Development of acoustic nozzle for 3D printing

Sriphutkiat, Yannapol

2019

Sriphutkiat, Y. (2019). Development of acoustic nozzle for 3D printing. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/83153>

<https://doi.org/10.32657/10220/47997>

NANYANG TECHNOLOGICAL UNIVERSITY

School of Mechanical and Aerospace Engineering

Development of acoustic nozzle for 3D printing

Submitted by

Yannapol Sriphutkiat

Supervisor: Asst.Prof. Zhou Yufeng

A thesis submitted in partial fulfillment
of the requirement for the degree of
Doctor of Philosophy

Year 2019

School of Mechanical and Aerospace Engineering

Nanyang Technological University

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research, is free of plagiarised materials, and has not been submitted for a higher degree to any other University or Institution.

[4/4/2019]

.....

Date

[ ]

.....

[Yannapol Sripitukiat]

Supervisor Declaration Statement

I have reviewed the content and presentation style of this thesis and declare it is free of plagiarism and of sufficient grammatical clarity to be examined. To the best of my knowledge, the research and writing are those of the candidate except as acknowledged in the Author Attribution Statement. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

[4/4/2019]

.....

Date

I

.....

[Zhou Yufeng]

Authorship Attribution Statement

This thesis contains material from 11 paper(s) published in the following peer-reviewed journal(s) / from papers accepted at conferences in which I am listed as an author.

Chapter 3 is published as

- Yannapol Sriphutkiat and Yufeng Zhou, Particle accumulation in a microchannel and its reduction by a standing surface acoustic wave (SSAW), *Sensors*, 17(1), 106, 2017
- Yannapol Sriphutkiat and Yufeng Zhou, Particle Accumulation in Microchannel and Its Reduction by Surface Acoustic Wave (SAW), *Proceedings of the 2nd International Conference on Progress in Additive Manufacturing (Pro-AM 2016)*, Singapore

The contributions of the co-authors are as follows:

- Asst.Prof. Zhou Yufeng provided initial project direction and edited the manuscript

Chapter 4 is published as

- Yannapol Sriphutkiat, and Yufeng Zhou, Particle manipulation using standing acoustic waves in the microchannel at dual-frequency excitation: effect of power ratio, *Sensors & Actuators: A. Physical*, 263, 521-529, 2017
- Yufeng Zhou and Yannapol Sriphutkiat, Microparticle Manipulation by Standing Surface Acoustic Waves with the Dual-Frequency Excitations, *Journal of Visualized Experiments*, 138, e58085, 2018
- Yannapol Sriphutkiat and Yufeng Zhou, Particle Manipulation using dual-frequency excitation of standing surface acoustic wave, *EAC Lab-on-a-chip Conference A*Star 2016*, Singapore

The contributions of the co-authors are as follows:

- Asst.Prof. Zhou Yufeng provided initial project direction and edited the manuscript

Chapter 5 is published as

- Yannapol Sriphutkiat and Yufeng Zhou, Accumulation of microparticles along radial axis of cylindrical tube using low and high frequency acoustic wave, Regional Conference on Environmental Engineering 2017, Hanoi, Vietnam
- Yannapol Sriphutkiat and Yufeng Zhou, Separation of microparticles along radial axis of cylindrical tube using low and high-frequency acoustic wave, 11th Regional Conference on Chemical Engineering 2018 (RCChE 2018)
- Yannapol Sriphutkiat and Yufeng Zhou, Acoustic manipulation of microparticle in a cylindrical tube for 3D printing, Under Revision

The contributions of the co-authors are as follows:

- Asst.Prof. Zhou Yufeng provided initial project direction and edited the manuscript

Chapter 6 is published as

- Yannapol Sriphutkiat and Yufeng Zhou, Accumulation of microparticle in 3D printed construct using acoustic nozzle, Proceedings of the 3rd International Conference on Progress in Additive Manufacturing (Pro-AM 2018), Singapore
- Yannapol Sriphutkiat and Yufeng Zhou, Patterning of microparticles/cells through the acoustic-assisted nozzle for 3D printer, Regional Conference on Electrical and Electronics Engineering (RCEEE 2018), Penang, Malaysia
- Yannapol Sriphutkiat and Yufeng Zhou, Cell alignment and accumulation using acoustic nozzle for 3D printing, Under Revision

The contributions of the co-authors are as follows:

- Asst.Prof. Zhou Yufeng provided initial project direction and edited the manuscript

[4/4/2019]

[ ]

.....
Date

.....
[Yannapol Sriphutkiat]

Acknowledgement

First and foremost, I would like to express my sincere gratitude to my advisor, Asst.Prof. Zhou Yufeng, for his continuous support of my PhD study and related research with his patience and immense knowledge. His guidance helped me throughout the research, publication and writing of this thesis. I could not have imagined having a better advisor and mentor for my PhD study.

Besides my advisor, I would like to thank the examiners for my confirmation examination and advisors for their insightful, constructive, and supportive comments.

My sincere appreciation also goes to Prof. Dipen Sinha, Dr. Gregory Goddard, and Dr. David Collins, who gave important guidance to me on the structural vibration of cylindrical tube and fabrication of SSAW device. Without their precious support, it would not be possible for me to complete this research.

I thank my fellow labmates (Mr. Surasak and Dr Liu Chenhui, and Dr Wang Mingjun) for the useful, constructive yet enjoyable discussions on the biological aspects, sterilization technique for cell culture and for all the fun we have had in the previous years.

Most importantly, I would like to thank Dr. Vincent Chai for his guidance and support on CFD simulation of particle deposition in the nozzle constriction which proves of concept or shows feasibility of this work before any experiment is done. It is really a crucial point to start for my PhD. work.

Lastly, I would like to thank my family for all their love and encouragement particularly to my parents who raised and supported me in all my pursuits. And most of all for my loving, supportive, encouraging, and patient sweetheart, Sasithorn, whose faithful support during the final stages of this PhD is so appreciated. Thank you.

Table of Contents

Statement of Originality.....	i
Supervisor Declaration Statement.....	ii
Authorship Attribution Statement.....	iii
Acknowledgement.....	v
Table of Content.....	vi
List of Figures.....	x
List of Tables.....	xvii
List of Symbols.....	xviii
Summary.....	xx
Chapter 1 Introduction	1
Chapter 2 Literature Review	10
2.1 Microparticle clogging in the microchannel	10
2.1.1 DLVO Theory and Non-DLVO Forces	10
2.1.2 Mechanism of clogging.....	13
2.2 Numerical simulation of microparticle deposition	18
2.2.1 Eulerian microparticle tracking.....	19
2.2.2 Lagrangian microparticle tracking.....	19
2.2.3 Microparticle behavior in the deposition model	19
2.2.4 Particle-wall interaction	20
2.2.4.1 Microparticle attachment	20
2.2.4.2 Microparticle Detachment	21
2.3 Microparticle manipulation using surface acoustic wave (SAW)	21
2.3.1 Standing surface acoustic wave (SSAW) in microfluidics channel.....	22
2.3.2 Single pressure node SSAW	23
2.3.3 Multiple pressure nodes SSAW	23
2.3.4 Tilted SSAW	24
2.3.5 Two-dimensional SSAW	24
2.3.6 SSAW from a single IDT.....	25
2.4 Microparticle/cell manipulation using structural acoustic vibration in cylindrical tube	25
2.4.1 Concentration of microparticles by acoustic wave	25

2.4.2 Numerical simulation of microparticle accumulation by structural-acoustic vibration in the cylindrical cavity	27
2.4.3 Governing equations	28
2.5 acoustic manipulation in the nozzle-based 3D printer	30
Chapter 3 Microparticle accumulation in microchannel and its reduction by a Standing Surface Acoustic Wave (SSAW)	31
3.1 Introduction.....	31
3.2 Simulation of microparticle accumulation by SSAW in microchannel.....	33
3.2.1 Governing Equation	35
3.2.2 Numerical Simulation Results	37
3.3 Experiment of microparticle accumulation and its reduction by SSAW	41
3.3.1 Experiment setup	41
3.3.2 Effect of flow rate and concentration of microparticles	45
3.3.3 Concentration of sodium alginate in the solution	46
3.3.4 Agglomeration area of microparticle and alginate solution.....	47
3.3.5 Standing Surface acoustic wave (SSAW).....	48
3.3 Microparticle accumulation and its reduction by SSAW in tapered microchannel	49
3.3.1 Clogging from microparticle accumulation	50
3.3.2 Reduction of microparticle accumulation and clogging by SSAW	53
3.4 Discussion	55
3.5 Summary.....	61
Chapter 4 Microparticle manipulation using Standing Surface Acoustic Wave (SSAW) at dual-frequency excitation: effect of power ratio.....	62
4.1 Introduction.....	63
4.2 Materials and Methods.....	66
4.2.1 Governing equation used in numerical simulation	66
4.2.2 Microparticle motion by 1D model.....	67
4.2.3 Microparticle motion by 2D model.....	67
4.2.4 Fabrication of microchannel and IDTs	68
4.2.5 Experiment setup	69
4.3 Results and discussion	70
4.3.1 Comparison of 1D and 2D simulation models.....	70
4.3.2 IDTs and PDMS microchannel	71
4.3.3 Simulation of microparticle motion by dual-frequency SSAW.....	72

4.4 Experiment validation	76
4.5 Summary	79
Chapter 5 Acoustic manipulation of microparticle in a cylindrical tube for 3D printing	81
5.1 Introduction	81
5.2 Material and Methods	84
5.2.1 Numerical simulation	84
5.2.2 Experimental setup	86
5.2.3 Printing evaluation	87
5.2.4 Statistical analysis	89
5.3 Results	89
5.3.1 Vibration modes	89
5.3.2 Accumulation of microparticles in the glass tube	91
5.3.3 Microparticle distribution in the printed structure	93
5.3.4 High orders of structural vibration	96
5.3.5 Progress of microparticle accumulation in the nozzle	98
5.3.6 Reduction of microparticle accumulation by acoustic excitation	100
5.3.7 Printing Structure and microparticle distribution	102
5.4 Discussion	104
5.5 Summary	107
Chapter 6 Cell Alignment and accumulation using acoustic nozzle for 3D printing	109
6.1 Introduction	109
6.2 Materials and Methods	112
6.2.1 Numerical Simulation Model	112
6.2.2 Cell culture, harvest, and differentiation	112
6.2.3 GelMA preparation	113
6.2.4 Experimental setup and evaluation of biological cell distribution	113
6.2.5 MHC-immunofluorescence of aligned C2C12 in printed construct	114
6.3 Results	114
6.3.1 Accumulation of biological cells by acoustic excitation	114
6.3.2 Accumulation and growth of cells undergone acoustic excitation	117
6.3.3 Width of cell microconstruct	118
6.3.4 Orientation of C2C12 cells undergone acoustic excitation	119
6.3.5 Immunofluorescent staining	121

6.4 Discussion	124
6.5 Summary	127
Chapter 7 Conclusion and Future work	128
7.1 Conclusion.....	128
7.2 Future Work.....	131
Reference	134
List of Publications, Patents and Awards.....	147

List of Figures

Figure 1.1. The outflow masses from the silo, with an exit orifice $D = 2.42d$, as a function of time, for four different vibration accelerations	6
Figure 2.1. Magnitude of particle-particle force and particle-wall interaction force with respect to the surface separation distance	13
Figure 2.2. Schematic diagram of shadow effect of deposited microparticles and its influence to the deposition of incoming microparticles. (a) deposition on spherical collector, (b) shadow effect on side view, and (c) shadow effect on top view	16
Figure 2.3. The deposition of incoming microparticle behind the deposited microparticle	17
Figure 2.4. The number of microparticles flown through the channel prior to clog (N^*)	18
Figure 2.5. Variation of the average clogging time t^* and N	18
Figure 2.6. (a) Acoustic streaming flow at $\kappa < 1$ and (b) acoustic radiation and scattering at $\kappa > 1$	22
Figure 2.7. Schematic diagram of SSAW consisting of (a) PDMS-LiNbO ₃ and (b) superstrate-LiNbO ₃	25
Figure 3.1. (a) Y-velocity of microparticle under laminar flow in the microchannel at the constriction angle of 90°, inlet of 100 μm , and outlet of 50 μm ; and (b) maximum velocity under different constriction angles from 3.6° to 90.0° and diameter ratios of the inlet to the outlet at the inlet of 100 μm	37
Figure 3.2. The motion of 30 microparticles at $t = 1.2$ s (left column) in top view and the Y-velocity of microparticles presented as mean $\pm$ standard deviation (STD) in $\mu\text{m/s}$ (right column) in a 100 μm microchannel at the constriction angle of 15° with the fluid viscosity of (a) 8.9×10^{-4} Pa·s (1×) without acoustic excitation; and (b) 8.9×10^{-4} Pa·s (1×); (c) 8.9×10^{-3} Pa·s (10×); (d) 4.45×10^{-2} Pa·s (50×); and (e) 8.9×10^{-2} Pa·s (100×) with the excitation of the standing surface acoustic wave at the vibration amplitude of 0.94 nm.....	40
Figure 3.3. Y-velocity of microparticle after 10 ms of the SSAW activation under (a) different vibration amplitudes and fluid viscosities of 2 μm	

microparticles 20 μm away from the microchannel center, and (b) different distances from the center for different microparticles at the vibration amplitude of 0.94 nm and a viscosity of 0.89 mPa·s	41
Figure 3.4. Schematic diagram of experimental setup.....	43
Figure 3.5. (a) The micrograph of microchannel structure: Zone A is the inlet reservoir, Zone B and C are the two consideration areas and (b) microparticles accumulation on the microchannel wall.....	44
Figure 3.6. Schematic of (a) microchannel constriction geometry, and (b) microchannel with inter digital transducers (IDTs)	45
Figure 3.7. The effect of microparticle concentration on the accumulation rate at flow rate of 2 $\mu\text{l}/\text{min}$ and 10 $\mu\text{l}/\text{min}$, respectively ($n = 20$).	46
Figure 3.8. The effect of sodium alginate concentration on the accumulation rate at micro-particles concentration of 1% and flow rate of 2 $\mu\text{l}/\text{min}$	47
Figure 3.9. Agglomeration of microparticles and alginate at the entrance of the microchannel.....	48
Figure 3.10. The effect of sodium alginate concentration on the agglomeration area.....	48
Figure 3.11. Comparison of the accumulation rate of microparticle in normal condition and under SAW excitation.....	49
Figure 3.12. Comparison of the agglomerate size of microparticles with and without SSAW excitation.	49
Figure 3.13. The representative photos of a gradual microparticle clogging around the constriction region (15°) in a microchannel with an inlet of 100 μm and outlet of 50 μm . The blue dashes represent the area of permanently-deposited microparticles while the yellow dots show the detachment of initially-deposited microparticles at $t = 18.5$ min.	51
Figure 3.14. Time-dependent accumulation area of microparticles in the microchannel at a constriction angle of 15° , 30° , and 45° , and 1% microparticle concentration in deionized water.	51
Figure 3.15. The clogging process of 5% alginate solution in the microchannel at the microparticle concentration of 1% and the constriction angle of 15° . The blue dotted line contours the agglomerated microparticles.	53

Figure 3.16. Time-dependent accumulation area of microparticles in the microchannel at the constriction angle of 15° and 45° with 1% microparticles in (a) 3%, and (b) 5% alginate solution.	53
Figure 3.17. The distribution of microparticles in the microchannel (a) before, and (b) after, the activation of the SSAW.....	54
Figure 3.18. Progressive microparticle accumulation in the microchannel at the constriction angle of 15° , 30° , and 45° with 1% microparticles in water and excitation of the standing surface acoustic wave (SSAW)	54
Figure 3.19. Progressive microparticle accumulation in the microchannel at the constriction angle of 15° with 1% microparticles in (a) 3%; and (b) 5% alginate solution, without and with the excitation of the standing surface acoustic wave (SSAW).	55
Figure 3.20. The attractive force on an 8- μm suspending microparticle from the wall and deposited microparticles at the various distances.	58
Figure 4.1. Schematic diagram of PDMS microchannel and IDTs on the LiNbO_3 substrate and boundary conditions used in the finite element method, Γ_i : impedance boundaries, Γ_d : Dirichlet actuation boundary.....	68
Figure 4.2. Schematic diagram of the experimental setup.....	70
Figure 4.3. (a) The initial uniform distribution of 4- μm microparticles in the cross-section of a microfluidic microchannel with the height of 50 μm and width of 300 μm , (b) their steady-state positions by SSAW simulated using the 2D model, and (c) the comparison of microparticle positions predicted by 1D (dash line) and 2D models (symbols).	71
Figure 4.4. (a) Photograph of a pair of interdigital transducers (IDTs) aligned with a 300- μm Polydimethylsiloxane (PDMS) microchannel and (b) S_{11} signal of IDTs measured by an impedance analyzer.	72
Figure 4.5. (a) The pressure waveform and (b) the corresponding acoustic radiation force applied to the 4- μm microspheres in a 300- μm microfluidic microchannel by dual-frequency excitation at the varied power ratios of $P_I = 100\%$ (purely fundamental frequency), 95%, 91%, 90%, 85%, and 0% (purely third harmonic) at the total acoustic power of 146 mW.	73

