 5000 ppm PEO solutions are $4 \times 10^{-3}$, $6 \times 10^{-3}$, $2 \times 10^{-2}$ Pa·s, respectively.

The relaxation time for 500 ppm, 1000 ppm, 5000 ppm PEO solutions were calculated as 9.1ms, 14.1ms, 40.2 ms [47]. $W_s$ was calculated as 170, 263, 750 for 500 ppm, 1000 ppm, 5000 ppm PEO solutions respectively when the flow rate ratio $\alpha$ was set as 4 ($Q_s = 5 \mu l/min$, $Q_{sh} = 20 \mu l/min$) in this straight channel.

6.3.3. Particle preparation

Particle suspensions were prepared by diluting 4.8 μm of internally green dyed fluorescent polystyrene microspheres (product no. G0500, CV < 5%, Thermo Fisher Scientific, USA) particles with a concentration of ~10$^7$ particles ml$^{-1}$ using DI water and PEO solution, respectively. To observe the particle migration better, the sample fluid stream was mixed with red fluorescent dye because it has different fluorescence spectra than the fluorescent particles, and therefore, the distinct trajectory of the particles and sample medium could be seen by different fluorescent light. Before commencing each experiment the particles were shaken by a vortex mixer to guarantee a uniform suspension.

6.3.4. Experimental setup

The dyed sample solution and sheath solution were transferred to 1 ml syringes and then introduced into the microfluidic chip through silicon tubes by syringe pumps (Legato 100, Kd Scientific). The microfluidic chip was then placed onto an inverted microscope (CKX41, Olympus, Japan), and images of the fluorescent particles and fluids were observed and captured by a CCD camera (Optimos, Q-imaging, Australia), and then the fluorescent images were post-processed and analysed with Q-Capture Pro 7 (Q-imaging, Australia) software.
The flow rate of sample flow $Q_s$ was fixed at 5 µl/min and the flow rate of the sheath flow $Q_{sh}$ varied from 5 µl/min to 40 µl/min, so that the ratio of the flow rate of sheath flow to sample flow ($\alpha = Q_{sh}/Q_s$) ranged from 1:1 to 8:1 and the total flow rate ranged from 10 µl/min to 45 µl/min. Fluorescent images and profiles of fluorescent intensity were taken laterally at 10 mm downstream from the inlet to examine migration performance.

6.4. Results

6.4.1. Effects of channel length

![Figure 6-3 Particle lateral migration phenomenon at (a) the inlet; (b) 5 mm downstream; (c) 10 mm downstream from the inlet; (d) the corresponding normalised fluorescent intensity profiles.](image)

The particle lateral migration also depends on the distance from the inlet of the channel. Figure 6-3 shows the lateral migration of particles at the inlet (Figure 6-3 (a)), at 5 mm downstream (Figure 6-3 (b)), and at 10 mm downstream from the inlet (Figure 6-3(c)), respectively, in a 1000 ppm PEO sample solution-DI water sheath flow. At the inlet (Figure 6-3(a)), the overlapping green and red fluorescence means that the particles are randomly distributed in the PEO solution. At 5 mm downstream from the inlet (Figure 6-3 (b)) most particles begin to migrate from the PEO solution to the DI water and focus...
into a tight streak. At 10 mm downstream from the inlet (Figure 6-3 (c)), almost all the particles have migrated from the PEO solution to the DI water and are focused tightly. The profiles of particle fluorescent intensity taken at three different distances (Figure 6-3 (d)) indicate that the particles have shifted from the upper side to the centreline of the channel along its length, and the width of the focusing streak has also been reduced.

The mixture of both 4.8-µm and 13-µm particles are focused to a tight stream at the 1st stage; after the initialized particles enter the 2nd stage, 4.8 µm particles (β = 0.13) migrate to the center of the channel, while 13 µm particles (β = 0.35) are attracted near the channel walls. As mentioned above, the fluid tends to flow through the larger gap between the particle and the wall when the particle migrates from the centerline, and the enhanced compressive normal stress at the near center side of the particle induced by the intensified shear rates will drive particles towards the side walls. In this channel, the existence of an asymmetrical expansion–contraction cavity arrays in the 1st stage, results in the secondary flow which pushes the particles to the opposite side of cavities. Therefore, only one equilibrium position exists along the channel wall for the 13-µm particles in straight channel section at the 2nd stage.

6.4.2. Effects of flow rate

In this section, the effects of the flow rate on particles lateral migration in sample-sheath flow of PEO solution-DI water are investigated. The sample flow rate remained constant at $Q_s = 5 \, \mu l/min$, while the sheath flow rate $Q_{sh}$ varied from 5 µl/min (i.e., $\alpha = 1$) to 40 µl/min (i.e., $\alpha = 8$). Figure 6-4 (a) shows the fluorescent images 10 mm downstream from the inlet, and the corresponding fluorescent intensity profiles along the width of the channel are shown in Figure 6-4 (b).

When the flow rate ratio $\alpha$ is low (e.g. $\alpha=1:1$ and 2:1, Figure 6-4 (ai, aii, bi and bii), the red-dyed PEO solution occupies almost half of the width of the channel after 10 mm, and although the particles can be transferred into the DI water solution the lateral distance between the particle streak and the edge of the PEO solution is relatively small. As the flow rate ratio $\alpha$ increases (e.g. $\alpha=4,5,6$, Figure 4 (aiv) ~ (avi), Figure 6-4 (biv) ~ (bvi)), the red-dyed PEO solution is forced into a much narrower width through the channel and it’s observed distinctly that the particles move away from the PEO solution and are
focused into a tight streak around the centre of the channel. As the flow rate ratio $\alpha$ increases further (Figure 6-4 (avii), (aviii), (bvii), and (bviii)), the particle’s focusing streaks expand significantly, but particle transfer can still occurred because the total flow rate is too high for the particles to focus properly. In order to analyse the performance of particle lateral migration, the difference between the lateral positions corresponding to a 0.2 normalised fluorescent intensity of the red-dyed PEO solution and the peak fluorescent intensity of the green-dyed particles, respectively, are shown in Figure 6-4 (c) (Here we suppose the 0.2 normalised fluorescent intensity is low enough, and the PEO in the fluid can be neglected at that position). It’s observed that when the flow rate is small, any differences in the lateral position are small which means that most of the particles are still inside the PEO solution, and these differences in the lateral position are enhanced as the flow rate increases, indicating a more efficient lateral migration of particles. In the following studies, a flow rate ratio of $\alpha=4$ ($Q_s = 5 \, \mu l/min$, $Q_{sh} = 20 \, \mu l/min$) was chosen for both better particle migration and better particle focusing performance.
6.4.3. Effects of PEO concentration

The effects of different PEO concentrations on the lateral migration of particles are shown in Figure 6-5 where PEO solutions with concentrations of 500, 1000, and 5000 ppm were tested. The flow rate ratio was maintained at $\alpha=4$ ($Q_s = 5 \mu l/min$, $Q_{sh} = 20 \mu l/min$). Particle migration from one solution to another in those cases involving 500 ppm and 1000 ppm PEO solutions (see Figures 6-5 (a) and (b)) and the particles were well focused. However, in the 5000 ppm PEO solution the lateral distance decreased and the particle focusing width increased, and this solution diffused faster, which resulted in a less effective particle lateral migration (see Figure 6-5 (c)).