Figure 4.6. Motion of microparticle initially at $y = 0 \mu\text{m}$ (a) with the diameter of $4 \mu\text{m}$ under the varied power ratios (88–91%) and total acoustic powers (73–648 mW) of dual-frequency SSAW and (b) with the varied diameters of 4, 6, 8 and $10 \mu\text{m}$ at the total acoustic power of 73 mW.	73
Figure 4.7. The motion of $4\text{-}\mu\text{m}$ microspheres in a $300\text{-}\mu\text{m}$ microchannel by dual-frequency excitation at the varied power ratio of (a) $P_1 = 100\%$ (purely fundamental frequency), (b) $P_1 = 95\%$, (c) $P_1 = 91\%$, (d) $P_1 = 90\%$, (e) $P_1 = 85\%$, and (f) $P_1 = 0\%$ (purely third harmonic) at the total acoustic power of 146 mW.	75
Figure 4.8. (a) microparticle position and microparticle concentration, (b) motion of microparticles initially at $y_0 = 0 \mu\text{m}$, and the microparticle accumulation time using the dual-frequency SSAW at the total acoustic power of 146 mW with varied power ratios, and (c) the accumulation time of microparticles at various acoustic power from 73 to 438 mW.....	75
Figure 4.9. The accumulation of $4\text{-}\mu\text{m}$ microspheres in a $300\text{-}\mu\text{m}$ microchannel at the pressure node by dual-frequency excitation at the varied power ratios of (a) $P_1 = 100\%$ (purely fundamental frequency), (b) $P_1 = 90\%$, (c) $P_1 = 85\%$, and (d) $P_1 = 0\%$ (purely third harmonic).	77
Figure 4.10. Comparison of simulation and experimental results of (a) the position of pressure node ($R^2 = 0.85$, $n = 37$) and (b) the microparticle concentration at each pressure node in the microchannel ($R^2 = 0.83$, $n = 31$) at the varied power ratios of P_1	77
Figure 5.1. Cross-section diagram of subdomains and boundary conditions in the FEM simulation.....	85
Figure 5.2. Schematic diagram of experimental setup to observe the motion of microparticles (a) along and (b) in the cross-section of the glass tube, and (c) representative photo of the accumulated microparticles in the glass tube under the acoustic activation.....	87
Figure 5.3. (a) The simulated radial stress of glass tube at the excitation frequency of 168 kHz in kPa, (b) comparison of simulated (172 kHz) and measured (168 kHz) normalized vibration velocity in the polar plot, (c) time-average acoustic pressure in kPa at 168 kHz, (d) the locations of $50\text{-}\mu\text{m}$ microparticles after 0.2 seconds of excitation in the simulation,	

and cross-sectional image of microparticles (e) without and (f) with the acoustic excitation.....	90
Figure 5.4. The representative photos of microparticles in the glass tube with 1% sodium alginate and 0.25% microparticle (a) before and (b) after the acoustic excitation, and the corresponding distributions of the normalized light intensity in (c) and (d), and (e) the change of the peak light intensity during the microparticle accumulation in the fluid with 1% alginate and varied microparticle concentrations.	92
Figure 5.5. Accumulation time and width of microparticles in the solution with (a) 1%, 2%, 3%, and 4% sodium alginate and 0.25% microparticles and (b) 0.25%, 0.5%, 1.0%, 1.5%, and 2.0% microparticles and 1% sodium alginate ($n = 6$ for each condition).....	93
Figure 5.6. (a) The printed structures with 2% sodium alginate and 0.5% microparticle on the petri dish, and zoomed photos illustrating the distribution of microparticle distribution inside them (b) without and (c) with an acoustic excitation during printing.....	94
Figure 5.7. Histogram (solid line) and fitted Gaussian curve (dashed line) of microparticle distribution in the printing structure using the ink with 1% sodium alginate and 0.5% microparticle concentrations (a) without and (b) with the acoustic excitation. Comparison of the distributed microparticle width (c) at the various sodium alginate concentrations from 1% to 4% and microparticle concentration of 0.5% and (d) at the sodium alginate concentration of 1% and various microparticle concentrations from 0.25% to 2%. * shown in the figure represents statistical differences between the experimental results of a group without and with the acoustic excitation ($p < 0.05$).....	95
Figure 5.8. Comparison of the (a) simulated acoustic pressure field in kPa, and (b) location of accumulated microparticles in the cross-section at 393 kHz (left column) and 563 kHz (right column), representative photos of accumulated microparticles (c) in the cross-section, (d) along the glass tube, (e) in the printed structure, (f) the histogram and fitted Gaussian curves for each accumulation lines under the acoustic excitation at 385 kHz (left column) and 657 kHz (right column).	98

Figure 5.9. Microparticle distribution in the cylindrical tube (a) without acoustic excitation, (b) at 2 seconds of acoustic excitation at 899 kHz and in the nozzle (c) without acoustic excitation, (d) with acoustic excitation.....	99
Figure 5.10. The progress of accumulation of microparticle on the nozzle over time (a) 7:00 mins, (b) 7:28 mins, (c) 7:36 mins, (d) 8:01 mins, and (e) 10:27 mins.....	100
Figure 5.11. Progression of accumulation area and medium discharge from the nozzle with and without acoustic excitation at the alginate concentration of (a), (b) 1% and (c), (d) 2% and (e), (f) 3%.	101
Figure 5.12. Printed structure of square shape (a) without, (b) with the acoustic excitation and particle distribution inside the printed structure (c) without, (d) with the acoustic excitation, and histogram of particle distribution along the width of printed structure (e) without, (f) with the acoustic excitation, and (g) color contrast analysis of the printed structure after crosslinked. * shown in the figure represents statistical differences between the experimental results of a group without and with the acoustic excitation ($p < 0.05$).	103
Figure 6.1. Numerical simulation of (a) time-averaged acoustic pressure field in kPa and (b) cell distribution in the cylindrical nozzle at 871 kHz.....	115
Figure 6.2. Representative micrograph of C2C12 cells in 3D printed construct of 5% GelMA (a) without and (b) with the acoustic excitation, and cell distribution fitted Gaussian curve in dashed line (c) without and (d) with the acoustic excitation, also (e) plot of mean values and standard deviations from fitted Gaussian curve, $n=6$	116
Figure 6.3. Morphology and distribution of the C2C12 cells in 5% GelMA without the acoustic excitation on the (a) day 1, (b) day 4, (c) day 7, and with the acoustic excitation on the (d) day 1, (e) day 4, (f) day 7.	118
Figure 6.4. Cell density in the printed GelMA construct on day 1, 4 and 7 (a) without, (b) with acoustic excitation and (c) width cell microconstruct without and with the acoustic excitation over period (days) of cell culture in GelMA.	118
Figure 6.5. Elongation and alignment of cells in the GelMA construct (a) without and (b) with the acoustic excitation, and a normalized number	

of cell in each orientation angle (c) without and (d) with the acoustic excitation, each value was represented with mean $\pm$ std.	120
Figure 6.6. Immunofluorescence (IF) against myosin heavy chain (green color) and cell nuclei counterstained by DAPI (blue color) of the cell construct (a) without, (b) with acoustic excitation, (c) histology of skeletal muscle tissue, (d) zoomed-in area obtained from the white window, and normalized number of cell nuclei in each orientation angle (e) without, (f) with acoustic excitation, each value was represented with mean $\pm$ std, and (g) standard deviation of fitted Gaussian curve and cell nuclei circularity index.	122
Figure 7.1. Accumulation of microparticles in cylindrical glass tube at (a) 0.28 W and, (b) 0.63 W.....	132

List of Tables

Table 2.1. The schematic of clogging mechanism [40]	14
Table 3.1. Material parameters at Temperature = 27 °C	34
Table 3.2. Time-dependent accumulation area in the microchannel with 1% microparticle concentration in alginate solution fitted by AeBt and the accumulation area at 25 min of circulation.....	52
Table 5.1. Material properties used in the numerical simulation.....	85

List of Symbols

F_{Rad}	Acoustic radiation force
p_{ac}	Acoustic pressure
Ω	Angular frequency
Φ_{p-p}	Attraction potential between particles
Φ_{p-w}	Attraction potential between particle and wall
κ_p	Compressibility of particle
κ_f	Compressibility of fluid
e_M	Coupling matrix
σ_w	Charge density on the wall surface
C_u	Cunningham correction factor
ρ_p	Density of particle
ρ_f	Density of fluid
ρ_g	Density of glass
ρ_{LNB}	Density of LiNbO ₃
ρ_{PDMS}	Density of PDMS
ϵ_m	Dielectric constant of medium
ϵ_p	Dielectric constant of particle
f_{dip}	Dimensionless scattering coefficients for dipole
f_{mono}	Dimensionless scattering coefficients for monopole
σ_{dir}	Direct stress on the material
ϵ_{dir}	Direct strain on the material
d_{p-w}	Distance between particle and wall
d_{p-p}	Distance between particles
δ	Distance to the boundary layer
F_{Drag}	Drag force
η	Dynamic viscosity
c_M	Elasticity matrix
K_c	El-Batsh parameter
D_E	Electric displacement
E	Electric field
k_{31}	Electromechanical coupling factors on 31 direction
k_{33}	Electromechanical coupling factors on 33 direction
F_{stick}	Force of particle to stick to the wall
f	Frequency
f_1	Fundamental frequency
f_3	Third harmonic frequency
H	Hamaker constant
ϵ_M	Permittivity matrix
ϵ	Poisson's ratio
p_f	Pressure of fluid
Φ_{prop}	Propagating potential
r	Radius of particle
n_{pi}	Reflective index of particle
n_{mi}	Reflective index of media
Γ	Root mean square of acceleration from vibration
Φ_{scat}	Scattering potential

v_L	Speed of longitudinal wave
v_s	Speed of shear wave
c_f	Speed of sound in fluid
c_p	Speed of sound in particle
c_{PDMS}	Speed of sound in PDMS
c_{LiNbO_3}	Speed of sound in LiNbO ₃
γ	Surface tension
T	Temperature
W_A	Work of particle stick to the wall
Y	Young's modulus
Z	Zeta potential

Summary

A random distribution of microparticles/cells increases the chance of deposition on the inner surface of the nozzle, leading to obstruction/clogging of nozzle constriction. Nozzle clogging in 3D printers and other high-resolution machines is a common problem resulting in the loss of time, budget, productivity, part uniformity, and integrity of the printed part. Nozzle clogging is mainly due to the deposition of microparticles, which affects the accuracy and reliability of printing as well as the choice of printable materials. In jetting and extrusion, the surface tension and viscosity of fluid medium and the concentration of solid microparticles/cells are limited to a certain range. In addition, the obstruction of flow path could increase the mechanical stress in that region. High mechanical stress on biomaterials and cells during bioprinting decreases the viability of cells on the printing scaffold. Current methods (e.g. surfactant, limited volume fraction and size of aggregates) cannot reduce clogging effectively and have limitations. Addition of surfactant can damage cells resulting in low cell proliferation. In the circuit printing (inkjet-based), the volume fraction of aggregates should be controlled to avoid clogging. But low volume fraction causes impairment in the electrical performance of the printed structure. In this study, a method of utilizing ultrasound was proposed, developed, and evaluated in order to reduce the nozzle clogging, improve the printing stability and accuracy of the nozzle-based 3D printer. Such method could also be applied to other high-resolution machines with little modification.

The proposed method is to use acoustic waves to align microparticles/cells through the constriction/nozzle and, subsequently, in the printed construct. Also, it is to evaluate the effect of acoustic waves to suppress nozzle clogging. In the first part of this study, the effect of standing surface acoustic wave (SSAW) on the reduction of microparticle accumulation was studied in a microchannel. The fluid medium consists of deionized water and sodium alginate with different concentrations ranging from 0% to 5%. The experimental results had a good

agreement with the numerical simulation. The experimental results showed that SSAW is capable of reducing the microparticle accumulation area effectively in the low alginate concentration solution. Meanwhile, such capability decreased in the high concentration of alginate. Additionally, to enhance the tunability of SSAW, a dual-frequency excitation was utilized in the microchannel. The dual-frequency excitation method utilizes a superposition of SSAW at the fundamental (f_1) and third harmonic (f_3) frequencies allowing the number and location of the pressure node to be controlled more flexibly.

In the later part of this study, an acoustic excitation of the structural vibration was used for focusing the microparticles/cells towards the center of the cylindrical tube. It was found that the focusing time and width of microparticles in the cylindrical tube increase with the concentration of sodium alginate and microparticles in the ink. Subsequently, the ink was printed from the nozzle consistently. Most of the microparticles are distributed in the central part of the printing structure. In comparison to the conventional printing strategy, acoustic excitation could significantly reduce the width of accumulated microparticles in the printing structure (p -value < 0.05). In addition, the microparticle motion at the higher harmonics (385 kHz and 657 kHz) was also studied. Lastly, the C2C12 cells (myoblast muscle cells) were printed out from the nozzle through the cylindrical tube using the acoustic excitation. The acoustically-patterned C2C12 cells in the three-dimensional printed gelatin methacrylate (GelMA) construct were monitored for 7 days for their growth and morphology. Overall, the acoustic approach is able to accumulate microparticles/cells in the printed construct at a low cost, simple configuration, and low power, but high biocompatibility. In the future, acoustic patterning of various biological cell types in printed construct could be investigated. As acoustic method has a capability to manipulate the microparticle/biological cells depending on their physical properties (compressibility, density and size).

Chapter 1 Introduction

Three-dimensional (3D) object manufacturing in which materials such as plastic, fluid, and metal are deposited onto one another in layers is called 3D printing or additive manufacturing (AM). Its applications include automotive parts, aerospace parts, apparel, and artificial organs [1, 2]. Three-dimensional printing has the advantages of generating a free-form structure, enlarge the component size, and reduce the equipment cost in comparison to other manufacturing techniques. The market for the additive manufacturing is expected to reach US\$16.8 billion globally by 2022, leading by metal, biomedical, electronic, and consumer printers. Most of the printers are nozzle-based which rely on the extrusion/drop/injection of the printed material through the nozzle. Additionally, based on the American Society for Testing and Materials (ASTM) classification, nozzle is a necessary component for material jetting and extrusion printers. These two types are the majority of the printers used in the market. The material extrusion printer includes fused deposition and bioprinter (extrusion-based) whereas material jetting printer includes all inkjet-based printers [3]. The diameter of nozzle can range from a few micrometers to centimeters depending on the application required resolution and printing material. However, a nozzle used in the 3D printing is restricted to the physical properties of the printing material and a limited concentration of microparticles/cells suspended in the printing medium. With the use of an ordinary nozzle, the distribution of solid microparticles/cells in the printed construct is random.

Meanwhile, patterning of microparticles/cells in the printed construct could enhance the mechanical strength, functionality, and growth of biological cells. For instance, proper alignment and orientation of the fibers in a polymer matrix could transfer the loads away from the critical locations for the improved performance [4]. Hierarchically ordered materials at the nano- and micro-scale levels that exploit material composition and capabilities can expand the applications of AM techniques. The ability to print 3D scaffolds with a controlled hierarchical

structure could enhance the high mechanical strength, which is desirable for load-bearing bone defect repair and regeneration [5]. Decorating the surface of carbon nanotubes with particular antibodies enables the detection of specific antigens as functional materials [6]. Nonetheless, application of multifunctional nano-composites with respective printing media may have certain limitations, such as nozzle clogging [7]. Printing multiphase fluid medium consisting of microparticles/cells through a narrow nozzle could cause an obstruction or a clogging at the nozzle constriction. Microparticles/cells flowing in the nozzle could deposit on the inner surface of the nozzle especially at the constriction region. This deposition builds up slowly and shrinks the flow path in the nozzle constriction [8]. Subsequently, the nozzle could be clogged which affects the printing performance and causes damage to the nozzle.

In general, clogging is a result of the consecutive deposition of substances or arch formation of the solid microparticles/cells. In 3D printing, clogging or blocking of the nozzle is a common problem for nozzle-based printers. It can cause non-uniformity of the printed part, loss of time and material. Clogging of the nozzle is difficult to predict accurately and dependent on the physical properties of the printing solution [9]. There is a limitation of the viscosity and mechanical properties (i.e., surface tension) of the printing solution for inkjet-based and extrusion-based printers [10]. For instance, in the inkjet-based printer, surface tension is generally above 28 mN/m and viscosity is limited to be below 40 mNs/m². In comparison, the surface tension and viscosity of pure water are approximately 73 mN/m and 1 mNs/m², respectively, at the standard temperature and pressure [11]. For extrusion-based printing, it is difficult to build a rigid free-form three-dimensional structure with low mechanical strength using the solution of low viscosity. The ability to form a three-dimensional hydrogel scaffold and cell with high spatial resolution and reliability has a great potential for the artificial organ printing [12]. The tissue and other components (e.g. vessel and tube) need precise location and continuity of printing, delivering a specified quantity of cells and hydrogel at the exact time

and location [13]. In 3D bioprinting research, clogging and aggregation of cells is still an obstacle, which limits the concentration and density of cells in the bio-ink and also the use of highly viscous hydrogel. Suspensions are likely to sediment and aggregate in the cell reservoir, tube and nozzle of the printing system [14], the sedimentation reduces the width of the flow path which may also lead to clogging within the narrow geometry of the inkjet nozzle. The clogging could significantly increase the normal stress and shear stress applied to the cells, which may decrease the cell viability and proliferation rate, and decrease formation of non-uniform droplet sizes of bioink [13, 15]. To explore the process of clogging, the mechanism of clogging and related theory is discussed in the literature review in Chapter 2.

Even though clogging problem is found in various areas, this phenomenon is still far from being fully understood. The attempt to reduce or delay the clogging is still under the investigation. There are various techniques to suppress nozzle clogging depending on the state of the printing ink (e.g. homogeneous fluid ink, non-homogeneous ink and solid particles/grains). For instance, printing material in the homogeneous fluid state (e.g. casting, molten metal, glass and homogeneous ink) usually needs solidification. In casting, the clogging can be reduced by optimizing the nozzle design [16, 17] and increasing the fluctuation of the flow [18]. The fluid stagnation zone in the nozzle has low temperature and energy circulation rate which may solidify the molten liquid and clog the nozzle. Hence, the design of nozzle which minimizes the flow stagnation zone could suppress the nozzle clogging. Another state of the printing ink is non-homogeneous ink including electronics/circuit printing, bioprinting, and polymer printing. For this type of ink, the surfactant can be added to modify the contact angle of the deposited drops on the platform surface and subsequently reduce the surface tension of the printing material [19, 20]. Besides, surfactant could be used to reduce the size of microparticle agglomeration. As a result, the fluid with too high surface tension may be able to be printed easily and reduce the chances of nozzle obstruction. However, there are three major

limitations of using a surfactant. Firstly, some surfactants can change the medium properties and cell membrane resulting in the decrease of cell proliferation in a long-term [13, 15]. Parsa et al. printed HepG2 cells with pluronic (biocompatible and FDA approved surfactant) using 100 μm inkjet printer. The HepG2 cells with pluronic showed a slight decrease in the viability and proliferation in the first few days. Nevertheless, it caused 50% decrease in cell proliferation over 13 days [13]. Secondly, the surfactant changes the physical properties significantly. In case of non-ultraviolet curable material, printing material needs enough physical properties to maintain the 3D structure on the platform. Lastly, only a limited range of the surface tension could be reduced using the surfactant, depending on the type of fluid, substrate, and surfactant [21, 22]. The relationship between the concentration of surfactant and the surface tension in the solution is described [23]. The reduction of surface tension is because surfactant molecules attach to the surface of the solution and form a spherical aggregate, where the hydrophobic chain is pointing to the center by polar groups. Between point A and B, the surface tension of the solution changes sharply. However, beyond the point B, the surfactant can no longer change the surface tension of the solution. Nevertheless, the ability of the surfactant to reduce the clogging is still under research. Furthermore, great attention is paid to the multiphase system (fluid medium with solid microparticles), which includes colloidal microparticle, filtering, and non-homogeneous ink.

To date, the number of studies to tackle clogging problem in 3D printing is limited. For instance, Kim et al. [9] studied the fuse deposition modelling (FDM) extrusion head and predicted the status of printing by tracking the motor supply current. The resistance of motor used in FDM to feed filament material increases, when the filament is clogged in the nozzle. Therefore, higher torque is required to feed the filament and could be detected directly from the motor supply current. Another study is for the inkjet-based printer. Lee. et al. [24] used the ring-slit device to detect the microparticle aggregation and tried to suppress the nozzle clogging

by applying higher driving voltage, varying volume fraction, and optimizing the contraction zone. In order to avoid the nozzle clogging, volume fraction of aggregates, $\phi_{\text{aggregates}}/\phi_{\text{initial}}$, should not exceed 5×10^{-4} , and a total number of aggregated microparticles should be below 7×10^5 per mL. For the circuit printing, ZnO microparticle is used in the ink. Limitation of volume fraction and the number of microparticles cause impairment in the electric performance of the printed structure [24]. To optimize the contraction zone, the printing condition and Z (Zeta potential) value need to be considered ($Z = \frac{(d\rho_f\gamma)^{1/2}}{\eta}$), where d is the nozzle diameter, ρ_f is the density of fluid, γ is the surface tension, and η is the dynamic viscosity [24]. Therefore, the optimum design is material-dependent.

Among other states of the printing ink, clogging of solid particles/grains is the best understood (e.g. salt, pepper powder in the shakers and solid grains in an industrial silo). Clogging of grains mainly depends on the width of the outlet opening and the grain size, and there is no grain deposition to the hopper wall. One of the effective techniques with the minimal side effects is to apply external vibration to the nozzle. Janda et al. [25] proposed that external vibration at the eccentrically shaped opening of the hopper could reduce the probability of clogging. The minimum outlet opening size decreases with the increase of the vibration acceleration, where Γ is the root mean square (RMS) acceleration from vibration normalized by the gravity acceleration (g). Additionally, high vibration acceleration facilitates the stable outflow (see Fig. 1.2.). As the vibration decreases, the jam will be delayed. The anomalous dynamics of the outer wall of the nozzle is able to disturb the clogging behavior [26]. From these studies, it could be implied that the use of the external vibration applied to the nozzle could facilitate the stability of the solid grain outflow and subsequently reduce the probability of clogging.

Figure 1.1. The outflow masses from the silo as a function of time, for four different vibration accelerations [25].

Therefore, our proposed method is to apply external vibration from acoustic waves to the nozzle for patterning of microparticle/cells and suppression of nozzle clogging. Firstly, to simplify the clogging problem, the microparticle accumulation and clogging is studied in two-dimensional polydimethylsiloxane (PDMS) microchannel. To apply the external vibration, standing surface acoustic wave (SSAW) was used for manipulation of microparticles/cells in the microchannel. SSAW is formed by an interference of travelling waves either from multiple SAW sources or the interdigital transducers (IDTs). SSAW has been widely used for the manipulation of microobjects/particles and cells [27-31]. Single pressure node SSAW can be created at the center of microchannel along its axis by setting the width of the microchannel to be half of the wavelength. A pair of IDTs was aligned parallel to the microchannel with one on each side. Hence, the microparticles could be pushed towards the location of pressure node by the acoustic radiation force. The current techniques of SSAW in the microchannel are discussed in Chapter 2 (Literature Review). Besides, the acoustic excitation of the structural vibration of the cylindrical tube was proposed. Briefly, acoustic excitation is generated from the transducer(s) attached along the longitudinal axis of the cylindrical tube and contributed to the whole solid tube structure. The first order structural mode was excited to align the microparticles at the center of the cylindrical tube filled with liquid and the nozzle at the end [32, 33]. Hence, microparticles/cells could be acoustically patterned along the center of the

cylindrical tube and subsequently printed out from the nozzle. The patterning of microparticles/cells still remains in the printed construct after printing.

The purpose of this work is to utilize the acoustic manipulation in order to pattern/align microparticles/cells and suppress nozzle clogging. This could improve the printing stability and accuracy of the nozzle-based 3D printer and other high-resolution machines. Additionally, the choice of printable material is expanded, and the part functionality is improved with controlled pattern/arrangement of microparticles/cells in the printed construct. Printing of biological cells suspended in the biomaterials is currently on the research attention because of its capability to fabricate the living cells in 3D free-form scaffold [15, 34, 35]. Hence, developing a nozzle to suppress the clogging issue and pattern the microparticles/cells is worth to be explored.

Clogging of bioprinter nozzle is still far from being fully understood. To mimic the clogging phenomenon in the nozzle, a progressive accumulation and clogging of microparticles in the PDMS microchannel were investigated. Microparticles flowed through a narrow constriction in the microchannel. The accumulation of microparticles on the inner surface of the microchannel constriction was studied in Chapter 3. Subsequently, SSAW was used for the manipulation of microparticle numerically and experimentally. The effect of the acoustic radiation force on microparticle in the fluid medium with different viscosities was taken into consideration. Then, microparticle accumulation and its reduction by SSAW in the microchannel were observed experimentally. The effects of constriction angle in the microchannel, alginate concentration and SSAW on the microparticle accumulation/clogging were studied. SSAWs move microparticles away from the wall, towards the center of the microchannel, and therefore, reduce the chance of microparticle accumulation/clogging.

In Chapter 4, a dual-frequency excitation was utilized to enhance the tunability of SSAW. The dual-frequency excitation method utilizes a superposition of SSAW at

fundamental (f_1) and third harmonic (f_3) frequencies allowing the location of pressure node to be controlled flexibly. Changing the power ratio, the amplitude and distribution of resultant acoustic radiation force on microparticles/cells lead to reconfigurable patterns, such as the number and position of the pressure nodes and the corresponding percentage of microparticles accumulated at each pressure node. Besides, with an optimum power ratio of f_1 to f_3 ($\approx 9:1$), the accumulation time from SSAW could be reduced significantly (~ 2 -fold).

In Chapter 5, the acoustic method is applied to the nozzle of 3D printer. Acoustic excitation from the structural vibration was used for focusing the microparticles/cells towards the center of the cylindrical tube. The nozzle was connected to the end of the cylindrical tube. The measured location of pressure and the excitation frequency of the cylindrical glass tube (172 kHz) agreed well with the numerical simulation (168 kHz). At this excitation frequency, acoustic excitation could effectively and consistently focus the microparticles. It was found that the focusing time and width of microparticles in the tube increase with the concentration of sodium alginate and microparticles in the ink. The microparticles are concentrated in the central part of the printed structure. In comparison to the conventional printing strategy, acoustic excitation could significantly reduce the width of accumulated microparticles in the printed structure ($p < 0.05$). In addition, the microparticle motion excited at higher harmonics (385 kHz and 657 kHz) was also studied.

In Chapter 6, the C2C12 cells (myoblast muscle cells) were patterned by acoustic waves in the cylindrical tube and printed out from the nozzle. The acoustic-patterned C2C12 cells in the three-dimensional printed gelatin methacrylate (GelMA) construct were monitored for 7 days. Since myoblast cells differentiate into smooth muscle cells, this type of cells usually prefers the dense cell-cell condition for initiating the differentiation process. The use of acoustic excitation gathers the cells, which supports cell-cell interaction and could promote the cell differentiation. Overall, the acoustic approach is able to promote the differentiation of

biological cells in the printed construct at a low cost, simple configuration, low power, but high biocompatibility.

Lastly, in Chapter 7, the key findings from each study in this dissertation are concluded, and the suggestions for the future work are described. Our proposed acoustic method for the nozzle was found to be effective in patterning the microparticles/cells in the printed construct as well as reducing the microparticle accumulation which suppresses nozzle clogging. In the future, the acoustic nozzle could be tested with different materials and types of nozzle-based printers. Other types of solid microparticles/cells and fluid media could be patterned and printed theoretically. In addition, this acoustic nozzle has a potential for commercialization as it has distinct advantages over other dispensing nozzles currently available in the market. For commercialization, this acoustic nozzle should be further improved for the ease of manufacturing, installation, and tunability.

Chapter 2 Literature Review

Previous studies related to this thesis are reviewed and discussed in this chapter, and their topics are organized as follows;

- Microparticle clogging in microchannel, related forces, and clogging mechanism
- Numerical simulation of microparticle deposition
- Microparticle manipulation using surface acoustic wave (SAW)
- Microparticle/cell manipulation using structural acoustic vibration in the cylindrical tube
- The use of acoustic manipulation in the nozzle-based 3D printer

2.1 Microparticle clogging in the microchannel

Microparticle clogging in the nozzle of a printer occurs in a scale of micrometers. Clogging usually starts from deposition/agglomeration of the solid microparticles. Subsequently, deposited microparticles continue to form a single layer and end up with the clog. However, this process is unstable as the deposited microparticles could detach from the nozzle wall due to the forces acting on it. Hence the possible forces involved in this process and mechanism of clogging were discussed in this section.

2.1.1 DLVO Theory and Non-DLVO Forces

The Derjaguin, Landau, Verwey and Overbeek (DLVO) theory can explain the interparticle repulsion force from an overlapping of ion in each particle, in the event that two microparticles get close to each other. This theory analytically describes both microparticle agglomeration and interaction forces between charged surfaces and fluid medium with a consideration of the van der Waals attraction, electrostatic and Born's repulsion [36]. The electrostatic repulsion is formed by the electrical double layer of different types of ion. A short distance between microparticles could significantly increase the van der Waals attraction and the potential of electrostatic repulsion. In the stationary system, the resultant potential energy calculated from the van der Waals and the electrostatic repulsion potential is the key predictor

for the particle's behavior. In other words, electrostatic repulsion hinders microparticle agglomeration creating the energy barrier, but the van der Waals attraction pulls the particles. Under the flow, if the microparticle collision and drag have sufficient force to overcome the barrier, van der Waals attractive force causes the microparticles to stick to each other irreversibly [37].

2.1.1.1 van der Waals attraction force

The fluctuation of dipoles from each atom (interparticle) form the attraction potential (van der Waals) which can be expressed as [38, 39];

$$\phi_{p-p} = - \frac{H}{12d_{p-w} + (1 + \frac{11.12d_{p-w}}{L})} \quad (2.1)$$

where r is the atom radius, d_{p-w} is a minimum distance between microparticle surface and wall, H is the Hamaker constant ($H = 10^{-20}$ J, PS microparticles in water), L is the length of retardation. In order to calculate the Hamaker constant, McLachlan's equation which consider interaction between particle, wall and medium is applied. But for only interparticle force, the Hamaker constant can be simplified as [38, 40]

$$H = \frac{3}{4} K_B T \left(\frac{\epsilon_p - \epsilon_m}{\epsilon_p + \epsilon_m} \right)^2 + 1.89 \pi \hbar v_e \frac{(n_{pi}^2 - n_{mi}^2)^2}{(n_{pi}^2 + n_{mi}^2)^{1.5}} \quad (2.2)$$

where n_{pi} is microparticle reflective index, n_{mi} is medium reflective index, v_e is the electron's orbiting frequency, K_B is Boltzmann constant which is equal to 1.38×10^{-23} J/K, ϵ_p is microparticle dielectric constant, ϵ_m is medium dielectric constant and T is temperature. The attraction potential between particles (ϕ_{p-p}) is proportional inversely to the separation distance. The irreversible attachment could occur if internal potential energy is less than the attraction potential. In the microchannel, there is a high chance of interaction of microparticle with the wall. The van der Waals potential of microparticle and wall is expressed as,

$$\phi_{p-w} = - \frac{H}{6d_{p-w}} \quad (2.3)$$

2.1.1.2 Electrostatic Repulsion

For the ionic solution, charged ions can form layers on the surface of the colloidal microparticle. Thus, there is a formation of a double layer of counter-ions around the colloid. As every microparticle has surrounding double layers, the surface contact of another microparticle is obstructed by the repulsive electrostatic force from the surrounding double layers. The electrostatic repulsion potential (ϕ_e) could be calculated by the Derjaguin approximation [40, 41];

$$\phi_{e(p-p)} = 2\pi H \frac{\sigma_p^2}{\epsilon_p \kappa^2} \ln(1 - e^{-\kappa d_{p-p}}) \quad (2.4)$$

where σ is a charge density of the particle, κ is the inverse of the screening length (the size of double layer). The double layer length changes inversely with the square root of counter ions concentration. Thus, by adding more electrolytes such as NaCl or KCl to increase the concentration in solution and subsequently, modify the electrostatic force, the double-layer length would be decreased. Ions are able to accumulate on the surface of microparticle and wall. The electrostatic repulsion arises from the same mechanism, and the electrostatic potential between microparticle and wall can be expressed as [42, 43]

$$\begin{aligned} \phi_{e(p-w)} = \frac{2\pi r}{\kappa \epsilon_p} \{ & (\sigma_p^2 + \sigma_w^2) \left(d - \frac{\ln(e^{2\kappa d_{p-w}}) - 1}{2\kappa} \right) \\ & + \frac{\sigma_w \sigma_p}{\kappa} \ln\left(\frac{1 + e^{-\kappa d}}{1 - e^{-\kappa d}}\right) \} \end{aligned} \quad (2.5)$$

where σ_w is the charge density on the wall surface. The comparison between interparticle interaction and particle-wall interaction is shown in Fig. 2.1. The interaction of microparticle with the wall has significantly lower magnitude. In a short distance, microparticle tends to deposit on the wall rather than stick with the other particles. In addition, once the first

microparticle is deposited on the wall, suspended microparticles are even preferable to stick to the deposited microparticle [40].

Figure 2.1. Magnitude of particle-particle force and particle-wall interaction force with respect to the surface separation distance [44].

2.1.1.3 Non-DLVO forces

Two main non-DLVO forces are hydrophobic interaction force and hydration. In a short distance, a surface separated by the water has a strong repulsive force (5 MP or higher). Thus, this force is capable of overcoming the electrostatic double layer repulsion [45]. Hydrophobic force is a strong interaction force between water and non-polar molecule in the microscale and normally stronger than the DLVO force at the same separation distance. This force is able to predict the solubility of the chemical. Hydrophobes are not dissolved in water-based solution if interaction force between hydrophobes-water is smaller than those of water-water and hydrophobes-hydrophobes [46, 47].

2.1.2 Mechanism of clogging

Previous studies showed that clogging occurs when the microparticles flow into a confined space [48-55]. In the case of porous medium, the clogging either happens at the entrance or within the first few rows of pore throats which depends on the degree of

confinement (W/D , where W is the channel width and D is the microparticle diameter) and volume fraction. In the microscale, consecutive deposition is more frequent than immediate clogging. Dersoir [40] classified the mechanisms of clogging into three types: complete blocking, bridging and standard clogging (see Table 2.1).

Table 2.1. The schematic of clogging mechanism [40]

Particle diameter > Channel width	Particle diameter < Channel width	
Complete Blocking	Bridging	Standard Clogging

The complete blocking is the situation where the diameter of microparticle is larger than the channel size whereas bridging and standard clogging occur when channel size is larger than the microparticle diameter. In the sieving process, which is a separation of microparticles in different sizes, the clogging occurs by complete blocking mechanism. Hassan et al. [56] observed the growth of filter clogging region on a sieve infiltration mode. Structure, porosity, and thickness of the filter clogging region were studied and related to the decreased pressure during the formation of clogging region [56]. Brans et al. [57] observed the mixture of colloidal microparticles in the liquid flow between parallel surfaces. The results showed a higher chance of large microparticles to be clogged in the middle of the channel, and smaller microparticles deposited along the microchannel edge.

The bridging is a clogging mechanism where a bridge or an arch is established once the microparticle size is smaller than the channel size. Therefore, microparticles adhere to one another and on the pore walls at the same time and then form in this way an arch. The bridging

mechanism is commonly found in the microparticles flow without liquid medium. In liquid, bridging happens especially at the incline wall/constriction and high microparticle volume fraction. If many microparticles in the stabilized forward flow pass through a channel simultaneously, they may get stuck at the entrance of the channel to establish an arch. One of ways to diminish the bridge clogging is to rapidly change the flow direction. Sharp and Adrian [50] observed the arch pattern inside the transparent tube flowed with 50- μm microparticles. Ramachandran and Fogler [58] used 0.2- μm microparticles and found the bridge or arch pattern with the same degree of confinement. There is a critical value of microparticle concentration and flow rate to form the channel clog, above which the formation of the bridge should not occur. Nevertheless, near the entrance of the channel, hydrodynamic forces are able to dominate the microparticle trajectories. Bacchin et al. studied the clogging of latex microparticles in a rectangular elongated Polydimethylsiloxane (PDMS) microchannel (the ratio of width to depth is equal to four) at the varied flow rates and volume fraction. It was found that the critical volume fraction is 5×10^{-4} . However, the increase of velocity shows lower volume fraction for bridge clogging [59, 60].

The standard clogging is a gradual mechanism and less severe than the others. However, it is difficult to unclog due to the adhesion and accumulation of microparticles on the surface of microchannel wall. The accumulation could lead to the gradual reduction of the cross-sectional area of the channel and finally the complete occlusion. The progressive process can be divided into three consecutive stages: deposition of the first microparticle near the entrance of microchannel, a layer of more microparticles, and clogging. Electrostatics force forms the barrier to prevent the agglomeration and blocks the microparticles from moving closer to each other. With the absence of electrostatic barrier, the microparticles can form multilayers. But the monolayer would be formed if the microparticles flow below the critical velocity and electrostatic barrier are presented [61, 62]. While the first microparticle is deposited, it

influences the area nearby as a shadow zone as described in Fig. 2.2 [63]. The chance of suspended microparticles to get captured in this area would be increased. The growth of shadow zone is mainly due to the increased microparticle velocity, weak ionic strength, large microparticle size and strong microparticle electrostatic repulsion [64, 65].