With 500 ppm and 1000 ppm PEO solutions, the elastic force ($F_E$) experienced by the particles, overcame the drag force to enable an effective lateral transfer of particles, but the viscosity of the 5000 ppm PEO solution was much higher and diffusion occurred more rapidly due to stronger elastic turbulence effect [148, 149], which means it is much harder to force particles across streamlines. From the rheological data of PEO solutions, the higher ratio of loss modulus to storage modulus (damping ratio) corresponds to a faster diffusion (Figure 6-6 (a)). We believe that there must be an intrinsic linkage between polymer fluid diffusion speed and its rheological property (e.g. damping ratio (Figure 6-
6 (b)), which requires a dedicated and systematic further investigation. From above experimental investigations, PEO solution with 1000ppm concentration was chosen for particle lateral migration.

Figure 6-5 The effects of different PEO concentrations (a) 500 ppm; (b) 1000 ppm; (c) 5000 ppm on particle lateral migration and the corresponding fluorescent intensity profiles.
Figure 6-6 Rheological properties of 500 ppm, 1000 ppm, 5000 ppm PEO solutions. (a) the amplitude dependence of the viscoelastic modulus (G’ and G”) of each viscoelastic fluid, where G’ and G” represent storage modulus and loss modulus, respectively; (b) the damping ratio (the ratio of loss modulus and storage modulus, G”/G’) of each PEO solution.

6.5. Conclusion

In this work, particle lateral migration in sample-sheath flow with different properties (viscoelastic fluids and Newtonian fluids) was investigated. By using non-Newtonian viscoelastic fluids, particles can be transferred laterally across streamlines in sample-sheath flow of viscoelastic PEO solution-DI water. This method employs the elastic force, and the particle migration direction can be selectively controlled by the proper pair of co-flowing solutions. The effects of channel length, flow rate, and PEO concentration on the particle migration were also studied. This lateral particle migration technique does not require any external force field or complex channel structures, and can deliver selective particle lateral migration by simply adding PEO molecules to particle suspension. This selective particle lateral migration technique can be potentially used in solution exchange, automated cell staining, and washing in microfluidic platforms.
7. On-chip microparticle and cell washing using co-flow of viscoelastic fluid and Newtonian fluid *

7.1. Introduction

Washing of microparticles and cells have broad applications in biology, medicine and clinical analysis [172-174]. Washing is often necessary for the preparation of samples prior to experimentation or analysis [169, 175, 176]. For example, the extraction of stained leukocytes from whole blood samples requires conventional steps such as mixing reagents, centrifugation, and resuspension or microfluidic washing steps to remove cell lysates or other cell components [120, 168, 177]. Transferring particles or cells from a high background to a low background can improve the measurement accuracy in flow cytometry [168].

Centrifugation is the conventional cell washing method. Although simple and ubiquitous, centrifugation has many limitations. For example, the centrifugation processes are often carried out in batches and are not continuous in nature. This method is also time-consuming, labour-intensive, and the high speed rotation may alter the analytes of interest. The recovery rate and purity of the collected cells is limited because of the manual pipetting steps, which varies significantly based on experience and skill of operators.

Microfluidic techniques, which can manipulate particles and control fluids at micron and submicron dimensions [8], have been used in various biomedical and biochemical fields [178-181], and present alternative methods for particle/cell washing. According to their operating principle, microfluidic techniques are classified as active and passive methods. Active methods rely on external force fields such as dielectrophoresis (DEP) [166, 182], optical [21], magnetic [183, 184] and acoustophoresis [167, 185-187]. Whereas passive methods rely on intrinsic hydrodynamic forces induced in microchannels with specialized geometry or structures [169, 177, 188] such as deterministic lateral displacement [120, 169], pinched-flow fractionation [189], differential inertial focusing in the channels with

* Results of this chapter are published in: Dan Yuan, Say Hwa Tan, Ronald Sluyter, Qianbin Zhao, Sheng Yan, Nam-Trung Nguyen, Jinhong Guo, Jun Zhang, Weihua Li. On-chip microparticle and cell washing using co-flow of viscoelastic fluid and Newtonian fluid. Analytical chemistry, 2017, 89(17): 9574-9582.
shifting aspect ratios [168, 170, 190, 191], trapping particles or cells by vortex technology using expansion and contraction cavity arrays [192, 193], inertial flow deformation induced by sequences of simple micro-pillar arrays to switch fluid streams [171, 194], and fluid transfer around particles induced by particles rotation [195].

The above microfluidic methods for particle/cell washing are all performed in Newtonian fluids, which employ either external force fields or specially designed complex channels. Recently, the interest in particle manipulation in viscoelastic fluids has been growing because of its superior focusing performance in the relatively simple channel geometry. In viscoelastic fluids, the dilute polymer within the fluid can induce the first normal stress difference ($N_1$). The suspended particles or cells flowing in the micro-channels can migrate laterally under the effect of $N_1$ [35, 121, 122]. Many researchers have studied particle/cell focusing or separation in viscoelastic fluids with or without the aid of sheath flow [47, 58, 66, 71, 196]. In those experiments that with the aid of sheath flow to do particles/cells separation [61, 66, 81], the sample and sheath streams are both viscoelastic fluids. The separation mechanism is that all the particles/cells pre-focused by sheath flow are prone to migrate to the centre of the channels in the viscoelastic fluids, while the migration speed is different according to their sizes. However, the particles/cells lateral migration behaviours in sample-sheath flow with different properties are rarely studied.