Figure 2.2. Schematic diagram of shadow effect of deposited microparticles and its influence to the deposition of incoming microparticles. (a) deposition on spherical collector, (b) shadow effect on side view, and (c) shadow effect on top view [63].

The interaction between the suspended and deposited microparticles is the main factor of forming multilayers. If the hydrodynamic forces of the deposited microparticles are able to overcome the electrostatic barrier between two microparticles, the suspended microparticles can be captured. However, in the microscale, the inter-particle lubrication force governs the microparticle separation [66]. Interestingly, the distance of the second layer deposition is approximately same as the distance of dominant lubrication force with consideration of van der Waals attraction force. Ramachandran et al. [58] explained that ionic strength of the solution affects the critical velocity and multilayer formation. In that work, the destination of the microparticles to be captured was not mentioned. Whittle et al. [67] showed that the incoming microparticle can be stopped by rolling upon the immobile microparticles until they get stuck just downstream (see Fig. 2.3).

Figure 2.3. The deposition of incoming microparticle behind the deposited microparticle [67].

The final stage of this process is clogging. Retardation effect was used to explain microparticle accumulation and clogging near the constriction by Wyss et al. [48]. Microparticle tends to continue its trajectory as long as the fluid streamlines do not bend. Wyss et al. found that suspending microparticle is captured once it moves close to the deposited microparticle, which is mainly caused by van der Waals attraction force [48]. In addition, the sparse microparticle concentration regime was observed. A parameter corresponding to the incidence of clogging is the average number of microparticles, N^* , which flow through the microchannel before the clogging [48]. In Fig. 2.4, the solid line is a power law fit with an exponent, which is equal to four. The rescaled data are independent of the ratio between pore and size of microparticles. The clogging can occur by standard clogging ($d_{particle} < width_{channel}$), complete blocking ($d_{particle} > width_{channel}$), or combination of both [52].

Figure 2.4. The number of microparticles flown through the channel prior to clog (N^*) [48].

Figure 2.5. Variation of the average clogging time t^* and N^* [48].

2.2 Numerical simulation of microparticle deposition

Even though the simulation of microparticle deposition has been performed previously, the simulation of deposition on the nozzle is few and far between. The coupling between microparticle deposition and fluid-structure interaction is quite challenging. In ANSYS FLUENT, Eulerian and Lagrangian are two different approaches for microparticle deposition which have been studied in the numerical simulation.

2.2.1 Eulerian microparticle tracking

The Eulerian microparticle tracking is a favorite approach for closed environments as shown by Zhao et al. [68, 69] and Murakami et al. [70]. Friedlander and Johnstone [71] and Davies [72] developed the first deposition model based on it. Dehbi [73] showed that the Eulerian approach is suitable only for flows with dense microparticle suspensions where the particle-particle interaction is too large to ignore. A strong coupling between the phases has to be accurately defined for a proper Eulerian simulation.

2.2.2 Lagrangian microparticle tracking

Unlike Eulerian approach, microparticles are treated as a dispersed phase and individual microparticles are tracked in the Lagrangian approach. Guha [74] deduced that the turbulence and fluctuation of flow have a significant effect on microparticle motion so that the Lagrangian calculation is suitable for this situation. This method is valid for all microparticle sizes as microparticles are treated individually. Moreover, it provides information about microparticle collision on the surface which is helpful incorporation into the sticking model. El-Batsh et al., [75] pioneered the Lagrangian discrete phase method (DPM) model in the CFD software, FLUENT, by developing a deposition model based on Eulerian-Lagrangian approach and successfully demonstrated the model for various experimental cases.

2.2.3 Microparticle behavior in the deposition model

This microparticle deposition model is to simulate the interaction between microparticles and wall. Computational software has built-in boundary conditions of the wall when microparticle strikes the wall. But the interaction between microparticle and wall is not able to simulate the microparticle deposition on the nozzle wall accurately in Fluent. Thus, a user-defined function (UDF) is applied to the wall boundary condition for modelling the particle-wall interaction.

2.2.4 Particle-wall interaction

The rate of microparticle accumulation is calculated by the rate of microparticle deposition minus that of microparticle detachment. The simulation involves microparticle deposition, microparticle detachment, and location of microparticle accumulation on the wall.

2.2.4.1 Microparticle attachment

The van der Waals attraction and electrostatic repulsion are two main forces contributing to the microparticle deposition. The model of Johnson et al. [65] provides the sticking force based on the microparticle size and material properties.

$$F_{\text{stick}} = \frac{3}{4} \pi W_A d_p \quad (2.7)$$

where W_A is the work of sticking, a constant which relies on material properties of the microparticle and surface (unit: J/m²), d_p is the diameter of the particle. During the flow, the normal velocity of the microparticle decreases due to the sticking force. When the microparticle hits the wall, it rebounds and its normal velocity direction reverses. After that, the microparticle can be pushed back to the wall by drag and interparticle collision forces. The process iterates till the particle's normal velocity is too low to bounce away from the wall. Eventually, the microparticle is deposited on the wall. This velocity at which capture of a microparticle occurs is known as the capture/critical velocity. Brach and Dunn [76] formulated an expression to calculate the capture velocity of a microparticle using a semi-empirical model from the experimental data,

$$v_{cr} = \left(\frac{2E_p}{d_p} \right)^{1.43} \quad (2.8)$$

where E_p is Young's modulus of the microparticle. If the normal velocity of the microparticle is less than the capture velocity ($v_n < v_{cr}$), the microparticle will deposit on the surface. Otherwise ($v_n > v_{cr}$), microparticle will rebound. Rebounding microparticle from the surface

continues on its trajectory until it escapes the nozzle or impacts the surface again. The El-Batsh parameter (K_c) is based on the Young's modulus of the microparticle and the surface,

$$K_c = 0.51 \left(\frac{5\pi \left(\frac{1 - v_s^2}{E_s} + \frac{1 - v_p^2}{E_p} \right)}{4\rho_p^{1.5}} \right)^{0.4} \quad (2.9)$$

where v_{cr} is the microparticle capture velocity, E_s is the Young's modulus of the surface material, V_s is the Poisson's ratio of the surface material, E_p is Young's modulus of microparticle material, V_p is the Poisson's ratio of microparticle material, d_p is the microparticle diameter and ρ_p is the microparticle density.

2.2.4.2 Microparticle Detachment

Applying an external force which overcomes the sticking force can cause microparticle to detach from the surface. Microparticles may lift off from the surface, slide over or roll on the surface. Wang [77] have discussed this process. Soltani & Ahmadi [78] determined the detachment of microparticle from the surface. Then, the critical wall shear velocity is defined as:

$$u_{\tau c} = \frac{C_u W_A}{\rho d_p} \left(\frac{W_A}{d_p K_c} \right)^{\frac{1}{3}} \quad (2.10)$$

where $u_{\tau c}$ is the critical wall shear velocity, C_u is the Cunningham correction factor, d_p is the diameter of the microparticle, and K_c is the El-Batsh parameter. The microparticle is removed

from the surface, in case the turbulent flow has a wall friction velocity $u^* = \sqrt{\frac{\mu \left(\frac{du}{dy} \right)_{wall}}{\rho}}$ which

is larger than $u_{\tau c}$. If $u^* < u_{\tau c}$, microparticle will not detach; otherwise, it will detach.

2.3 Microparticle manipulation using surface acoustic wave (SAW)

Noninvasive actuation and rapid movement of the microparticles in the microchannel are useful in the microscale experiment. One of the noticeable methods in the microparticle

actuation and manipulation is to use surface acoustic wave (SAW) which is able to mix, atomize fluid, or manipulate microparticle. For the microparticle manipulation, losing the energy of a high-frequency acoustic wave in liquid via viscous damping of the wave induces acoustic streaming flow, and imparts an acoustic radiation force to the suspended microparticle like polystyrene beads [79]. In Fig 2.6, microparticles are influenced by the travelling acoustic waves-based acoustic streaming flow or acoustic radiation force, depending on $\kappa = \frac{\pi d}{\lambda}$ where d is the microparticle diameter and λ is the wavelength of the SAW. If $\kappa < 1$, the microparticle will not be affected by the acoustic streaming force. However, the microparticles could still be trapped in the vortices of acoustic streaming flow. For microparticles with larger diameters ($\kappa > 1$), acoustic radiation and scattering dominate the acoustic streaming. Therefore, the microparticles are pushed in the same direction as the acoustic wave propagation. Microparticle properties (e.g. diameter, shape, and density) and the sound speed influence the acoustic radiation forces, whereas the acoustic streaming is mainly influenced by the viscosity and density of the fluid.

Figure 2.5. (a) Acoustic streaming flow at $\kappa < 1$ and (b) acoustic radiation and scattering at $\kappa > 1$ [79].

2.3.1 Standing surface acoustic wave (SSAW) in microfluidics channel

An interference between two travelling surface acoustic waves (SAWs) from two IDTs leads to a formation of SSAW on a substrate surface and the presence of pressure nodes and anti-nodes. The microparticles/cells are subsequently pushed toward the pressure node of

SSAW [80]. In contrast, Yeo and Friend [30] argued that microparticles are actually aligned and aggregated in the pressure nodes of bulk acoustic wave (BAW). SAW radiates into the fluid domain resulting in the formation of BAW and reflected wave from both sides of the channel wall. Subsequently, standing waves are established in the microchannel. To use SSAW in microfluidic device, it could be applied in various configurations including SSAW single pressure node, multiple pressure nodes, tilted-angle, single IDT SSAW, two-dimensional SSAWs and SSAW generated by a single IDT.

2.3.2 Single pressure node SSAW

A single pressure node SSAW can be created by setting the width of the microchannel to be half of the wavelength of BAW in water, and a pair of IDTs aligned parallel to the microchannel with one on each side. In this setup, a single pressure node is located at the center of the microchannel along its longitudinal axis. Chen et al. [28] proposed the SSAW device for flow cytometry to detect and count microparticles or cells by focusing microparticles into a single stream along the center of the microchannel and by integrating a laser-induced fluorescence detection system. Huang et al. [29] proposed the microparticle separation using a single pressure node SSAW across three laminar liquid streams, the center of the microchannel being filled with deionized water or sheath fluid, and two side streams of mixture solution flowing along the sides. Microparticles with the same density and compressibility but different in size are influenced by the acoustic radiation force with different magnitudes [29]. Larger microparticles experience greater acoustic radiation forces and displacement than smaller ones towards the pressure node.

2.3.3 Multiple pressure nodes SSAW

Multiple pressures nodes SSAW may be created across the width of a microchannel. For example, two pressure nodes SSAW are located near each side of the wall to push

microparticles away from the center of the microchannel. Different types of cells could be sequentially patterned at different locations in the channel by adjusting the phase difference between signals applied to the IDTs and subsequently, shifting the SSAW pressure nodes. Ai et al. [80] isolated *E.coli* bacteria, 0.25 and 2 μm in radius and length, respectively, successfully from the peripheral blood mononuclear 7- μm cells. Chen et al. [81] used this setup and a phase-shift approach to align the cells along the pressure nodes parallel to the length of the microchannel by SSAW. The cells were settled down and adhered to the substrate surface after the termination of SSAW [81]. This method demonstrated a capability of achieving a 100-fold concentration of highly diluted blood cells, with a recovery efficiency of up to 99% [28], which is more biocompatible than centrifugation [28].

2.3.4 Tilted SSAW

By using single pressure node SSAW, the microparticle separation distance is determined by a quarter of the wavelength [79]. It is possible to increase the separation distance by positioning the microchannel at an angle to the IDTs [82]. A 10-fold increase in the separation distance was achieved, and separation efficiency was ensured by the presence of several pressure nodes within the channel against microparticles escaping in the tilting range of 10°-15°. The tilted SSAW achieved 20 times higher separation of circulating tumor cells in a blood sample from a breast cancer patient with 80% of recovery rate at the low concentrations than the conventional SSAW devices [83].

2.3.5 Two-dimensional SSAW

Two-dimensional SSAW were created by superimposition of two orthogonal SSAWs at the same frequency on a piezoelectric substrate. 1D SSAW was used to focus or separate micro-objects whereas two-dimensional SSAW could pattern the micro-objects or even single microparticles in the two-dimensional space [79]. Furthermore, with this method, a distance between microparticles or clusters of microparticles could be manipulated by applying pulse

signal on the orthogonal pair of IDTs. It could be done by firstly arranging microparticles in lines with one pair of IDTs, thus; standing waves are established along one axis only. Then, a modulated RF signal could be applied in the orthogonal direction by activating the orthogonal pair of IDTs to push particles toward one another. The modulated signal was set to a pulse signal with 0.5-s duration and 2-s interval which microparticles are gradually moved toward the pressure node. Once the microparticles reach required distance between other microparticles, the pulse signal is stopped [84].

2.3.6 SSAW from a single IDT

Witte et al. reported the use of a single IDT to generate SSAW in the microchannel with superstrate. Unlike the SSAW conventional method (Fig. 2.7a), the SAW travelled along the LiNbO₃ substrate and propagated into the coupling layer. The superstrate vibrates in Lamb wave mode. (Fig. 2.7b). Leaked BAW waves radiated into a liquid in SU-8 microchannel. Reflected wave within the microchannel then superposed with the incident wave and eventually created a standing wave [85].

Figure 2.6. Schematic diagram of SSAW consisting of (a) PDMS-LiNbO₃ and (b) superstrate-LiNbO₃ [85].

2.4 Microparticle/cell manipulation using structural acoustic vibration in cylindrical tube

2.4.1 Concentration of microparticles by acoustic wave

About 30 years ago, acoustic waves were used for accumulating the suspended microparticles at the acoustic pressure nodal or antinodal planes in fluid [86]. Later on, it was

applied for various applications including concentrating [87], fractionating [88], positioning [89, 90], orientation or alignment of microparticles [91, 92]. The advantage of acoustic separation over conventional separation method is to sort microparticles by their different physical properties (e.g. compressibility and density). This is useful for polymer waste recycle because different types of polymers have similar size and shape but different densities and compressibilities. Therefore, the acoustic waves could be applied for polymer separation effectively. This separation is achievable by a transducer, a reflector and a flow splitter. The transducer and the reflector were placed in parallel. Standing waves were formed between the transducer and the reflector. Hence, the microparticles with positive acoustic contrast moved toward the pressure node while those with negative acoustic contrast moved to the antinode. The low- and high-density polyethylene microparticles of overlapping size distributions could be sorted using a periodically swept frequency of driving signal [93]. Several years later, the effect of travelling waves on the accumulating microparticles in the cylindrical cavity was investigated. The travelling waves could be generated by two transducers attached at both ends of the cylindrical cavity. The two travelling waves propagating in different directions form the standing wave in the cylindrical cavity. Acoustic radiation force is generated from a combination of Rayleigh streaming and inhomogeneity of the beam field in the cylinder [94]. Another method to enhance the tunability of microparticle accumulation is to apply the sinusoidal excitation signals at the same frequency to a pair of IDTs but in different phases. As a result, the location of standing wave nodal position could be adjusted. Subsequently, the microparticles were pushed to the controlled nodal position [90, 95]. Lastly, focused standing wave is an alternative method for acoustic manipulation. The experimental setup consisted of a transducer, a reflector and a capillary tube filled with microparticle mixture. Unlike the other methods mentioned earlier, the fluid flowed in the same direction as the wave propagation. Since the capillary tube was placed in between the transducer and the reflector, multiple

pressure nodes appeared in the capillary tube. Using this method, microparticles in the required size were captured at the pressure nodes allowing Ar-ion laser for sensing, which could enhance the sensitivity of the optical sensor [96, 97].

One of the most effective methods was studied by Goddard et al. [32, 33]. Acoustic manipulation is realized by exciting the transducer(s) attached along the longitudinal axis of the cylindrical tube for the structural vibration of the whole part. The first order structural mode was excited to align the microparticles at the center of the cylindrical tube filled with liquid. The structural vibration could also be tuned with the excitation frequency, material properties, and aspect ratio and cause the localized vibration of the outer surface of the cylindrical tube which could then be transferred throughout the whole cylindrical structure. At a specific excitation frequency and structural vibration mode, microparticles were pushed toward the center of the cylinder. Thus, the use and accurate alignment of an additional reflector or second transducer is not required. In comparison to the other methods, energy density at the transducer excitation region is lower because the energy was converted to vibration and transferred to the entire structure resulting in the reduced cavitation, convection, and thermal gradient. Goddard et al. [98] incorporated this setup with flow cytometry to enhance its performance effectively instead of using hydrodynamics force from the sheath fluid in a conventional flow cytometer. In order to optimize the accumulation efficiency, the piezoelectric material behaviour, structural-acoustic vibration, acoustic propagation, and trajectory of the microparticles in the fluid were included in the numerical model of the system, and experiment validation were implemented.

2.4.2 Numerical simulation of microparticle accumulation by structural-acoustic vibration in the cylindrical cavity

Finite element model (FEM) divides the systems into interconnected elements. The system consists of multiple elements and each element is influenced by the adjacent elements.

In this case, piezoelectric material is compressed from the electrical stimulation. The mechanical stress acting on the material causes the element in the region to deform. The deformed elements influence the adjacent elements to deform. The finite element model simulates this behaviour by solving PDEs for each element. FEM is capable of simulating complicated meshes (e.g. irregular and unstructured meshes) and material properties (e.g. inhomogeneous and anisotropic materials). The set of points filling up the interested domain are required. The points are connected into sub-elements by an mesh generation algorithm. The quadrilateral is the most fundamental structured mesh whereas unstructured mesh does not have any predefined element size or shape. In two dimensions, the unstructured mesh can include triangles or other shapes as well as the quadrilaterals used by structured grids. To model the cylindrical tube system, structural network analysis was performed [99, 100]. The elastodynamic field is decomposed into the azimuthal harmonics, and the shell is simplified as a set of first-order PDEs. The elastodynamic state in the cylindrical shell and piezoelectric behavior in the transducer are identified.

2.4.3 Governing equations

The linear behaviour of the piezoelectric material is presented in the stress-charge and strain-charge forms

$$\sigma_{dir} = c_{EM}S - e_ME, \quad D = e_M\varepsilon_{dir} + \varepsilon_ME \quad (2.11)$$

$$\varepsilon_{dir} = s_{EM}\sigma_{st} - dE, \quad D = d\sigma_{dir} + \varepsilon_ME \quad (2.12)$$

where σ_{dir} is direct stress, ε_{dir} is direct strain, E is the electric field, D is the electric displacement, c_{EM} is the elasticity matrix, e_M is the coupling matrix, ε_M is the permittivity matrix. Then the propagation of acoustic wave in the liquid is expressed using Helmholtz equation,

$$\nabla \cdot \left(-\frac{1}{\rho} \nabla p_{ac} \right) - \frac{\omega^2 p_{ac}}{\rho c^2} = 0 \quad (2.13)$$

where the acoustic pressure (p_{ac}) is a harmonic quantity ($p = p_0 e^{i\omega t}$), ρ_c is the density, ω is the angular frequency, and c_f is the speed of sound in the fluid.

FEM was used to find the approximate solution of partial differential equations (PDEs). The main components consist of piezoelectric material and cylindrical glass tube filled with fluid. The electrical signal is applied to the piezoceramic plate attached to the glass tube. One side of the piezoceramic was defined as the free boundary while the other side was attached to the glass tube. The boundary of the glass tube was considered as hard wall and assumed to be reflective

$$\vec{n} \cdot \left(-\frac{1}{\rho} \nabla \vec{p} + \vec{q} \right) = 0 \quad (2.14)$$

where $\vec{n}$ is the normal vector pointing inward the center of the tube. Hence the acoustic standing waves could be formed in the fluid surrounded by the hard wall.

Because of the different travelling velocities of microparticle and fluid, the Stokes drag force from the fluid acted on the microparticles is commonly described as [101]

$$F_{Drag} = 6\pi\mu r (v_f - v_p) \quad (2.15)$$

where r , v_f , and v_p refer to the radius of the microparticle, the velocity of the fluid, and the microparticle, respectively.

In the acoustic field, monopole and dipole scattering from oscillation and pulsation of the microparticle result in the acoustic radiation force that is described using the Gauss's theorem [102].

$$F_{rad} = \frac{4}{3}\pi r^3 \nabla \left[f_{mono} \frac{1}{2} k_0 p_{prop}^2 - f_{dip} \frac{3}{4} \rho_0 v_{prop}^2 \right] \quad (2.16)$$

$$f_{mono} = 1 - \frac{k_p}{k_f}, \quad f_{dip} = \frac{\rho_p - \rho_f}{\rho_p + \rho_f/2} \quad (2.17)$$

where ρ_p and ρ_f are the density of microparticle and fluid, k_p and k_f are the compressibility of microparticle and fluid, f_{mono} and f_{dip} are the dimensionless scattering coefficients for monopole and dipole, respectively, and k_0 is the acoustic wave number. In the viscous fluid,

Prandtl–Schlichting and acoustic boundary layer could be taken into account by adding the viscosity-dependent correction into the dipole scattering coefficient [103].

2.5 Acoustic manipulation in the nozzle-based 3D printer

There have been few attempts to incorporate acoustic manipulation in the nozzle of a 3D printer. Acoustic waves were used for patterning the microparticles in the rectangular nozzle of the extrusion printer. A pair of piezoelectric transducers were attached to the side of the rectangular cavity. The width of the rectangular cavity was about half-wavelength of acoustic waves. The pressure node was located at the center of the rectangular cavity. Printed ink consists of 30- μm glass microparticles suspended in a mixture of epoxy, fumed silica and acetone. During printing, the microparticles were moved to the pressure node by an acoustic radiation force. The alignment of microparticles in the printed parts was similar to that in the rectangular cavity [104]. However, the rectangular nozzle is uncommon and needs complicated manufacturing at a high cost, which may limit the practice of such technology. The acoustic focusing of microparticles at the center of the cylindrical tube might be possible using the method proposed by Goddard et al. in the Section 2.4.1. Till now, such approach was not applied to the printing structure. In addition, high structural vibration modes and the effects of experiment parameters (e.g., the concentration of microparticles and hydrogel) for printing (e.g., accumulation time and width) have not been investigated to completely evaluate the potential of this approach.

Chapter 3 Particle accumulation and reduction by Standing Surface Acoustic Wave (SSAW)

Accumulation of microparticles on the wall of the microchannel is a common phenomenon in a colloidal fluid. A gradual accumulation of microparticles could eventually obstruct the fluid flow and lead to clogging, which seriously affects the accuracy and reliability of nozzle-based printing and causes damage to the nozzle. In the line of the issue mentioned above, this chapter describes the use of acoustic wave to reduce the accumulation of microparticles and delay clogging in the microchannel. In the early section of this chapter, background and motivation of this research are explained. Besides, the accumulation of microparticles under an excitation of SSAW was simulated around the constriction area of the microchannel. In the later section, a study of microparticles accumulation and clogging in the microchannel is presented. The main parameters tested in this experiment were fluid flow rate, microparticle concentration, and alginate concentration. Finally, reduction of microparticle accumulation by SSAW and hydrodynamic parameters were studied further. The reduction of microparticles accumulation under the excitation of SSAW was investigated.

3.1 Introduction

Inkjet printing has been used widely in recreating a digital image by propelling droplets onto paper, plastic, or other substrates using either continuous or drop-on-demand technology since the late 1970s [105]. Its advantages include low cost and noise, but high resolution. Meanwhile, this versatile computer-aided tool can also be applied in many manufacturing fields with high-throughput, such as the fabrication of functional and structural materials [106], all-polymer transistor circuits [107], organ/tissue printing [12], and recombinant proteins microarrays [108]. However, the accumulation of microparticles usually occurs during nozzle-based printing, especially in small nozzles for extrusion of fine drops. This phenomenon is a

progressive process and may cause an obstruction of the upstream fluid flow, either temporarily or permanently, and finally lead to the clogging. The clogging problem would result in non-uniformity of the printed part, loss of material, long printing time, and excessive time devoted to printing quality, and it is also difficult to predict. For dense micro-particle mixtures, the microparticle accumulation and the corresponding printability time before the occurrence of clogging are highly dependent on the microchannel geometry and hydrodynamic parameters, such as the fluid viscosity, concentration of microparticles, and flow rate [59, 81]. However, it is difficult to build a stable three-dimensional scaffold freeform structure from the bio-ink with such low viscosity and mechanical strength. Thus, there is a great need to reduce the clogging problem in the nozzle-based printing system and increase its printability.

Until now there have been few methods to effectively reduce clogging during the nozzle-based printing. To reduce the interaction force between the liquid and solid layer and the surface tension of printing material, a surfactant is usually added. However, surfactants could change the properties of the cell membrane and decrease cell proliferation. The printed Hep G2 hepatocytes onto hydrogels with the addition of 0.05% pluronic (a biocompatible and Food and Drug Administration approved surfactant) decreased the cell viability from > 95% after two days to 50% over 13 days [13]. Electromagnetic force generated by either injecting a DC current or electromagnetic induction (i.e., 1000 A at 5000 Hz) can modify the turbulent flow in the nozzle entry region and reduce the recirculation zone in a cylindrical tundish nozzle and, subsequently, the potential of trapping oxide microparticles for clogging [109].

Another solution is an acoustic approach, such as using bulk acoustic waves (BAW) and surface acoustic waves (SAW) or travelling SAW (TSAW) which have been applied for microparticle/cell sorting, separation [110, 111], and encapsulation [112, 113] in the microfluidic microchannel. A typical BAW-based microfluidic microchannel is made of silicon and glass, which are challenging to implement with the fast-prototyping method.

Standing waves that are obtained from the leakage of surface acoustic waves into the microchannel from a pair of SAWs propagating in the opposite directions has promising results in cell/particle manipulation. Thus, it is reasonably hypothesized that the acoustic radiation or acoustophoretic force applied to the microparticles and the subsequent motion may decrease the deposition of microparticles on the microchannel wall or even break the bonding between the already-deposited small and isolated microparticles and the microchannel wall. Standard micro-electro-mechanical systems (MEMS) and soft-lithography procedures permit easy fabrication, miniaturization, and integration of SSAW, making it highly cost-effective for mass production. In addition, characteristics of SSAW-induced microparticle manipulation can be adjusted by tuning the applied power, wavelength, flow rate, and microchannel geometry.

In this study, the accumulation behavior of microparticles in water and hydrogel solution in a PDMS microchannel with varied constriction angles was observed under a light microscopy in order to understand the mechanism of clogging. The effect of the acoustic radiation force on the microparticle in the microchannel with different hydrodynamic parameters (i.e., fluid viscosity and microchannel geometry) was numerically simulated. Then, a pair of IDTs were fabricated on the piezoelectric substrate which is LiNbO_3 to generate SSAW in the PDMS microchannel. The excitation of SSAW was found to reduce the area of microparticle accumulation and postpone the onset of clogging. The performance of SSAW was further evaluated at varied alginate concentrations (fluid viscosity) and constriction angles.

3.2 Simulation of microparticle accumulation by SSAW in microchannel

Microparticle manipulation device via surface acoustic wave (SAW) consists of PDMS microchannel filled with a fluid and the interdigital transducers (IDTs) on a piezoelectric material substrate (LiNbO_3). Each IDT is capable of generating travelling surface acoustic

waves (TSAWs). Therefore, standing surface acoustic wave (SSAW) is formed from a superposition of TSAWs. Numerical simulation was performed in COMSOL 5.0. Two simulation domains were PDMS and water whose material properties are listed in Table 3.1. The fluid flow was assumed to be fully developed laminar flow (with $Re < 1$, as fluid velocity ranges from 2.0-100.0 $\mu\text{m/s}$, a characteristic length (rectangular duct) is $\frac{2 \times \text{Width} \times \text{Height}}{\text{Width} + \text{Height}} = 0.67 \times 10^{-4}$). Effect of particle is neglected in the calculation of Reynold number as the volume fraction is lower than 1%. The fluid field influenced microparticle motion through drag force. The acoustics field affected microparticle through the acoustophoretic force. The effect of acoustophoretic force on the microparticle in this simulation was validated by Muller et al. [114]. In our model, the effect of acoustic radiation and viscous drag forces were taken into consideration.

Table 3.1. Material parameters at Temperature at 27 °C

Water	density, ρ_w	997 kg/m
	speed of sound, c_w	1497 m/s
	viscosity, μ_w	0.890 mPa.s
	compressibility, κ_w	448 TPa^{-1}
microparticle	density, ρ_p	1050 kg/m
	speed of sound, c_p	2350 m/s
	Poisson's ratio, ε_p	0.35
	compressibility, κ_p	249 TPa^{-1}
poly-dimethylsiloxane (PDMS, 10:1)	density, ρ_{PDMS}	920 kg/m
	speed of sound, c_{PDMS}	1076.5 m/s
lithium niobate (LiNbO ₃)	speed of sound, c_{LNB}	3990 m/s
	wavelength, λ	200 μm
	frequency, f	19.95 MHz

3.2.1 Governing Equation

3.2.1.1 Governing equation for Acoustic field and acoustophoresis

The acoustophoresis is a method to manipulate microparticles in the acoustic field. Due to the difference between acoustic properties of microparticle and medium, the acoustic waves scatter and push microparticles toward the pressure node [115, 116]. Total acoustic waves near the surface of microparticle represent a summation of scattering waves and propagating waves. Firstly, wave equation with total potential velocity (ϕ_{total}) is described as

$$\nabla^2 \phi_{total} = \frac{1}{c_0^2} \partial_t^2 \phi_{total} \quad (3.1)$$

And the total acoustic waves around the surface of the microparticles are a sum of propagating and scattering waves in terms of propagating potential (ϕ_{prop}) and scattering potential (ϕ_{scat})

$$\phi_{total} = \phi_{prop} + \phi_{scat} \quad (3.2)$$

Monopole and dipole components play an important role on far-field boundary which f_{mono} and f_{dip} are the dimensionless scattering coefficients for monopole and dipole, respectively. Together with Gauss's theorem, the resulting radiation force can be obtained [102]

The less effect from SAW on the microparticles in highly viscose fluid is expected which might be explained by putting an additional term on dipole scattering coefficient, viscosity-dependent correction [103, 117]. With a consideration of Prandtl–Schlichting boundary layer theory and acoustic boundary layer, the effect of viscosity does not affect body mass (monopole) scattering, but it has an effect on the dipole scattering. Thus, the correction term is added in the dipole coefficient (Eq. 2.16) as following,

$$f_{dip} = \frac{2(1 - \gamma)(\rho_p - \rho_0)}{2\rho_p + \rho_0(1 - 3\gamma)} \quad ; \quad \gamma = -\frac{3}{2} \left[1 + i \left(1 + \frac{\delta}{r} \right) \right] \left(\frac{\delta}{r} \right) \quad (3.3)$$

where δ is a thickness of boundary layer, i indicates the complex number $\sqrt{-1}$

3.2.1.2 Governing equation for Laminar flow and drag force

With the low Reynold number and low Mach number, the fluid motion model is classified to be incompressible laminar flow, described by the following equation,

$$\rho(\vec{u} \cdot \nabla)\vec{u} = \nabla \cdot [-p_f I + \mu(\nabla\vec{u} + (\nabla\vec{u})^T)] + \vec{F} ; \rho \nabla \cdot (\vec{u}) = 0 \quad (3.4)$$

$\vec{u}$ is the fluid velocity in vector form, μ is dynamic viscosity, ρ is fluid density, p_f is pressure on the fluid body, I is an identity matrix, $\vec{F}$ is an external force [23, 118]. Drag force is an effect of fluid on object which relies on the difference of velocity between fluid and microparticle. For a sphere in the radius of r in a low Reynold's number flow, the Stokes drag is showed in Eq. 2.15 [101, 119].

3.2.1.3 Governing equation for microparticle motion and interaction

van der Waals attraction potential which includes microparticle-particle and microparticle-wall interaction force is expressed

$$\phi_{p-w} + \phi_{p-p} = - \left(\frac{H}{6d} + \frac{rH}{12d + \left(1 + \frac{11.12d}{L} \right)} \right) \quad (3.5)$$

where ϕ_{p-w} is the attraction potential between microparticle and wall, ϕ_{p-p} is the attraction potential between microparticle and microparticle, H is the Hamaker constant, d is the distance between microparticle-particle or microparticle-wall.

3.2.2 Numerical Simulation Results

Two domains are PDMS (solid phase) and water (liquid phase). The triangular mesh with 6971 elements were generated. For boundary condition, the inlet and outlet were set on the right and left edges, respectively. The edges in contacted with PDMS were set as walls for fluid domain with dimension indicated in Fig 3.1a. The particle motions were solved in transient as particles were effected by drag forces and acoustic radiation force. Microparticle streamlines were usually converged at the constriction of the microchannel. The microparticle streamlines flowed from the inlet to the outlet is shown in Fig. 3.1a. The maximum vertical (Y-axis) velocity increased with the angle of constriction. In addition, it was also sensitive to the diameter ratio of the inlet to the outlet, increasing from 6.75 $\mu\text{m/s}$ at 2:1 to 131.7 $\mu\text{m/s}$ at 10:1 at a constriction angle of 90° (see Fig. 3.1b).

Figure 3.1. (a) Domain and mesh generated on the geometry, (b) Y-velocity of microparticle under laminar flow in the microchannel at the constriction angle of 90° , inlet of 100 μm , and outlet of 50 μm ; and (c) maximum velocity under different constriction angles from 3.6° to 90.0° and diameter ratios of the inlet to the outlet at the inlet of 100 μm .

Microparticle trajectory in the field of SSAW was simulated with a consideration of drag and acoustophoretic forces. The motion of microparticles towards the center of the microchannel was dependent on the fluid properties (i.e., dynamic viscosity and density), microparticle properties (i.e., radius and shape), and microparticle location. Both microchannel walls were considered as reflected boundaries where planar acoustic waves were reflected. Meanwhile, the inlet and outlet were considered as an open boundary for acoustics. At a vibration amplitude of 0.94 nm, the maximum magnitude of standing wave in the microchannel was 0.18 MPa, and the pressure node was located at the center of the microchannel. The motion of 30 microparticles, which were initially distributed uniformly at the inlet, was simulated by a time-transient analysis. The average and standard deviation of Y-velocities of these microparticles at 1.2 second of SSAW activation with four various fluid viscosities which were 8.9×10^{-4} Pa·s (water viscosity, 1×), 8.9×10^{-3} Pa·s (10×), 4.45×10^{-2} Pa·s (50×) to 8.9×10^{-2} Pa·s (100×) are shown in Fig. 3.2. Excitation of the SSAW increased the average Y-velocity of microparticles in water from 0.8 $\mu\text{m/s}$ to 86.6 $\mu\text{m/s}$ at $t = 100$ ms, which confirmed our hypothesis that the acoustophoretic force could effectively push the microparticles away from the microchannel wall. However, the average Y-velocity at $t = 100$ ms decreased with the fluid viscosity of the solution to 12.3 $\mu\text{m/s}$ (10×), 3.1 $\mu\text{m/s}$ (50×), and 1.9 $\mu\text{m/s}$ (100×). The microparticle motion in a highly-viscous medium (e.g., 100× in Fig. 3.2e), even with the SSAW, was similar to the free motion in water.

The reason of large error bars of average Y-velocity is because this parameter is calculated from Y-velocity of all particles within the microchannel domain, and so the particles were initially located at the different Y-location in the fluid domain. Particle moves closed to the constriction region the particles were squeezed through this such a narrow constriction, the each particle has different value of Y-velocity. The particle which is away

from the constriction (Y-location) has high Y-velocity while the particle closed to the constriction has low Y-velocity (indicated by the color bar in Fig.3.2, left column).