Recently, our group [141] and others [102] have proposed and explored the lateral migration of particles using the co-flow configuration of viscoelastic and Newtonian fluids. Ha et al. implemented particle separation using $\lambda$-DNA viscoelastic and Newtonian fluids [102]. $\lambda$-DNA is linear, doubled-stranded DNA from an *Escherichia coli* bacteriophage. Instead of using expensive $\lambda$-DNA as the diluted polymer to form viscoelastic fluids, our group has investigated particle lateral transfer from poly(ethylene oxide) (PEO)-containing viscoelastic fluids to a Newtonian fluid [141]. In the present work, we further characterise particle manipulation properties using this configuration, and apply them for on-chip microparticle and cell washing. The phenomenon of lateral migration of particles with different sizes using co-flow of viscoelastic fluids and Newtonian fluids is studied, and the critical blockage ratio for efficient particle washing is determined. Secondly, the continuous on-chip washing of leukaemic Jurkat cells is demonstrated using co-flow of viscoelastic fluids and Newtonian fluids. Finally, the cell
washing quality was tested by using hemocytometry and flow cytometry.

Compared with other particle/cell washing methods, our method can be performed in simple straight channels, without any external force. The simplicity of the method is due to the viscoelastic force that is induced by the medium intrinsic property. Although inertia-based cell washing can work in straight channel as well [168, 170], with higher flow rate, the particles/cells size for efficient migration and washing is limited. However, our method can work with smaller and wider range of particles/cells sizes. This work investigates a new method for particles/cell washing, and can deepen the understanding of particle behaviour in coflows with different properties. Moreover, the PEO polymer, which is added to the medium to tune its elasticity, is biocompatible and of low cost. In summary, the technique presented here can be safer, simpler, cheaper and more efficient than the tedious conventional medium exchange and washing methods. This method has the potential to allow for direct processing of various native biofluids and may be suitable for a wide range of biomedical applications.

7.2. Schematic diagram of particles’ on-chip washing using co-flow of viscoelastic fluid and Newtonian fluid

Figure 7-1 shows the concept of on-chip particle washing using co-flow of a viscoelastic fluid and a Newtonian fluid. The mixture of particles with two different sizes in the viscoelastic fluids is injected from one inlet, while the Newtonian fluids is introduced from the other inlet as a sheath flow. The flow rate ratio is 1:3 with the flow rate of sample flow $Q_s$ and sheath flow $Q_{sh}$ at 2 µl/min and 6 µl/min, respectively. At the inlet section of the channel, the particles are dispersed in the original viscoelastic fluids, and are squeezed to the upper side of the channel due to the Newtonian sheath flow. As the fluid moves, the particles with their blockage ratio above a certain value are focused into a tight streak, and transported laterally. Progressively, all the particles move across the boundary of the PEO medium (viscoelastic fluids) and migrate into deionized water (Newtonian fluids). However, the particles with their blockage ratio below the threshold cannot migrate to the Newtonian medium, and stay in their original viscoelastic medium. The migration phenomenon is attributed to the elastic force $F_E$ in viscoelastic fluids. The migration direction is from viscoelastic fluids to Newtonian fluids, which has been validated in our
Figure 7-1 Schematic diagram of on-chip particle washing using co-flow of a viscoelastic fluid and a Newtonian fluid.

previous work [141]. The particles are affected by elastic force $F_E$ (points to the other Newtonian fluids) and drag force $F_D$ with the opposite direction of elastic force. For particles with a blockage ratio above a threshold, the elastic force is strong enough to push the particles laterally across streamlines and enter the other fluids, as the elastic force is proportional to particle size. After the particles transfer across the viscoelastic fluids and Newtonian fluids interface, and completely migrate out of PEO solution, the elastic force disappears. However, particles can still migrate a certain lateral distance within the deionized water. This phenomenon might be caused by the inertia of the particles. For particles with their blockage ratio below a threshold (e.g., nanoscale molecules in the culture medium), the elastic force is not strong enough to drive particles to the other medium, so they remain within the viscoelastic fluids stream. Therefore, the lateral migration and on-chip washing of particles or cells can be achieved. The critical particle size for efficient particle or cell washing can be determined. For the particles that can migrate out of viscoelastic fluids, the lateral migration distance in Newtonian fluids highly depends on the particle size. Larger particles migrate further in the Newtonian fluids than smaller particles do.
7.3. The particle migration processes in sample viscoelastic fluid and sheath Newtonian fluid

The particle migration process in sample viscoelastic fluids and sheath Newtonian fluids are analysed in detail according to Newton’s second law. Using the same method, particle separation according to different blockage ratios can be realized using this co-flow configuration of viscoelastic fluids and Newtonian fluids.

In our current work, the maximum particle Reynolds number $R_P (\approx 0.09)$ is much smaller than 1, therefore, we neglect the effect of fluid, and inertial lift force is negligible on the particles.

The particle migration processes are shown in Figure 7-2. There are four stages as particle migrates from viscoelastic fluids to Newtonian fluids:

(1) $y_c < y_0 - a/2$: the particles are fully immersed in viscoelastic fluids. In this stage, particles are affected by the elastic force and drag force. The elastic force directs to the centre of the channel, while the drag force is the opposite direction of the elastic force. The particles are accelerated by a combination of elastic and drag force. As the acceleration process is very fast, particles can quickly reach their equilibrium velocities. The acceleration process is extremely fast according to the following analysis, therefore, this acceleration process can be negligible.
(2) $y_0 - a/2 \leq y_c \leq y_0$: the lower side of the particle is at the interface and the elastic lift forces direct toward the centreline due to the compressive elastic stresses acting from the sidewall. The elastic lift forces become stronger as they migrate towards the channel centre due to the offset effects of the compressive elastic stresses acting from the lower particle side vanishes.

(3) $y_0 \leq y_c \leq y_0 + a/2$: the near-wall side of the particle is at the interface and the elastic lift forces become weaker as the particle migrates out of the viscoelastic fluids gradually.

(4) $y_0 + a/2 < y_c \leq w$: the particles are fully immersed in the Newtonian fluids. They can still migrate laterally due to the inertia of the particle. In this stage, particles are affected only by the drag force, and their lateral velocity is decelerated by drag force, and they reach their equilibrium positions when the velocity reduces to zero.

Here, we ignore the complex process particle experience the interface, and only analyze in detail migration process when particles are immersed fully in viscoelastic and Newtonian fluids according to Newton’s second law as follows.

(I) When particles are immersed in the viscoelastic sample flow:

$$F_E + F_D = m_p \frac{dv_p}{dt}$$

$$F_E = C_{el} a^3 \nabla N_1$$

$$F_D = 3 \pi \mu_j a (v_f - v_p)$$

where $F_E$ is the elastic force, $F_D$ is the Stokes drag force, $m_p$ is the particle mass. where $v_f$, $v_p$ are the velocities of the fluid element and particles, respectively. $C_{el}$ is the non-dimensional elastic lift coefficient, $a$ is the particle size. $N_1$ is the first normal stress difference. $\mu_j$ is the fluid dynamic viscosity.