(a)

(b)

(c)

Figure 3.2. The motion of 30 microparticles at $t = 1.2$ s (left column) in top view and the Y-velocity of microparticles presented as mean $\pm$ standard deviation (STD) in $\mu\text{m/s}$ (right column) in a $100\ \mu\text{m}$ microchannel at the constriction angle of 15° with the fluid viscosity of (a) 8.9×10^{-4} Pa·s (1 $\times$) without acoustic excitation; and (b) 8.9×10^{-4} Pa·s (1 $\times$); (c) 8.9×10^{-3} Pa·s (10 $\times$); (d) 4.45×10^{-2} Pa·s (50 $\times$); and (e) 8.9×10^{-2} Pa·s (100 $\times$) with the excitation of the standing surface acoustic wave at the vibration amplitude of 0.94 nm.

The effects of fluid viscosity, the vibration amplitude of the SSAW, microparticle size, and distance to the microchannel center on the maximum Y-velocity were further investigated (see Figure 3.3). It was found that the microparticle's Y-velocity increased almost linearly with the vibration amplitude or acoustic pressure but decreased with the fluid viscosity. In water, the microparticle Y-velocity was increased by 3.2-fold from 51.1 to 164 $\mu\text{m/s}$ with an increment of vibration amplitude by two-fold (from 0.48 to 0.96 nm). With the increment of fluid viscosity by two-fold (from 0.89 mPa·s to 1.78 mPa·s), the microparticle velocity was reduced from 164 to 91 $\mu\text{m/s}$. Therefore, high acoustic power was required to push

microparticles in the highly-viscous medium. In addition, the Y-velocity increased almost linearly with the distance away from the microchannel center and then became saturated with small and slow oscillations at the vibration amplitude of about 10 μm . The maximum Y-velocity of the 1- μm microparticle was $9 \times 10^{-3} \mu\text{m/s}$ and 6.7 $\mu\text{m/s}$ at 0.01 μm and 10 μm away from the center, respectively. Large microparticle resulted in high velocity towards the pressure node at the microchannel center at Y-velocity of 10 $\mu\text{m/s}$ and 163 $\mu\text{m/s}$ for 1- μm and 4- μm microparticle at 20 μm away from the center, respectively. There is no error bar here as it is numerically calculated from a single particle.

Figure 3.3. Y-velocity of microparticle after 10 ms of the SSAW activation under (a) different vibration amplitudes and fluid viscosities of 2 μm microparticles 20 μm away from the microchannel center, and (b) different distances from the center for different microparticles at the vibration amplitude of 0.94 nm and a viscosity of 0.89 mPa·s.

3.3 Experiment of microparticle accumulation and its reduction by SSAW

3.3.1 Experiment setup

The experimental setup is shown in Fig. 3.4. Microchannels were fabricated using soft-lithography techniques. PDMS (Sylgard 184, Dow Corning, Midland, MI, USA) was mixed with an elastomer base in a ratio of 10:1. The mixture was degassed in a vacuum oven (3608-1CE, Thermo Scientific, Waltham, MA, USA) and poured on a silicon wafer (SI8PSPD, Bonda Technology, Singapore) with a negative tone photoresist (SU-8, Microchem, Westborough,

MA, USA) pattern on the top. Then the patterned silicon wafer was degassed again and heated at 70°C for 3 h in an incubator (BD 56, Binder, Bohemia, NY, USA) for PDMS solidification. The length, width, and height of the microchannel were 1 cm, 50–100 μm , and 30 μm , respectively.

To apply SSAW to the system, a pair of IDTs were fabricated. Twenty nanometers of Cr and 400 nm of Al were deposited on a substrate of a four-inch double-side-polished LiNbO₃ wafer (Y-128° propagating, University Wafer, Boston, MA, USA). Twenty strips with a width of 50 μm and 2 cm aperture were patterned on the plastic mask (Infinite Graphics, Singapore) for photolithography by coating the positive photoresist (AZ 9260, Microchemicals, Ulm, Germany) on the LiNbO₃ wafer. Eventually, the Cr-Al layer on the non-exposed area was removed by acetone. Oxygen plasma (Harrick Plasma, Ithaca, NY, USA) was used to treat the surface of PDMS and LiNbO₃. PDMS was aligned on the LiNbO₃ and heated at 80 °C in the vacuum chamber. The IDTs were driven by sinusoidal waves at their resonant frequency of 19.95 MHz from a function generator (AFG3000, Tektronix, Beaverton, OR, USA) and then amplified by a power amplifier (0.3–1.0 W, 25A250A, Amplifier Research, Souderton, PA, USA). In order to maximize the power conversion, the impedance of the IDTs was tuned to about 50 Ω using an impedance matching unit built in the lab.

The polyethylene tubing with an inner diameter of 1 mm was inserted into the microchannels to supply the circulation. Since the cross-sectional area of tubing was significantly larger than that of the microchannel, the hydrodynamic resistance could be neglected. The microparticles (SiO₂ MS-7.75, 8–10 μm , Cospheric, Santa Barbara, CA, USA) were mixed with deionized (DI) water. In order to increase the solution viscosity, sodium alginate powder (180947, Sigma-Aldrich, Singapore) was diluted in DI water by heating to 80°C and stirred. As sodium alginate is a common hydrogel [120], its concentration of 1%–5% was used and its viscosity is in the range of 2.54–41.7 cPs under atmospheric pressure (around

3–50 times that of the viscosity of water). Before each experiment, the solution was spun by vortex (Barnstead Thermolyne Vortex, Dubuque, IA, USA) for 5 min and then put in an ultrasound sonicator (8892, Cole-Parmer, Vernon Hills, IL, USA) for 15 min to disrupt any agglomeration and achieve a uniform distribution of microparticles. Then the mixture and a small magnetic bar (Z329207, Sigma-Aldrich) were filled into a 3 mL syringe driven by a syringe pump (NE-1000, New Era Pump Systems, Farmingdale, NY, USA) at a flow rate of 4 $\mu\text{L}/\text{min}$. The dynamic behavior of microparticles in the microchannel was observed under a light microscope (CKX-41, Olympus, Tokyo, Japan) using 40 $\times$ magnification and captured by a digital camera (DP70, Olympus), from which the images were quantitatively analyzed using ImageJ software (National Institute of Health, Bethesda, MD, USA). Accumulation area was used to quantify the behavior of microparticle accumulation up to 30 min or until complete obstruction.

Figure 3.4. Schematic diagram of experimental setup.

Accumulation area, rate, and agglomeration size were three main parameters used to quantitatively describe the behavior of microparticle accumulation in the clogging. They were processed by Matlab (Mathworks, Natick, MA) and ImageJ. The accumulation area was defined as the total area of accumulated microparticles. The accumulation rate was defined as

the percentage of the area of accumulated microparticles over the total area of microchannel over time. The agglomeration size represented the area of each group of accumulated microparticles on the microchannel wall. A large agglomeration area implied that microparticles tend to aggregate with others nearby, which mostly occurred in Zone A. Therefore, a chance of blocking the arch or the bulk at the entrance was higher.

Figure 3.5. (a) The micrograph of microchannel structure: Zone A is the inlet reservoir, Zone B and C are the two consideration areas and (b) microparticles accumulation on the microchannel wall.

For later part of the experiment, the constriction angle is varied, and the constriction is narrower. The solution was flowed from 100-μm width region to 50-μm width region (see Fig. 3.6b) of the microchannel which has constriction angle of 15°, 30° and 45°.

In order to reduce clogging in the microchannel, a pair of IDTs were fabricated to generate the SSAW (see Fig. 3.6a). Twenty nanometers of Cr and 400 nm of Al were deposited on a substrate of a four-inch double-sided-polished LiNbO₃ wafer (Y-128° propagating, University Wafer, Boston, MA, USA). Twenty strips with a width of 50 μm and 2 cm aperture were patterned on the plastic mask (Infinite Graphics, Singapore) for photolithography by coating the positive photoresist (AZ 9260, Microchemicals, Ulm, Germany) on the LiNbO₃ wafer. Eventually, the Cr-Al layer on the non-exposed area was removed by acetone. Oxygen plasma (Harrick Plasma, Ithaca, NY, USA) was used to treat the surface of PDMS and LiNbO₃. PDMS was aligned on the LiNbO₃ and heated at 80. °C in the vacuum chamber. The IDTs were driven by sinusoidal waves at their resonant frequency of 19.95 MHz from a function generator

(AFG3000, Tektronix, Beaverton, OR, USA) and then amplified by a power amplifier (0.3–1.0W, 25A250A, Amplifier Research, Souderton, PA, USA). In order to maximize the power conversion, the impedance of the IDTs was tuned to about $50\ \Omega$ using an impedance matching unit built in the lab.

Figure 3.6. Schematic of (a) microchannel constriction geometry, and (b) microchannel with inter digital transducers (IDTs).

3.2.2 Effect of flow rate and concentration of microparticles

Microchannel dimension used in this experiment has a cross-section of $100 \times 50\ \mu\text{m}^2$ with 90° constriction angle. For experiment parameters, two flow rates were used which were 2 and $10\ \mu\text{l}/\text{min}$. As a result, at the flow rate of $2\ \mu\text{l}/\text{min}$, the accumulation rates were 1.5, 2.1, 2.8, and $3.1\ \%/ \text{min}$ for 0.2%, 0.6%, 1.0%, and 1.4% concentrations of microparticles, respectively (see Fig. 3.7). The accumulation rates at the flow rate of $10\ \mu\text{l}/\text{min}$ were 0.05, 0.29, 0.60, and $1.03\ \%/ \text{min}$, for 0.2%, 0.6%, 1.0%, and 1.4% concentrations of microparticles, respectively. At both flow rates, the relationship between the accumulation rate and microparticle concentration could be fit quite well in a linear model. Because the accumulation occurs randomly along the microchannel wall, the standard deviation was large at low flow rate (i.e., $2\ \mu\text{l}/\text{min}$). In comparison, the microparticles are hard to accumulate at the wall at high flow rate (i.e., $10\ \mu\text{l}/\text{min}$). Sometimes the accumulated microparticles were stripped from the wall by the inside flow according to the microscopic observation. However, whether the stripped parts had high propensity of another accumulation in the downstream was unknown

due to the limited viewing range. The experimental observation may also illustrate the mechanism of plaque formation in the vascular wall and blood circulation obstruction in the lower extremity for chronic arterial insufficiency or in the middle cerebral artery (MCA) for stroke.

Figure 3.7. The effect of microparticle concentration on the accumulation rate at flow rate of 2 µl/min and 10 µl/min, respectively (n = 20).

3.2.3 Concentration of sodium alginate in the solution

In contrast, there was no monotonic relationship between microparticle accumulation and the concentration of sodium alginate. At micro-particles concentration of 1% and flow rate of 2 µl/min, the sodium alginate concentration was varied from 1 to 10% which covers the range of alginate concentration used in bioprinting applications [121-125]. Below the alginate concentration of 4%, the accumulation rate increased rapidly from 2.09 to 5.90 %/min at 0% and 3% alginate concentration (see Fig. 3.8). This may be because particles and alginate at low concentration could form a small aggregates which make it stability deposits on the wall. On the other hand, the accumulation rate dropped significantly at above 4% alginate concentration in Zone B and C because the micro-particles and alginate agglomerated to form bridge structure in Zone A. At the high alginate concentration, a large lump of alginate microparticles was

formed because the alginate has a strong intermolecular force for adhesion [132]. A 5% alginate solution tended to form aggregates as large as 30 μm . Such a large aggregates may be detached from the wall with a fluctuation of flow.

Figure 3.8. The effect of sodium alginate concentration on the accumulation rate at micro-particles concentration of 1% and flow rate of 2 $\mu\text{l}/\text{min}$.

3.2.4 Agglomeration area of microparticle and alginate solution

A group of agglomerated micro-particles and alginate blocked the flow at the entrance of the microchannel (see Fig. 3.9). From microscope observation, this structure of clogging is classified as bridge formation clogging. It was found that the agglomeration area increased from $96.4 \pm 57.3 \mu\text{m}^2$ to $215.3 \pm 146.4 \mu\text{m}^2$ by the increasing alginate concentration from 1% to 10% at the flow rate of 2 $\mu\text{l}/\text{min}$ and the microparticle concentration of 1.0% (see Fig. 3.10). There was no turning point found in this range of concentration.

Figure 3.9. Agglomeration of microparticles and alginate at the entrance of the microchannel.

Figure 3.10. The effect of sodium alginate concentration on the agglomeration area.

3.2.5 Standing Surface acoustic wave (SSAW)

SSAW was generated from interdigitated transducers (IDTs) made of Cr-Al electrodes on the LiNbO₃ substrate. A pair of IDTs was used to form standing wave at the center of the microchannel. The continuous sinusoidal waves at the driving frequency of 19.95 MHz were generated from function generator and amplified to the power of 0.8-1.0 watts by the power amplifier before being delivered to both IDTs. The surface waves were produced by IDTs, and propagated along the LiNbO₃ surface [126]. The excitation of SSAW showed significant reduction of microparticle accumulation rate. For 1.2% microparticles concentration, the accumulation rate dropped 5- to 10-fold with the excitation of SSAW (from $0.05 \pm 0.01\%/min$ at 0.2% microparticle to $0.22 \pm 0.08\%/min$ with 1.0% microparticle, see Fig. 3.11) at the flow

rate of 2 $\mu\text{l}/\text{min}$. Moreover, the average agglomeration size also dropped moderately from $253.6 \pm 48.9 \mu\text{m}^2$ to $149.0 \pm 32.1 \mu\text{m}^2$ ($p < 0.05$, see Fig 3.12). In the future, reduction on the agglomeration area could be even more significant with optimal ultrasound parameters or excitation mode.

Figure 3.11. Comparison of the accumulation rate of microparticle in normal condition and under SAW excitation.

Figure 3.12. Comparison of the agglomerate size of microparticles with and without SSAW excitation.

3.3 Microparticle accumulation and its reduction by SSAW in tapered microchannel

In this section, microparticles were flowed from 100- μm width region to 50- μm width region with varied constriction angles which were 15°, 30°, and 45°. The microparticle

accumulation area was calculated. The suspension was stirred continuously by the magnetic stirrer bar. SSAW was activated at the frequency of 19.95 MHz with the power of 0.8-1.0 Watt.

3.3.1 Clogging from microparticle accumulation

Formation of microparticle clogging in the microchannel was monitored, and the deposition was found to start at about 12 min (see Fig. 3.13). The blue dashed line surrounds the microparticles deposited at the microchannel constriction. However, some deposited microparticles were not stable so that they were detached from the wall (shown as yellow dots at about 18 min). In contrast, these stable microparticles at the constriction expanded continuously and quickly at 18.5 min). Eventually, the microchannel was almost blocked, microparticles accumulated rapidly the inlet, and the clogged area was densely packed. Overall, the growth of microparticle accumulation area over time can be fitted exponentially by Ae^{Bt} , where t is the time (see Fig. 3.14 and Table 3.2). The accumulation area was initially quite small and then increased significantly after approximately 12 min. It was found that a small constriction angle resulted in a smaller accumulation area and a delay of clogging. About 25 min of circulation, the accumulation areas with constriction angles of 45° and 15° were $9.4 \times 10^3 \pm 2.6 \times 10^3 \mu\text{m}^2$ and $6.3 \times 10^3 \pm 2.2 \times 10^3 \mu\text{m}^2$, respectively, as listed in Table 3.2.

Figure 3.13. The representative photos of a gradual microparticle clogging around the constriction region (15°) in a microchannel with an inlet of $100\ \mu\text{m}$ and outlet of $50\ \mu\text{m}$. The blue dashes represent the area of permanently-deposited microparticles while the yellow dots show the detachment of initially-deposited microparticles at $t = 18.5\ \text{min}$.

Figure 3.14. Time-dependent accumulation area of microparticles in the microchannel at a constriction angle of 15° , 30° , and 45° , and 1% microparticle concentration in deionized water.

Table 3.2. Time-dependent accumulation area in the microchannel with 1% microparticle concentration in alginate solution fitted by AeBt and the accumulation area at 25 min of circulation

Angle	SSAW	Alginate	Accumulation Area (μm^2)	A	B	R^2
15°	No	0%	$6.3 \times 10^3 \pm 2.2 \times 10^3$	6.2×10^1	1.8×10^{-1}	1.00
30°	No	0%	$8.1 \times 10^3 \pm 2.5 \times 10^3$	1.3×10^2	1.6×10^{-1}	0.97
45°	No	0%	$9.4 \times 10^3 \pm 2.6 \times 10^3$	1.5×10^2	1.6×10^{-1}	0.98
15°	Yes	0%	$1.7 \times 10^3 \pm 2.7 \times 10^2$	5.0×10^1	1.4×10^{-1}	0.99
30°	Yes	0%	$2.2 \times 10^3 \pm 5.0 \times 10^2$	5.0×10^1	1.5×10^{-1}	0.99
45°	Yes	0%	$2.6 \times 10^3 \pm 5.7 \times 10^2$	6.1×10^1	1.5×10^{-1}	1.00
15°	No	3%	$5.4 \times 10^3 \pm 2.9 \times 10^3$	1.5×10^2	1.4×10^{-1}	0.97
15°	No	5%	$8.2 \times 10^3 \pm 4.1 \times 10^3$	6.3×10^2	9.8×10^{-2}	0.95
15°	Yes	3%	$2.0 \times 10^3 \pm 5.2 \times 10^2$	3.9×10^1	1.6×10^{-1}	0.99
15°	Yes	5%	$4.1 \times 10^3 \pm 2.0 \times 10^3$	2.7×10^2	1.1×10^{-1}	0.97
45°	No	3%	$6.2 \times 10^3 \pm 2.7 \times 10^3$	2.1×10^2	1.3×10^{-1}	0.96
45°	No	5%	$9.9 \times 10^3 \pm 4.2 \times 10^3$	9.0×10^2	9.2×10^{-2}	0.95

The fluid viscosity affected microparticle accumulation. The progressive microparticle clogging in 5% sodium alginate with 1% microparticle is shown in Fig. 3.15. It was found that microparticles and alginate tended to form a lump. The accumulation on the wall occurred before clogging, but the deposited lumps on the wall did not expand until 12 min. The large lump of microparticles and alginate extended towards the constriction and got stuck at 14.5 min, showing the occurrence of clogging. However, the microchannel had not been fully clogged yet and microparticles were able to flow through the opposite side of the wall. After that, the lump continuously grew at the constriction with the deposition of more microparticles and the increase of its density (darkening in the image) and finally formed the complete clog at 17 min. The progressive growth of accumulation of 3% and 5% of alginate solution with 1% of microparticles in the microchannel at the constriction angle of 15° and 45° is shown in Fig. 3.16.