Substitute (10), (11) into (9), and solve the differential equation, we obtain the particle velocity:
\[ v_p = \frac{C_o a^2 \nabla N}{3 \pi \mu_f a} [1 - \exp(-\frac{3 \pi \mu_f a}{m_p} t)] = \frac{C_o a^2 \nabla N}{3 \pi \mu_f} [1 - \exp(-\frac{3 \pi \mu_f a}{m_p} t)] \quad (12) \]

The characteristic time scale for particle to reach steady state is then:

\[ \tau_p = \frac{m_p}{3 \pi \mu_f a} = \frac{a^2 \rho_p}{18 \mu_f} \quad (13) \]

Considering a particle with a diameter \( a = 5 \mu m \), and density \( \rho_p = 1.05 \times 10^3 \), \( \mu_f = 5 \times 10^{-3} \), the characteristic time is approximately 0.3 \( \mu s \). Since the acceleration time is extremely short, we can assume the acceleration process is negligible, and hence the particle equilibrium velocity can be expressed as:

\[ v_p \propto \pi \mu_f \quad (14) \]

(II) When particles are within Newtonian fluid, the particles are affected by viscous drag force only. According to Newton’s second law:

\[ F_D = ma \quad (15) \]

\[ F_D = 3 \pi \mu_f a (v_f - v_p) = -3 \pi \mu_f a v_p \quad (16) \]

\[ ma = m \frac{dv_p}{dt} \quad (17) \]

Substitute (8), (9) into (7), we can obtain:

\[ \frac{dv_p}{v_p} = -\frac{3 \pi \mu_f a}{m} dt \quad (18) \]

Assuming \( v_{p0} \) is the initial particle velocity when particle enter Newtonian fluid from the viscoelastic fluid, \( T \) is the time when the particle velocity reach zero, after integration:

\[ \int_{v_{p0}}^{0} \frac{dv_p}{v_p} = \int_0^T -\frac{3 \pi \mu_f a}{m} dt \quad (19) \]
We can get:

\[ T = +\infty \] (20)

The particle lateral velocity in Newtonian fluids is \( v \) at arbitrary time \( t \), after integration:

\[ \int_{v_p}^{v} \frac{dv_p}{v_p} = \int_0^\tau \frac{3\pi\mu_f a}{m} dt \] (21)

We can get:

\[ v = \frac{v_{p0}}{\frac{3\pi\mu_f a}{e^{m/\rho}} t} \] (22)

Therefore, when the particle velocity reaches zero, the particle reaches its equilibrium position.

The lateral migration distance after particle enters Newtonian fluids can be expressed as:

\[ L = \int_0^T v dt = \int_0^\tau \frac{v_{p0}}{e^{m/\rho}} dt \] (23)

When \( T \) approaches \( \infty \), the migration distance is:

\[ L = \frac{mv_{p0}}{3\pi\mu_f a} = \frac{a^2 v_{p0} \rho_p}{18 \mu_f} \] (24)

Neglecting the acceleration process that particles move across the interface of viscoelastic sample flow and Newtonian sheath fluids, then substituting equation (14) into equation (24), we can get the particle migration distance within Newtonian fluids:

\[ L = \frac{a^4 C_{el} \rho_p \nabla N_i}{54\pi \mu_f^2} \propto a^4 \] (25)

From equation (25), it can be concluded that the lateral migration distance in Newtonian fluids highly depends on the particle size. Larger particles migrate a much longer lateral
distance in the sheath flow than the smaller particles. This has been proven in our experiments.

For particles with their blockage ratio below a threshold, the elastic force is not strong enough to overcome drag force and drive particles to the other medium, so they remain within the viscoelastic fluids stream. For particles with a blockage ratio above a threshold, the elastic force is strong enough to overcome drag force and push the particles laterally across interface and enter the other fluids.

7.4. Methods

7.4.1. Design and fabrication of microfluidic device

The device is a simple straight rectangular channel with two inlets and two outlets. Its cross section is 30 × 50 μm (width × height), and length 5 mm. Standard photolithography and soft lithographic techniques were used to fabricate the device [142, 143].

7.4.2. Preparation and rheology of the PEO solution

For particle washing, PEO (2,000,000 Da; Sigma-Aldrich) was diluted to 1000 ppm in deionized water (DI water) containing 0.01% (v/v) Tween 20 (Sigma-Aldrich) to form the viscoelastic fluids, which acts as the sample flow. The sheath stream in particle washing experiment was DI water, which served as the Newtonian fluids. Tween 20 was added to both fluids to prevent particle aggregation. For on-chip Jurkat cell washing, PEO was added to a phosphate-buffered saline (PBS) (Sigma-Aldrich) at 2000 ppm. Before the cell experiments, the same volume of PBS containing 2000 ppm PEO was added to Jurkat cell culture medium (complete RPMI 1640 medium), thus the PEO concentration of the Jurkat cell culture medium is 1000 ppm, and PBS in sheath fluid worked as the Newtonian fluids. The viscosity of the Newtonian fluids and 1000 ppm PEO solution as a function of the shear rate were shown below (Figure 7-3).

The rheological property of the fluids was measured in a rotational rheometer (Antonpaar MCR 301) that has a parallel plate configuration and a diameter of 20 mm. The measurement was performed at room temperature (24 ± 1°C). The shear viscosity of the viscoelastic fluids was measured in shear rates ranging from 100 s⁻¹ to 10³ s⁻¹. The shear
The viscosity of the Newtonian fluid remains constant at about $3 \times 10^{-3}$ Pa·s. In this range, the PEO solutions show a slight shear thinning behavior. The average viscosity of 1000 ppm PEO solution within the tested shear rate region is $5 \times 10^{-3}$ Pa·s.

![Figure 7-3 Viscosity of Newtonian fluid and 1000 ppm PEO solution as a function of shear rate](image)

### 7.4.3. Particle and cell preparation

For particle washing experiments, particle suspensions were prepared by diluting the mixture of 0.8-µm internally red dyed fluorescent polystyrene microspheres (ThermoFisher Scientific, CV 5%) with 2-µm, 3-µm, 5-µm and 10-µm internally green dyed fluorescent polystyrene microspheres (Thermo Fisher Scientific, CV 5%) in the 1000 ppm PEO medium, respectively. The 0.8-µm particles have a different fluorescence spectrum than that of particles of other sizes, and are too small to migrate laterally. Thus, the 0.8-µm particles are used here as an indicator of the distribution of the original medium. The blockage ratio $\alpha$ for 0.8-µm, 2-µm, 3-µm, 5-µm and 10-µm particles is 0.02, 0.05, 0.08, 0.13, and 0.27, respectively.

For cell washing experiments, leukaemic Jurkat cells (ATCC), an immortalized human T cell line (average diameter of approximately 15 µm), were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (ThermoFisher Scientific) containing 10% fetal bovine serum (Bovogen Biologicals) and 2 mM L-glutamine (ThermoFisher Scientific) in a humidified incubator (Thermo Scientific) at 37°C and 95% air/5% CO₂. Before commencing each experiment, the particle mixture was re-suspended by vortex and cell...
samples were manually stirred to provide uniform suspensions in a complete medium containing PEO. Absorbance spectra of fluids were assessed using a microplate reader (SPECTROstar\textsuperscript{Nano}, BMG Labtech).

### 7.4.4. Experimental setup

The sample medium (the mixture of PBS containing 2000ppm PEO with the same volume of Jurkat cell and RPMI 1640 culture medium), and sheath medium (PBS) were transferred to the chip from two 1-ml syringes, with silicon tube connected between them. The flow rate was controlled by syringe pumps (Legato 100, Kd Scientific, USA). An inverted microscope (CKX41, Olympus, Japan) mounted with a CCD camera (Optimos, Q-imaging, Australia) was used to observe and capture the images of the fluorescent particles, cells and fluids. The fluorescent images were post-processed and analysed with Q-Capture Pro 7 (Q-imaging, Australia) software.

The volumetric flow rates of sample flow $Q_s$ and sheath flow $Q_{sh}$ were 2 µl/min and 6 µl/min, respectively, so that the ratio of the flow rate of sheath flow to sample flow ($\alpha = Q_{sh}/Q_s$) was fixed at 1:3. The absorbance spectra of the media from the two inlets and the two outlets were tested by plate reader.

### 7.5. Results

#### 7.5.1. On-chip particle washing

As described above, the particles are affected by an elastic force $F_E$ (pointing to the Newtonian fluids) and drag force $F_D$ which is in the opposite direction of the elastic force. The particle lateral migration for mixtures of either 0.8-µm ($\alpha =0.02$) + 2-µm ($\alpha =0.05$), 0.8-µm + 3-µm ($\alpha =0.08$), 0.8-µm + 5-µm ($\alpha =0.13$) or 0.8-µm + 10-µm ($\alpha =0.27$) particles at the inlet, 1.5 mm from the inlet, 3 mm from the inlet, and the outlet are shown in Figure 7-4. The red fluorescent streams in Figure 7-4 (a) and red curves in Figure 7-4 (b) indicate the distribution of 0.8-µm particle medium, while the green fluorescent streams in Figure 7-4 (a) and green curves in Figure 7-4 (b) indicate the distribution of particles with other sizes.
Particle mixtures of either 0.8-µm + 2-µm, 0.8-µm + 3-µm, 0.8-µm + 5-µm or 0.8-µm + 10-µm in 1000 ppm PEO medium were injected into one inlet, while Newtonian fluids was injected into the other inlet. From the fluorescent images and corresponding normalized fluorescent intensity profiles, the mixture of 0.8-µm + 2-µm particles remain mixed together in the viscoelastic fluids along the channel from the inlet to the outlet, and both exited together into outlet 1 [Figure 7-4 (ai) and Figure 7-4 (bi)]. For particle mixtures of 0.8-µm + 3-µm, the 3-µm particles started to focus and migrated laterally. Some of the 3-µm particles migrated out of the original medium, thus 3-µm particles moved in both the viscoelastic and Newtonian fluids, and exited from both outlets (Figure 7-4 (aii) and Figure 7-4 (bii). As the particle size continued to increase, the elastic force become strong enough to push the particles to migrate laterally and distinctively across streamlines to enter the Newtonian fluids. Figure 7-4 (aiii)/(biii) and (aiv)/(biv) show that 5-µm and 10-µm particles experienced stronger elastic forces, so that they began to be focused into a single streak and migrate laterally at 1.5 mm from the inlet. Further downstream, the particles continued to migrate laterally further, detaching from the original medium and enter the Newtonian fluids, which eventually exited into outlet 2. The 0.8-µm particles remained dispersed and followed in the original viscoelastic fluids and come out from outlet 1. Particle washing can be realized using co-flow of viscoelastic and Newtonian fluids. However, the separation or washing performance depends on the particle size or particle blockage ratio. In our device, the critical particle blockage ratio for efficient particle washing is 0.08; when the particle blockage ratio exceeds 0.08, lateral migration starts.

In Figure 7-4 (b), the two red curves in each sub-figure indicate the distribution of 0.8-µm particles at the inlet and the outlet, as well as the original medium. It can be seen that the red curve at the outlet shift slightly along the lateral direction compared with that at the inlet, indicating the 0.8-µm particles and original medium slightly diffuses as they flow through the whole channel.
Figure 7-4 On-chip particle washing. (a) Fluorescent images of particle migration processes for mixture of 0.8-µm + 2-µm, 0.8-µm + 3-µm, 0.8-µm + 5-µm, 0.8-µm + 10-µm particles at inlet, 1.5mm from inlet, 3mm from inlet, and outlet. (b) Corresponding normalised fluorescent intensity profiles.
In a co-flow of viscoelastic and Newtonian fluids, particles with different sizes in the viscoelastic fluids either migrate gradually to the other fluids or remain in the original fluid. The migration speed of particles with different sizes can differ. Using this rule of thumb, size-dependent separation can also be achieved besides particle washing. Figure 7-5 shows the normalized particle lateral positions along the channel length at the inlet, 1.5 mm from inlet, 3 mm from the inlet, and the outlet. As the particle size and channel length increase, the lateral migration distance increases correspondingly. According to the lateral positions of different particle sizes at different channel lengths, apart from 0.8-µm + 5-µm and 0.8-µm + 10-µm particle mixture separation at the outlet, separation of 0.8-µm, 2-µm or 3-µm particles with 5-µm or 10-µm particles can also be achieved after a proper channel length (1.5 mm or 3 mm from the inlet). By adjusting the channel length, multiple particle separation and washing steps can be achieved using co-flow of viscoelastic fluids and Newtonian fluids in simple straight channels.

Figure 7-5 Particle lateral positions along the channel length at the inlet (0 mm), 1.5 mm from the inlet, 3 mm from the inlet, and the outlet (5 mm).