Figure 3.15. The clogging process of 5% alginate solution in the microchannel at the microparticle concentration of 1% and the constriction angle of 15°. The blue dotted line contours the agglomerated microparticles.

(a)

(a)

(b)

(b)

Figure 3.16. Time-dependent accumulation area of microparticles in the microchannel at the constriction angle of 15° and 45° with 1% microparticles in (a) 3%, and (b) 5% alginate solution.

3.3.2 Reduction of microparticle accumulation and clogging by SSAW

The excitation of the SSAW was able to move microparticles in the microchannel towards the pressure node by the acoustophoretic force. The pressure node was located at the center of the microchannel (see Fig. 3.17). The effect of SSAW on the accumulation area was studied quantitatively (see Fig. 3.18). The reduction in the accumulation area using SSAW at

the varied constriction angles was quite similar, 3.6- to 3.7-fold (from $9.4 \times 10^3 \pm 2.6 \times 10^3 \mu\text{m}^2$ to $2.6 \times 10^3 \pm 5.7 \times 10^2 \mu\text{m}^2$ at 45° , from $8.1 \times 10^3 \pm 2.5 \times 10^3 \mu\text{m}^2$ to $2.2 \times 10^3 \pm 5.0 \times 10^2 \mu\text{m}^2$ at 30° , and from $6.3 \times 10^3 \pm 2.2 \times 10^3 \mu\text{m}^2$ to $1.7 \times 10^3 \pm 2.7 \times 10^2 \mu\text{m}^2$ at 15° , respectively, $p < 0.05$). Overall, the SSAW was able to reduce accumulation area and delay clogging.

Figure 3.17. The distribution of microparticles in the microchannel (a) before, and (b) after, the activation of the SSAW.

Figure 3.18. Progressive microparticle accumulation in the microchannel at the constriction angle of 15° , 30° , and 45° with 1% microparticles in water and excitation of the standing surface acoustic wave (SSAW).

SSAW excitation is capable of reducing the accumulation area for high viscosity solution, as shown in Fig. 3.19. Here the constriction angle of 15° was only investigated because the other configurations have a very short time of developing the complete obstruction. The accumulation area of 3% and 5% alginate after 25 min of circulation is $2.0 \times 10^3 \pm 5.2 \times 10^2 \mu\text{m}^2$ and $4.1 \times 10^3 \pm 2.0 \times 10^3 \mu\text{m}^2$, which corresponds to 2.62- and 1.99-fold reductions,

respectively. Statistical analysis showed a significant reduction in the accumulation area by the excitation of the SSAW ($p = 0.003$ and 0.019 , respectively).

Figure 3.19. Progressive microparticle accumulation in the microchannel at the constriction angle of 15° with 1% microparticles in (a) 3%; and (b) 5% alginate solution, without and with the excitation of the standing surface acoustic wave (SSAW).

3.4 Discussion

The behavior and accumulation of microparticles on the microchannel wall and the formation of clogging were observed under the light microscope. It was found that the microparticle deposition begins at isolated locations on the microchannel wall, followed by the accumulation of more microparticles and the coalescence of multiple accumulation sites. Once the growing accumulation from both sides of the microchannel wall made contact with each other, the flow blocking (maybe partial obstruction) occurred. Afterward, the accumulation extended toward the inlet, and its density increased for the complete obstruction. The progressive growth of the accumulation area could be fitted by an exponential curve ($R^2 > 0.9$) and increased with the concentration of alginate and the constriction angle.

In order to reduce the microparticle accumulation and postpone clogging, SSAW excitation in the microchannel was proposed and evaluated. A significant reduction in the accumulation area was found (2.0 to 3.7-fold) regardless of the constriction angle but decreased with the concentration of alginate or the fluid viscosity. The accumulation area of

microparticles and alginate increased over time, but there were several stages during the process. At the initial stage, microparticles occasionally and randomly deposited on the microchannel wall due to the attractive force from the solid boundary. Microparticles with high zeta potential were stabilized while those with low value tend to coagulate or flocculate [127]. The accumulation area was small and grew very slowly. Then the attractive force became larger with more deposited microparticles, and the accumulation area increased almost linearly. After that, the accumulation area increased exponentially, which may be due to several reasons. One is fluid blockage near the constriction. Initial expansion of deposited microparticles was permeable and allowed the liquid to pass through, but trapped the microparticles. Then the structure was packed so densely that the microchannel was completely clogged [128]. Another reason is that the van der Waals force and the deflection of the streamline from the deposited microparticles and the microchannel wall could overcome the electrostatic barrier to capture more incoming microparticles [8, 67, 129]. When the microparticles slid over the deposition layer, the induced shear field reduced their velocities along the wall so that they aggregated with the accumulated microparticles. Thus, initially accumulated microparticles may work as an accelerator in the microparticle accumulation [8, 52]. It is reasonably hypothesized that the reduction in the initial deposition would postpone the accumulation effectively, but may not completely avoid it. However, the large aggregate was not always stable due to the microparticle detachment. When the rolling moment derived from the fluid overcame the rolling resistance, the hydrodynamic detachment of colloids occurred [130]. Once the aggregate expanded and connected with the others or the opposite microchannel wall, clogging occurred. Then microparticles accumulated dramatically towards the inlet, and the density of accumulation increased correspondingly owing to the compressed inter-particle space.

The inter-particle force is critical for the microparticle accumulation and could be estimated by the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory. The attractive force

between the wall and suspending 8- μm microparticle and between the deposited microparticles and suspending ones is shown in Fig. 3.20, assuming that the deposited microparticles are located beside each other on the same layer and the suspending microparticle can contact with only a few deposited microparticles [8], which are valid at low ionic strength [59, 128]. The attractive force increases with the number of deposited microparticles and the closeness between them. Thus, the initial microparticle deposition could generate the attractive force for the accumulation of more microparticles, and an effective approach to reducing the accumulation should be performed at the initial stage of this phenomenon. microparticle deposition was usually found near the constriction of the microchannel [59, 60]. A small constriction angle achieves less microparticle accumulation, which may be due to the small forward flow stagnation surfaces available and the high permeability by inevitable funneling of microparticles into the constriction [128]. The low Y-velocity facilitates the streamline to converge almost linearly with less fluctuation, which may reduce the trapping probability of the microparticles. The supporting force from the microchannel wall at the constriction on the deposited microparticles should be greater than the drag force, which increases with the constriction angle, to avoid the detachment. The surface property also plays an important role in this phenomenon. The interaction energy barrier is significantly small for rough surfaces over a large range of relevant microparticle-wall separation distances to facilitate primary minimum deposition. Similarly, the rough surface has the decreased depth of the primary energy minimum so that the adhered microparticles are weak and even detach due to hydrodynamic forces and diffusion [131]. In addition, ionic strength of the medium determines the surface interaction between the wall and microparticle and, subsequently, the formation of clogging. Clogging is slow but robust and dense under low ionic strength while fast, but fragile and loose under high ionic strength [128]. More work is required to fully understand the phenomenon and mechanisms of clogging.

Figure 3.20. The attractive force on an 8- μm suspending microparticle from the wall and deposited microparticles at the various distances.

At the high alginate concentration, a large lump of alginate microparticles was formed because the alginate has a strong intermolecular force for adhesion [132]. The characteristics of clogging in the alginate solution were similar to those in water. Microparticles flow as single or small aggregates, whereas microparticles and alginate may form a large lump and deposit on the wall with much higher stability. A 5% alginate solution tended to form aggregates as large as 30 μm . Although alginate solution is highly viscous, it is a shear thinning material, its viscosity decreasing with the applied stress [133]. As extrusion increases the flow rate and normal stress at the tip of a narrow nozzle, the alginate solution has no problem through the nozzle. Thus, this characteristic makes the use of alginate popular in 3D extrusion-based bio-printing. In addition, the biological substance also has a strong intermolecular force for easy aggregation, such as cell adhesion molecules of selectins, integrins, syndecans, and cadherins [134]. Intermolecular interactions have already been utilized to induce the controlled assembly of macroscopic objects, such as molecular targeting using covalent bonding (dissociation energy of 30–260 kcal/mol), drug incorporation of the therapeutic agent with hydrogel, cell spheroids for pharmaceutical screening, and the investigation of cancer metastasis [135]. This

suggests that the clogging problem in 3D bio-printing may be more serious than that of microparticles investigated here.

SSAW has shown a significant reduction in microparticle accumulation in water and low alginate concentration medium because of two reasons. First, microparticles are pushed towards the pressure node (i.e., center of the microchannel) by acoustophoresis, whose force should be much larger than the van der Waals force from the wall. If both electrostatic and Born's repulsion forces are included, the pushing force will be even larger, but these interparticle forces are weak at a large distance [136, 137]. Second, the acoustic streaming generates viscous torque for the rotation of microparticles along the interphase boundaries [138, 139]. Such rotation may be able to reduce the aggregation between flowing microparticles and deposited ones on the wall by the slippery effect [128, 140]. The acoustic streaming generated by SSAWs has been used to remove nonspecifically bound proteins [141]. In highly viscous fluid, an additional term should be added to the dipole scattering coefficient as the viscosity dependent correction by considering Prandtl-Schlichting boundary layer theory and acoustic boundary layer [103]:

$$f_{dip} = \frac{2(1 - \gamma)(\rho_p - \rho_0)}{2\rho_p + \rho_0(1 + \frac{9}{2}[1 + i(1 + \frac{\delta}{r})](\frac{\delta}{r}))} \quad (3.8)$$

where δ is the distance to the boundary layer, i is the complex unit. The smaller r relative to δ , the larger the effect of viscosity. There is a strong enhancement proportional to $(kor)^{-3}$ in comparison to inviscid case due to non-vanishing interference between the incident and scattered waves. However, for SSAW and large nearly neutral buoyancy microparticles (i.e., cells), the acoustophoretic force in the inviscid medium is negligible (<1%). Therefore, less effect of SSAW in this study is mainly due to the viscous flow.

The motion of microparticles is determined by the resultant action of acoustic radiation and streaming force [142]. SSAW has a higher magnitude of acoustic streaming than the BAW

at the same driving frequency, and small microparticles are dominated by acoustic streaming. Critical microparticle size is about 10 μm at the excitation frequency of 6.65 MHz where radiation dominates the motion of microparticles [142]. At the frequency of 19.95 MHz as used in this study, acoustic radiation force would become more significant. Acoustic streaming induced by SSAW is found to be relatively strong near the channel side walls due to the inherent travelling wave component and increases with the height of the microchannel [143]. microparticle deposition mostly occurs where the fluid streamline deflects, such as at the constriction and entrance of the microchannel, along the side wall of the PDMS microchannel. However, only a few microparticles deposit on the top and bottom surfaces, theoretically. The acoustic streaming may be also beneficial in preventing microparticle deposition when it circulates microparticles above the bottom. In summary, the exponential growth of the accumulation area of microparticles in a microchannel is determined by both the geometry of the microchannel and the hydrodynamic parameters. The small constriction angle can moderately (~30%) reduce the accumulation area and delay the catastrophic clogging. The concentration of alginate (5%) leads to the lower increase of the accumulation area, but the stagnation of large aggregates at the constriction. A numerical model was established to simulate the microparticles' motion by SSAW with the consideration of fluid and microparticle properties, acoustic attenuation, acoustic impedance, laminar flow, drag, and acoustophoretic forces. The excitation of SSAW can reduce the accumulation area significantly in water by almost 3.7-fold. However, the increase of fluid viscosity (5% of sodium alginate) reduces the improvement of SSAW to two-fold. This acoustic approach provides a low-cost and effective solution to the microparticle accumulation and could delay clogging in the nozzle-based printing. The transparent microchannel allows the observation of the clogging phenomenon and understanding of the mechanisms. However, extrapolation to the nozzle in practice needs further investigation. Although the acoustophoretic force shows the ability to reduce the

clogging here, low acoustic power is usually produced by the current IDTs on rigid substrate for the lesser effect on the highly viscous fluid. However, the use of a flexible substrate or thin film with IDTs patterned on can be attached to the nozzle surface and activated at the input power up to 35 W [144-146]. The other option is the use of Bulk Acoustic Wave (BAW) from the curved transducer attached to the nozzle. Interference of the travelling wave and the reflected wave from the other side of the nozzle wall will form the standing wave. Piezoelectric ceramics can withstand high electric power.

3.5 Summary

Accumulation of particles on a microchannel wall was investigated. A gradual accumulation/deposition of particles can eventually obstruct the fluid flow and lead to clogging. In order to reduce the microparticle accumulation and suppress clogging, SSAW excitation in the microchannel was evaluated. A significant reduction in the accumulation area was found (2.0 to 3.7-fold) regardless of the constriction angle but decreased with the concentration of alginate or the fluid viscosity. The accumulation area of microparticles and alginate increased over time, but there were several stages during the process. At the high alginate concentration, a large lump of alginate microparticles was formed. The formation of clogging in the alginate solution were similar to those in water. Microparticles flow as single or small aggregates, whereas microparticles and alginate may form a large lump and deposit on the wall with much higher stability. The excitation of SSAW can reduce the accumulation area significantly in water by almost 3.7-fold. However, the increase of fluid viscosity (5% of sodium alginate) reduces the improvement of SSAW to two-fold. This acoustic approach provides a low-cost and effective solution to the microparticle accumulation and could delay clogging in the nozzle-based printing. The transparent microchannel allows the observation of the clogging phenomenon and understanding of the mechanisms. However, extrapolation to the nozzle in practice needs further investigation.

Chapter 4 Microparticle manipulation using Standing Surface Acoustic Wave at dual-frequency excitation: effect of power ratio

In this chapter, the effect of SSAW dual-frequency excitation on microparticle motion, location of pressure node and concentration of microparticle in each pressure node is presented. The SSAW dual-frequency excitation was used to enhance the tunability and accumulation efficiency of microparticles. Each IDT is capable of producing acoustic waves with multiple frequencies simultaneously while maintaining a high electrical-mechanical conversion efficiency. The dual-frequency excitation method utilizes a superposition of SSAW at fundamental (f_1) and third harmonic (f_3) frequencies allowing the location of pressure node of SSAW to be controlled flexibly in the microfluidic microchannel. By changing the power ratio between f_1 and f_3 , the amplitude and distribution of resultant acoustic radiation force on microparticles/cells lead to reconfigurable patterns, such as the number and position of the pressure nodes and the corresponding percentage of microparticles accumulated at each pressure node. It was found that if the power ratio is more than 90%, the accumulation time to the center of microchannel could be reduced by up to 2-fold. In comparison, if the power ratio is less than this threshold, three pressure nodes appear in the microchannel. With the decrease of the power ratio, two side nodal lines gradually shift toward the positions produced by f_3 only with more microparticles accumulated there. The experimental observation was in a good agreement with the numerical predictions. The advantages of this proposed method are wide tunability, non-invasiveness, and easy integration to a lab-on-a-chip system with few changes to the experimental setup.

4.1 Introduction

Microparticle manipulation, accumulation and separation play a critical role in the biological analysis and clinical diagnosis. Till now, many techniques have been developed in this field using different mechanisms, such as electro-osmosis [147], dielectrophoresis (DEP) [148], magnetophoresis [149], optical tweezers [150],[7] optoelectronic tweezer [151], hydrodynamic manipulation [152], and acoustophoresis [153-155]. Among them, acoustic methods seem to be ideal for on-chip manipulation or patterning of microparticles/cells as they can theoretically manipulate all types of microparticles. Compared to their optical, electrical and magnetic counterparts, acoustic approaches are inherently non-invasive to biological objects and work for most microparticles regardless of their optical, electrical, and magnetic properties [156]. Currently, there are two types of acoustic methods; bulk acoustic wave method and surface acoustic wave method. Microparticles exposed to standing acoustic waves tend to move towards nodal points. Although standing bulk acoustic waves (SBAWs) method has shown promising results [155, 157, 158], their non-planar structures and complex fabrication processes make it challenging to integrate with other planar lab-on-a-chip components. The microfluidic microchannel used in SBAWs is usually made of silicon and glass. The generation of SBAW highly depends on complicated resonator structure design and a stable temperature. Its resonant frequency is in the order of MHz. SSAWs can be generated by a pair of IDTs [27, 159-161]. A poly-dimethylsiloxane (PDMS) microchannel was aligned and bonded between two parallel IDTs. Upon the application of a radio-frequency (RF) signal to the IDTs, SSAWs were generated, and the suspended microparticles were focused at the pressure nodes located in the middle of the microchannel width by design. When microparticles/cells suspended in a fluid are exposed to an acoustic field, an acoustic radiation force acts on them due to the

differences in density and compressibility between microparticles/cell and the surrounding fluid. The magnitude of the acoustic force is dependent on the microparticle size and its acoustic contrast factor. Implementation of IDTs with the existing fast-prototyping methods, such as soft-lithography which is widely used in microfluidics, is much easier for mass production. The SSAWs are usually at high excitation frequencies (i.e., > 10 MHz), and they produce results in finer resolution in terms of microparticle manipulation compared to bulk acoustic waves. Hence, SSAW is becoming increasingly important in the fields of cell biology and medicine because of its simpler fabrication, experimental setup, higher manipulation flexibility, and better compatibility with optical microscopy for real-time observation.