In washing experiments, particles with a blockage ratio exceeding 0.08, will start lateral migration and exit at outlet 2. Figure 7-6 shows the fluorescent images of media from the inlet, and outlets 1 and 2 for particle mixture of 0.8-µm + 2-µm, 0.8-µm + 3-µm, 0.8-µm
+ 5-µm, and 0.8-µm + 10-µm visualized on a hemocytometer, respectively. For 0.8-µm + 2-µm particle mixture, the particles are too small. Thus, no particles migrate to the Newtonian fluids, and no particles come out from outlet 2, Figure 7-6 (a). However, for 3-µm particles, a small number of them exit at outlet 2, Figure 7-6 (b). For particle mixtures of 0.8-µm + 5-µm, and 0.8-µm + 10-µm, almost all of the 5-µm and 10-µm particles enter the Newtonian fluids and exit at outlet 2 (Figure 7-6 (c) and Figure 7-6 (d)). The recovery rates for 2-µm, 3-µm, 5-µm, and 10-µm particles at outlet 2 were 0%, 9%, 94.4% and 100%, respectively.

Figure 7-6 Fluorescent images of mediums from the inlet (left panels), and outlets 1 and 2 (as indicated) for particle mixtures of (a) 0.8-µm + 2-µm, (b) 0.8-µm + 3-µm, (c) 0.8-µm + 5-µm, and (d) 0.8-µm + 10-µm on a hemocytometer.
7.5.2. On-chip Jurkat cell washing

After studying the critical particle size for efficient particle washing, on-chip Jurkat cells washing was conducted as shown in Figure 7-7. The blockage ratio of the Jurkat cells was $\alpha = 0.4$, which exceeds the critical blockage ratio, thus Jurkat cells should have sufficient migration speed to transfer to the washing medium. Like the particles in viscoelastic fluids, Jurkat cells experience an elastic force which points to the Newtonian fluids and a drag force $F_D$ acting in the opposite direction. As the average diameter of Jurkat cells is 15 $\mu$m, the elastic force is dominant, and the cells start to migrate laterally at the position of 1.5 mm, Figure 7-7 (a) (Jurkat cells are labelled by blue circles). At the outlet, almost all Jurkat cells are transferred from the viscoelastic fluids to the Newtonian fluids, and exit at outlet 2. The medium from inlet 1 and the two outlets were both collected, Figure 7-7 (b). The viscoelastic fluids containing cells (cell culture medium + PEO) and the Newtonian fluids (PBS) were injected from inlet 1 and inlet 2, respectively. In this experiment, the flow rate ratio of sample and sheath flow is 1:3, and the width ratio of the two outlets is 1:2. As the fluids flow in this channel is laminar, the cell culture medium containing PEO at inlet 1 will exit at outlet 1, while the PBS medium at inlet 2 will flow into both outlets, and the cell culture medium will be diluted further. This phenomenon can be seen from the change in the colour of the medium, which becomes lighter at outlet 1 compared with the colour at inlet 1. However, Jurkat cells do not remain in the original cell culture medium, but migrate into the PBS medium and flow out from outlet 2. The hemocytometer test indicated that Jurkat cells in inlet 1 have been successfully transferred to outlet 2, Figure 7-7 (c). Flow cytometric data [Figure 7-7 (d)] displays a forward scatter (FSC-A: relative event size) and a side scatter (SSC-A: relative cell surface and intracellular complexity), validating the washing performance of this technique. Figure 7-7 (d) shows the events number in the two outlets, the dots inside the red gating area indicate the Jurkat cells (231 in outlet 1, 2958 in outlet 2). The recovery rate for Jurkat cell on-chip washing was 92.8%. The cells’ viability before and after solution exchange experiments were tested using 7AAD method. The cells’ viability before experiment is about 96.9%. The viability of cells from inlet after 2h is about 96.5%, while the viability of cells from outlet after PEO exposure is about 91%. It means that PEO has little effect on the cells’ viability.
In the co-flow of viscoelastic fluids and Newtonian fluids, diffusion is inevitable. Particles can migrate across the boundary of the two fluids when the velocity of the migrating particles is higher than the speed of diffusion for the PEO medium. Each substance in the fluid has a characteristic absorption spectrum. Therefore, according to the absorption spectrum, the substances in the fluid and if there are any contaminants can be determined. The heights of the peaks vary according to the concentration of the fluid. Scanning spectrophotometry was used to assess the diffusion extent of the co-flow. The absorbance spectra of the media from the two inlets and the two outlets were tested, Figure 7-8. The medium in inlet 2 is PBS and has minimal absorbance within the tested wavelength range, thus its absorbance spectrum is relatively flat. In contrast, the absorbance spectrum of the original cell culture medium in inlet 1 has two major peaks. These peaks show a reduction in the medium from outlet 1, indicating that the cell culture medium in outlet 1 was partially diluted by PBS. Moreover, the medium from outlet 2 displays a slight increase in absorbance compared to PBS indicating diffusion of the cell culture medium may have occurred. The relationship between absorbance and concentration of an absorbing species is linear. One of the molecules in the culture medium has the highest absorbance. The absorbance of this molecule in inlet 1 is about 0.175, while the absorbance of this molecule in outlet 2 (target washing outlet) is about 0.04. It means that the concentration of this molecule in culture medium has dropped by about 77% in the new Newtonian fluids after washing. It’s indicated that little culture medium diffused to the new medium.

Moreover, the 10nm and 100nm size molecule’s Peclet numbers were calculated as $3.84 \times 10^5$ and $3.84 \times 10^6$, respectively; and migration distance as 3.4µm and 1.1µm, respectively according to the following equations.

The diffusion coefficient is expressed as:

$$D = \frac{KT}{3\pi \mu a}$$

Where $D$ is the diffusion coefficient. $K$ is Boltzmann’s constant, which is about $1.3806488 \times 10^{-23}$ J/K. $T$ is the temperature, $\mu$ is viscosity of medium and $a$ is the particle diameter.
The mean square distance that a particle diffuses in time $t$ is expressed as $r$:

$$< r^2 > = 6Dt$$  \hspace{1cm} (27)

The Peclet number $Pe$ can be employed to evaluate the relative importance of convection to diffusion:

$$Pe = \frac{HU}{D}$$  \hspace{1cm} (28)

Where $H$ is the characteristic dimension of channel and $U$ is the characteristic velocity of fluid.

The sample flow rate is 2µl/min. As the molecule size is around tens to hundreds of nanometre, we calculate 10nm and 100nm size molecule’s Peclet number as $3.84 \times 10^5$ and $3.84 \times 10^6$, respectively, and migration distance as 3.4µm and 1.1µm, respectively.

The calculated results indicate negligible diffusion. However, in the actual experiments, this migration distance may be larger according to the Scanning spectrophotometry test.

Nevertheless, this spectra data reveal that diffusion of the co-flow is minimal, confirming efficient cell washing from culture medium to PBS medium.
Figure 7-7 On-chip Jurkat cells washing. (a) Jurkat cell migration processes at inlet, 1.5 mm from the inlet, 3 mm from the inlet, and the outlet. (b) The original Jurkat cell culture medium and the mediums exit from outlet 1 and outlet 2 after cell washing process. (c) Hemocytometer images of the inlet and the two outlets after cell washing. (d) Flow cytometer results of the two outlets.

Figure 7-8 The absorbance spectra of the mediums from the two inlets and the two outlets tested by plate reader.
7.6. Conclusion

In summary, we demonstrated on-chip washing of microparticles and cells using co-flow of viscoelastic fluids and Newtonian fluids. By simply adding a biocompatible polymer into the native biofluids, continuous on-chip particle and cell washing can be realized with a high recovery rate. After the investigations on the size-dependent particle lateral migration, the critical particle blockage ratio for efficient washing was determined as 0.08. Cell washing performance was verified by hemocytometry and flow cytometry. According to the absorbance spectra of the mediums from the two inlets and the two outlets, diffusion of the co-flow is negligible, indicating efficient cell washing from culture medium to PBS medium. The reported technique can be performed in a simple straight channel, without any external force fields. The technique is a more efficient alternative for tedious conventional medium exchange and washing methods. It has also huge potential to allow direct processing of various biofluids, and holds numerous biomedical applications.
8. Conclusion and future work

8.1. Conclusion

The main purpose of this research is to investigate particle migration in viscoelastic microfluidics, and develop microfluidic devices based on viscoelastic fluids to realize more efficient particle focusing, separation or solution exchange. These devices can be used as much easier particle manipulation methods in various biomedical or chemical fields. Based on the fluids viscoelasticity, two functionalities were improved.

(1) Particles can be 3D focused based on the dean-flow coupled elasto-inertial effects in an ECCA channel, and high purity plasma extraction from blood can be realized. Moreover, size-based separation can be realized in integrated ECCA straight channel.

(2) Particle can be transferred laterally by elastic force from viscoelastic fluids to Newtonian fluids in simple straight channels, thus simpler, more efficient on-chip cell washing can be realized.

8.1.1. Sheathless particle 3D focusing and separation

Using an ECCA channel, continuous, sheathless, and high throughput (>10000 s⁻¹) 3D particle focusing was realized in viscoelastic fluids. By properly controlling the flow rates to harmonize the inertial force, viscoelastic force and Dean-drag force, 3D particle focusing in non-Newtonian fluids along the opposite side of cavities was achieved. Particles in non-Newtonian fluids in an ECCA channel are more easily and better focused.

Continuous plasma extraction under viscoelastic fluids was achieved by exploiting the dean-flow-coupled elasto-inertial effects in an ECCA channel. 3 μm, 4.8 μm and 10 μm particles can be focused under the same flow rate, which indicates that this method can simultaneously focus platelets, RBCs and WBCs. The plasma purity can be 99.99% after second filtration using the same ECCA channel. This ECCA channel offers a continuous, sheathless, and high purity plasma extraction, which could potentially aid in the realization of lab-on-a-chip bioanalysis devices.
To extend the functionality, an innovative microfluidic device for sheathless particle focusing and separation in viscoelastic fluids is proposed in this work. Particles with different blockage ratios are firstly focused on the opposite cavity side by Dean-flow-coupled elasto-inertial effects at the first stage, then separated at the second stage based on differential focusing of particles with different sizes in viscoelastic fluids. This device was further applied for separation of Jurkat cells and yeast cells. The recovery rate, purity, and enrichment ratio for Jurkat cells can reach 99.6%, 24% and 11.9, respectively. The described device offers an efficient, continuous, and sheathless particle separation method in viscoelastic fluids. This device could be potentially used for clinical and biological applications, where biological particles with various sizes need to be separated, or for bio-sample enrichment to improve the sensitivity of the downstream detection unit.

8.1.2. Particle solution exchange

Particle lateral migration in sample-sheath flow with different properties (viscoelastic fluids and Newtonian fluids) was investigated. By using non-Newtonian viscoelastic fluids, particles can be transferred laterally across streamlines in sample-sheath flow of viscoelastic PEO solution-DI water. This method employs the elastic force, and the particle migration direction can be selectively controlled by the proper pair of co-flowing solutions. This lateral particle migration technique does not require any external force field or complex channel structures, and can deliver selective particle lateral migration by simply adding PEO molecules to particle suspension. On-chip washing of microparticles and cells using co-flow of viscoelastic fluids and Newtonian fluids was demonstrated. By simply adding a biocompatible polymer into the native biofluids, continuous on-chip particle and cell washing can be realized with a high recovery rate. The critical particle blockage ratio for efficient washing was determined as 0.08. The technique is a more efficient alternative for tedious conventional medium exchange and washing methods. It has also huge potential to allow direct processing of various biofluids, and holds numerous biomedical applications.

In summary, our study not only focuses on the investigation of particle migration behaviour in viscoelastic fluids, but also provides simpler and more effective chips for particle processing, including particle focusing and separation, plasma extraction from blood, and on-chip particle or cell washing.
8.2. Future work

8.2.1. More fundamental studies in viscoelastic fluids are needed.

Due to the complexity of the problem, the particle behaviors in viscoelastic fluids are strongly influenced by channel cross sectional shape, elasticity effects, inertial effects, shear-thinning viscosity, secondary flows, and blockage ratio etc. To uncover the principle of particle behaviors in viscoelastic fluids, both numerical and experimental studies have been extensively conducted. However, there are still many discrepancies between numerical results with experiments, or among experimental results from different literatures. For example, Del Giudice et al. [53] observed that particles were focused at the centerline of square microchannel in a aqueous solution of PVP (polyvinylpyrrolidone, 8% wt) when Deborah number (De) is 0.2. In contrast, for particles suspending in the PVP solution with similar rheological properties, Yang et al. found that particles migrates towards the centerline as well as four corners under the same Deborah number [47]. This indicates that, the exact relationship between fluid rheology and particle migration is still unclear. The hydrodynamic forces on particles, particle trajectories, the correlation of fluid rheology and particle migration, or particle behaviors in coflow of fluids with the same or different rheological properties needs further quantitative investigation.

Migration of sub-micro or nano-sized biological particles (DNA, protein, virus, bacteria, exosomes, etc.) is highly important in various biomedical and biotechnological applications, because these bioparticles are promising biomarkers in the early detection and diagnosis of many diseases. However, the migration of nano-scale particles in microfluidics is rarely investigated from both theoretical and experimental aspects because the Brownian motion effect may be significant once the particles size is reduced to nano-scale. Recently, researchers have demonstrated that viscoelastic fluids has the potential to realize sub-micro or nanoparticle migration, which may open a new gate through the territory of nano-scale particles (DNA, protein and virus etc.).
8.2.2. **Particle interaction and the associated particle dynamics in viscoelastic fluid need further investigation.**

Particle interaction in inertial manipulation has been extensively studied. The dynamics of the particle–particle interactions was studied, and a mechanism for the dynamic self assembly process was revealed [197]. It is found that inertial lift forces and a parabolic flow profile stabilize interparticle spacings that otherwise would diverge to infinity due to viscous disturbance flows. With increasing concentration of the suspension the spacing is influenced by particle crowding effects until stable trains are no longer observed [198]. Moreover, Humphry et al. presented that both the location and the number of focusing positions depend on the number of particles per unit length along the channel [199]. This axial number density is a function of both the channel cross-section and the particle volume fraction. Reece et al. have explored equilibrium focusing behavior as a function of channel geometry and particle concentration [200]. It is concluded that particle buckling scales weakly with concentration and strongly with channel geometry.

However, very few works have been reported on particle interactions in viscoelastic fluids. We previously investigated the effects of blood hematocrit on the viscoelastic focusing behaviour in an ECCA channel, and concluded that the higher the blood hematocrit, the more significant the interaction between blood cells, and the worse the cell focusing quality [71]. Holzner et al. [99] also found that as cellular concentration increases, the cell radial distributions become broader, and the cell focusing efficiency is decreased. They explained that for more concentrated cell suspensions, the inter-cell space on the centerline of the channel decreases, and they compete for the same space around the channel centreline. Besides, the variation in the dimension of cell population generates eventually random cell clumps, which destroy any order. It’s also found that the high volume fraction decreases the shear rate, weakening the effects of both shear thinning and elastic normal stresses, and the annular ‘particle-free zone’ becomes smaller as well [36].

Since the particle-particle interaction directly impairs the viscoelastic focusing and separation performance. Determining the suitable cell concentration to maximize the throughput of microfluidic device, as well as maintaining the proper functionality is still challenging. The theoretical models to evaluate the effects of particle-particle interactions
on particle viscoelastic focusing and extensive experimental quantification are still needed.

8.2.3. **Viscoelastic particle migration based on particle shape or deformability**

Shape is an important indicator for bioparticle separation as well, and it varies with cell type, cycle, and state, etc. It provides useful information in bioparticle identification, cell synchronization, disease diagnostics et al. Cell deformability is a promising label-free biomarker for the diagnosis of disease. The alteration in the deformability of cells compared with healthy cells may be utilized to diagnose blood disease, cancers and so on. Viscoelastic particle migration based on particle shape or deformability including the theoretical or numerical predictions, and experimental studies should be a promising research direction in the near future.

8.2.4. **Realize real “label-free” bio-particle migration**

The viscoelasticity-induced particle migration itself can be considered “label-free”, compared with the conventional molecular-based biomarkers, the viscoelasticity-induced particle migration does not require costly antibodies or dyes. The particle size, shape, or deformability is the intrinsic label-free biomarker, which is simpler and cheaper. However, as the viscoelastic fluids is formed by dissolving polymers in Newtonian fluids. The polymer molecules always contact with particles in the medium. Polymer has been entitled as biomaterial for a long time [201], and has been used for drug compounding [202, 203] and a wide variety of cosmetic and personal care products. In addition, it has also been used as a biocompatible modifier for a variety of enzymes and proteins to maintain their intrinsic activities [204] or to improve its blood-compatible properties [205]. Recently, aqueous solutions of polymer have been widely used in microfluidics and for a wide range of bio-medical experiments elaborated above [58, 66, 84]. Although it is noteworthy that aqueous solutions of polymer are hospitable to living cells, it is uncertain that any kind of polymer or polymer with any molecular weight is biocompatible and nontoxicity [206]. The influence of viscoelastic fluids on cells viability in microfluidics was rarely studied. Only in our previous experiments, the cells’ viability before and after solution exchange experiments were tested using viability assay method [85]. The cells’ viability before experiment is about 96.9%. The viability of cells from
inlet after 2h is about 96.5%, while the viability of cells from outlet after PEO exposure is about 91%. The results show that PEO has little effect on the cells’ viability in short term. Its influence on viability and phenotype of cells on long term and its interfere with reagents is still unclear, which deserves more attention from scientific community. However, the elimination of PEO from blood plasma, cell suspension or aqueous solution is another technical barrier for the wide application of viscoelastic fluids on cell manipulation and separation.

To minimize the effects of polymer molecules on bioparticles, the target particles should be isolated from the viscoelastic fluids immediately after the operation. A solution exchange technique which uses the coflow of viscoelastic fluids and Newtonian fluids may be one of the promising in-line methods to wash target particles. However, it is still facing the limitation of throughput and washing efficiency. Therefore, more novel microfluidic technologies to change the solution and wash bioparticles in a high throughput are still in demand.

Besides, various body fluids such as blood, saliva, DNA solutions, cytoplasm, et al., exhibit viscoelastic properties. By utilising the natural viscoelasticity of these body fluids, the polymer label problem may be avoided. Thus, investigation of particle/cell migration in the natural body fluids and tailoring the particle viscoelastic migration principle for biomedical applications may deserve attention.
References


X. Lu and X. Xuan, "Elasto-inertial pinched flow fractionation (ePFF) for continuous shape-based particle separation," *Analytical chemistry*, vol. 87, no. 22, pp. 6389-6396, 2015.


132


[206] Y. Ashihara, T. Kono, S. Yamazaki, and Y. Inada, "Modification of E. coli L-asparaginase with polyethylene glycol: disappearance of binding ability to anti-
Contributions to the thesis


5. **Dan Yuan**, Jun Zhang, Ronald Sluyter, Qianbin Zhao, Sheng Yan, Gursel Alici, Weihua Li. Continuous plasma extraction under viscoelastic fluid in a straight channel with asymmetrical expansion–contraction cavity arrays [J]. Lab on a Chip, 2016, 16(20): 3919-3928. **Cover page paper.**


Other publications


18. Jun Zhang, Sheng Yan, Dan Yuan, Gursel Alici, and Nam-Trung Nguyen, Weihua Li, “High throughput complete cell-free extraction of plasma by the integration of inertial microfluidics and membrane filter”, ASME 2016 5th Micro/Nanoscale Heat and Mass Transfer International Conference.