The acoustic radiation force applied to microparticles/cells is proportional to the driving frequency. The acoustic tweezers technique shares the same limitation as many other on-chip patterning ones; the pattern features cannot be modified easily. Once the IDT is fabricated with a certain pattern of the period, its driving frequency can be adjusted only within the small bandwidth (or high resonant Q factor). Thus, the IDTs usually operate in a static manner. In order to achieve a more flexible manipulation and enhance the focusing and separation efficiency, different strategies have been developed to change the acoustic fields in the microchannel. With a combination of an acoustic fractionation method and a split-flow lateral-transport thin (SPLITT) method, two acoustic standing waves, working at first (f_1) and second (f_2) modes, were used simultaneously in different parts of the microchannel. The technique was used to separate microparticles in different sizes based on the various rates at which the microparticles move to the nodal lines [162]. The f_1 and f_2 modes of the acoustic chamber could be switched alternatively in a continuous flow in the separation of microparticles [163, 164]. Three resonant modes of the microchannel were also used in the separation

of microparticles in a continuous flow. Firstly, f_1 was used for pre-concentration of all cells at the center of the microchannel. Subsequently, a switching acoustic field between f_2 and f_3 was used to separate cells of different sizes or contrast factors [165]. Because the piezoceramics in SBAWs is working in the thickness mode, f_1 and f_3 of the microchannel can be produced with high efficiency. Using a relay controlled by a rectangular control voltage, these two modes could be switched to separate the suspended constituents onto the side and center pressure nodal lines by tuning the duty cycle of the switching. This was found to be robust with respect to different microparticle position offset and time offset from the switching cycle [166]. However, several equipments were required due to significant differences in the electrical impedances at f_1 and f_3 thickness mode of piezoceramics [167]. The experiment setup consists of three function generators, two impedance matching units, and an electromagnetic relay were used for the signal generation, which increases the cost and complexity of the experimental setup. In order to achieve tunable cells and microparticles patterning by varying SSAW field, Slanted-Finger Interdigital Transducers (SFITs) were applied [156, 168]. SAWs are generated from the sub-channels where, the period of the slanted fingers satisfies the resonance condition, and the bandwidth of the excited SAW is inversely proportional to the number of slanted fingers. The SFITs allow dynamic control of the specific position of pressure node.

In this study, a new excitation strategy in the acoustic tweezer technique, dual-frequency excitation with f_1 and f_3 of IDTs simultaneously, was proposed and evaluated both numerically and experimentally. Because of the similar SAW generation efficiencies at these two modes, excitation signals could be forwarded to IDT through the power amplifier directly without individual impedance matching units. The amplitude and distribution of resultant acoustic radiation force applied to the cells and

microparticles are dependent on the power ratio. As a result, the microparticle accumulation in the microchannel could be tuned. Such a tunability is expected to be valuable in many on-chip cell studies, such as shortening the accumulation time, shifting the position of pressure node and the percentage of microparticle accumulation in each pressure node.

4.2 Materials and Methods

4.2.1 Governing equation used in numerical simulation

For a fluid with low Reynolds number (i.e., $Re = 0.55$), the motion of incompressible laminar flow was described in Eq. 3.5 in the previous chapter. Due to different velocities between fluid and microparticle (v_{fluid} and $v_{particle}$), the Stoke drag force produced on the object in the fluid is given in Eq. 3.6.

Acoustophoresis is due to the difference in momentum flux around the microparticle by acoustic waves [115]. When the acoustic wave propagates through the microparticle, it will cause the microparticle to oscillate and pulsate. Oscillation creates dipole scattering while pulsation produces monopole scattering. The resultant acoustic radiation force applied on the microparticle is described using the Gauss' theorem [102, 169] described in Eq. 3.3.

For the case of a sinusoidal standing wave, the acoustic radiation force acting on the sphere is simplified to

$$F_r = -\left(\frac{\pi r^3 \beta_f}{3}\right) p_0^2 k \phi \sin(2ky) \quad (4.1)$$

where p_0 is the acoustic pressure, r is the radius of the microparticle, and k is the acoustic wave number, ϕ is the acoustic contrast factor, given by

$$\phi = f_{mono} + \frac{3}{2} f_{dip} = \frac{5\rho_p - 2\rho_f}{2\rho_p + \rho_f} - \frac{\beta_p}{\beta_f} \quad (4.2)$$

where ρ_p and ρ_f are the density of microparticle and fluid, β_p and β_f are the compressibility of microparticle and fluid, p_{prop} and v_{prop} are pressure and velocity of propagating wave,

f_{mono} and f_{dip} are the dimensionless scattering coefficients for monopole and dipole, respectively,

For the dual-frequency excitation, the acoustic pressure may be expressed as

$$p(t) = p_1 \sin(2\pi f_1 t) + p_3 \sin(2\pi f_3 t) \quad (4.3)$$

Therefore, the corresponding acoustic radiation force is

$$F_r' = -\frac{1}{3} \pi r^3 \beta_f \phi [p_1^2 k_1 \sin(2k_1 y) + p_3^2 k_3 \sin(2k_3 y)] \quad (4.4)$$

In this study, f_1 and f_3 were activated simultaneously from a pair of IDTs. With the presence of two frequencies, the total radiation force field is calculated as a superposition of the radiation force from two standing waves of f_1 and f_3 .

4.2.2 Microparticle motion by 1D model

The trajectory of the microparticle is governed by Newton's second law. As the microparticle is small compared to the size of the microchannel, the longitudinal motion of a microparticle is assumed to follow the fluid streamlines. Thus, the transverse motion along y -axis under the action of the acoustic radiation force and the Stokes drag force is expressed as

$$m \frac{d^2 y}{dt^2} = F_r(y, t) + F_D(y, t) \quad (4.5)$$

Particle motion was simulated by solving the ordinary differential equation (ODE) above using the fourth order Runge-Kutta method in Matlab (MathWorks, Natick, MA, USA). Material properties used in the simulation are listed in Table 3.1. The simulated domain was assumed to be far away from wall boundaries. Hence, the effect of acoustic streaming in the other directions and reflected radiation force from the wall were ignored in this model. Also, it is in low volume fraction condition which the inter-particle interactions were neglected.

4.2.3 Microparticle motion by 2D model

In this model, the acoustic streaming and effect from the wall boundaries were taken into account. The motion of microparticles in the cross-section of the microchannel induced by

the SSAW was simulated through coupling electrostatic, solid mechanics, acoustic pressure, laminar flow and microparticle tracing modules in COMSOL Multiphysics (v5.2, Burlington, MA, USA). The piezoelectric effect, fluid flow and microparticle trajectory were studied under frequency domain, stationary, and transient response respectively. 8 pairs of IDTs made by aluminium electrodes were set on the surface of LiNbO_3 . Applying voltage to the IDTs, acoustic waves were produced, travelled along the surface of the LiNbO_3 wafer, and then leaked into the water to move the microparticles to the pressure node. The PDMS microchannel wall was modelled using impedance boundary conditions, Γ_i , while the boundary of the piezoelectric substrate was modelled using a displacement condition, Γ_d [142, 143]. All transmitted wave energy was assumed to be absorbed in the PDMS and there was no wave reflection at the interface of PDMS and air, which was only valid for a thick wall (i.e., > 2 mm). A total of 0.21 million triangular meshes were generated for the finite element analysis and were very dense near the electrodes (smaller than 50 nm). Meanwhile, multiple boundary layers were used for simulating leakage of waves from LiNbO_3 to water. In the simulation, the width and height of LiNbO_3 were 3000 μm and 500 μm , respectively. The PDMS was placed between a pair of parallel IDTs. The microchannel was enclosed and located at the center of PDMS, and its width and height are 300 μm and 50 μm , respectively (see Fig. 4.1).

Figure 4.1. Schematic diagram of PDMS microchannel and IDTs on the LiNbO_3 substrate and boundary conditions used in the finite element method, Γ_i : impedance boundaries, Γ_d : Dirichlet actuation boundary.

4.2.4 Fabrication of microchannel and IDTs

The PDMS microfluidic microchannel was fabricated using the soft-lithography and mould-replica techniques. PDMS (Sylgard 184, Dow Corning, Midland, MI, USA) was mixed

with elastomer base (Sylgard 184, Dow Corning) in a ratio of 10:1. The length, width, and height of microchannel were 2 cm, 300 μm and 50 μm , respectively. The mixture was degassed in a vacuum oven (3608-1CE, Thermo Scientific, Waltham, MA, USA) and poured on the silicon wafer (SI8PSPD, Bonda Technology, Singapore) with negative tone photoresist (SU-8, Microchem, Westborough, MA, USA) pattern on the top. Then the patterned silicon wafer was degassed again and heated at 70°C for 3 h in an incubator (BD 56, Binder, Bohemia, NY, USA) for solidification.

To fabricate the IDTs, positive photoresist lift off process was used 20 nm of Cr and 400 nm of Al were deposited on a substrate of 4" double-side-polished LiNbO₃ wafer (Y-128° propagating, University wafer, Boston, MA, USA). 20 strips with the width of 150 μm and aperture of 2 cm were patterned on a plastic mask (Infinite Graphics, Singapore) for photolithography by coating the positive photoresist (AZ 9260, Nicolaus-Otto-Straße, Germany) on the LiNbO₃ wafer. Eventually, the Cr-Al layer on the non-exposed area was removed by acetone. To bond and align PDMS microchannel on LiNbO₃, oxygen plasma (Harrick Plasma, Ithaca, NY, USA) was used to treat the surface of them. After the alignment, the device was heated at 80°C in the vacuum chamber for 10 min.

4.2.5 Experiment setup

The experimental setup is shown in Fig. 4.2 as per our previous study [170]. The IDTs were driven by its f_1 and f_3 simultaneously from a function generator (AFG3000, Tektronix, Beaverton, OR, USA) followed by a power amplifier (Model 25 Watt CW, 10 kHz - 250 MHz, Amplifier Research, Souderton, PA, USA). The solution in the concentration of 5.9×10^7 polystyrene beads (2106, 4- μm green fluorescent, Phosphorex, Hopkinton, MA, USA) per ml was spun by vortex (Barnstead Thermolyne Vortex, Dubuque, IA, USA) for about 2-3 min and then immersed in an ultrasound sonicator (8892, Cole-Parmer, Vernon Hills, IL, USA) for 10 min to disrupt any agglomeration before each experiment. The mixture was filled into a 3 ml

syringe that was driven by a syringe pump (NE-1000, New era pump systems, Farmingdale, NY, USA) at a flow rate of 3-5 $\mu\text{l}/\text{min}$. The accumulation of microparticles in the microchannel was observed under a light microscope (CKX-41, Olympus, Tokyo, Japan) at 40 $\times$ magnification and captured by a digital camera (QIC-F-CLR-12-C, QImaging, Surrey, BC, Canada), and then quantitatively analyzed using image processing software (ImageJ, National Institute of Health, Bethesda, MD, USA).

Figure 4.2. Schematic diagram of the experimental setup.

4.3 Results and discussion

4.3.1 Comparison of 1D and 2D simulation models

The positions of accumulated microparticles in the PDMS microchannel by SSAW were simulated by 1D and 2D models. The 1D model predicted that the microparticles accumulated at the pressure nodes in the standing wave field as described by Eq. 3.3. In comparison, the 2D model included the effect of microchannel height and acoustic streaming which may produce the migration and rotation of microparticles during their motions [143]. The differences in the numerical predictions were compared. It was found that the geometries of the microchannel and experimental conditions (i.e., frequency and acoustic power) used here lead to the difference in microparticle location for a few micrometers (see Fig. 4.3). Because of the limited microchannel height, there is only one accumulation layer along the z -axis in the 2D simulation. Three pressure nodes were at $y = 50\ \mu\text{m}$, $150\ \mu\text{m}$, and $250\ \mu\text{m}$ while the

corresponding accumulation positions were at 51.6-51.9 μm , 150.4-150.6 μm , and 247.9-248 μm , respectively, at the varied acoustic power of 73-648 mW. Therefore, the simple prediction by the 1D model had satisfactory accuracy and was used in this study due to a lower computational effort. The computation time of 2D model was about 162 min using a PC (3.2 GHz CPU, 24 GB memory) at the time step of 4 ns.

Figure 4.3. (a) The initial uniform distribution of 4- μm microparticles in the cross-section of a microfluidic microchannel with the height of 50 μm and width of 300 μm , (b) their steady-state positions by SSAW simulated using the 2D model, and (c) the comparison of microparticle positions predicted by 1D (dash line) and 2D models (symbols).

4.3.2 IDTs and PDMS microchannel

The alignment of a PDMS microchannel with a pair of IDTs is shown in Fig. 4.4a. Using a network analyzer (HP8510B, Agilent Technologies, Santa Clara, CA, USA), the S_{11} frequency response of IDTs shows several valleys in Fig. 4.4b, which corresponds to the different resonant modes. The values of 6.1 MHz and 17.8 MHz were close to f_1 and f_3 in the design of IDT (6.2 and 18.6 MHz) determined by the strip width. The S_{11} transmission coefficients at both resonant modes were quite similar (-7.52 dB vs. -6.3 dB). Therefore, the dual-frequency excitation simultaneously using the single IDT is possible to achieve similar acoustic energy output.

Figure 4.4. (a) Photograph of a pair of interdigital transducers (IDTs) aligned with a 300- μm Polydimethylsiloxane (PDMS) microchannel and (b) S_{11} signal of IDTs measured by an impedance analyzer.

4.3.3 Simulation of microparticle motion by dual-frequency SSAW

In the simulation, f_1 and f_3 were set as 6.2 and 18.6 MHz with the wavelength of 600 and 200 μm , respectively. The distribution of acoustic pressure and acoustic radiation force of SSAW on a 4- μm polystyrene microparticle in the 300- μm microchannel at the total acoustic power of 146 mW are shown in Fig. 4.5. The power ratio of P_I , which is defined as the percentage of the power of f_I to the total acoustic power, was varied from 0% (purely third harmonic) to 100% (purely fundamental frequency). It was found that if $P_I > 90\%$ the acoustic pressure distribution across the microchannel was always in phase. Therefore, there was only one pressure node at the center of the microchannel ($y = 150 \mu\text{m}$) that was same as the one under the excitation of the purely fundamental frequency ($P_I = 100\%$). However, when P_I is no larger than this threshold, the acoustic pressure in the central microchannel is always out of phase with respect to upper and lower microchannels. As a result, three pressure nodes occurred immediately after $P_I = 90\%$ at $y = 75, 150$ and $225 \mu\text{m}$, and two side nodes gradually moved to $y = 50$, and $250 \mu\text{m}$, respectively, with the decrease of P_I to 0%. Overall, this threshold ($P_I = 90\%$) was independent of the microparticle diameter (i.e., 4-10 μm), total acoustic power (i.e., 73-648 mW, see Fig. 4.6), and the driving frequency used in our study (i.e., 6.2-18.6 MHz, data not included) in our study.

Figure 4.5. (a) The pressure waveform and (b) the corresponding acoustic radiation force applied to the 4-μm microspheres in a 300-μm microfluidic microchannel by dual-frequency excitation at the varied power ratios of $P_1 = 100\%$ (purely fundamental frequency), 95%, 91%, 90%, 85%, and 0% (purely third harmonic) at the total acoustic power of 146 mW.

Figure 4.6. Motion of microparticle initially at $y = 0$ μm (a) with the diameter of 4 μm under the varied power ratios (88–91%) and total acoustic powers (73–648 mW) of dual-frequency SSAW and (b) with the varied diameters of 4, 6, 8 and 10 μm at the total acoustic power of 73 mW.

The motion of 4-μm polystyrene microparticles, which were assumed to initially distribute uniformly across the 300-μm microchannel by the dual-frequency excitations at the total acoustic power of 146 mW with the varied power ratios, is shown in Fig. 4.7. Due to a symmetry, only half of the microchannel ($0 \mu\text{m} \leq y \leq 150 \mu\text{m}$) was included. Around the critical power ratio of $P_1 = 90\%$, the microparticle concentration at each pressure node changed rapidly due to the accumulation of all microparticles only at the center of the microchannel or at three

pressure nodes. At $P_I > 90\%$, dispersed microparticles rapidly aligned around $y = 75 \mu\text{m}$ first and then slowly moved towards the central node because the resultant acoustic radiation force around the lower node towards the center is larger than the Stokes drag force. In contrast, at $P_I = 80\%$ the acoustic radiation force in the region of $61 < y < 89 \mu\text{m}$ is toward the lower node at $y = 61 \mu\text{m}$ where microparticles could be trapped by both drag and radiation forces. At the critical power ratio of $P_I = 90\%$, the microparticles within $75 < y < 255 \mu\text{m}$ were pushed toward the central pressure node and those within $0 \leq y \leq 75 \mu\text{m}$ towards the lower node. In comparison, at $P_I = 0\%$, the microparticles within the range of $100 < y < 200 \mu\text{m}$ and $0 \leq y \leq 100 \mu\text{m}$ accumulate at the central and lower nodes, respectively. As a result, the percentage of microparticles accumulated at the side node and central node slowly change from 25% to 33.3% and 50% to 33.3% with the decrease of the power ratio of P_I from 90% to 0%, respectively (see Fig. 4.8a). Thus, the position of pressure node, the microparticle concentration accumulated, and the microparticle motion towards the pressure node was found to be dependent on the power ratio, which provided more tunability in the microparticle manipulation. The motion of microparticles positioned at $y = 0 \mu\text{m}$ towards the corresponding pressure node at the varied power ratios is shown in Fig. 4.8b. At $P_I = 100\%$, the accumulation time of microparticle towards the pressure node, which is defined as the time to complete 99% of displacement, was about 1.95 s. In comparison, the corresponding value was reduced to 0.97 s, at $P_I = 95\%$. At $P_I = 95\%$, the reduction of microparticle accumulation time was slightly increased from 1.96-fold to 2.06-fold, at the acoustic power of 73 and 438 mW, respectively (see Fig. 4.8c). Overall, the effects of power ratio on the microparticle accumulation time at different acoustic powers were constant.

Figure 4.7. The motion of 4- μm microspheres in a 300- μm microchannel by dual-frequency excitation at the varied power ratio of (a) $P_I = 100\%$ (purely fundamental frequency), (b) $P_I = 95\%$, (c) $P_I = 91\%$, (d) $P_I = 90\%$, (e) $P_I = 85\%$, and (f) $P_I = 0\%$ (purely third harmonic) at the total acoustic power of 146 mW.

Figure 4.8. (a) microparticle position and microparticle concentration, (b) motion of microparticles initially at $y_0 = 0 \mu\text{m}$, and the microparticle accumulation time using the dual-frequency SSAW at the total acoustic power of 146 mW with varied power ratios, and (c) the accumulation time of microparticles at various acoustic power from 73 to 438 mW.

4.4 Experiment validation

With the full development of flow and generation of SSAW in the PDMS microchannel, the pressure nodes were produced by the synthesis of acoustic radiation force from SSAW. As a result, the initially distributed microparticles in the inlet streamline of the microchannel gradually moved and accumulated at the corresponding pressure nodes. Stabilized microparticle accumulation by the dual-frequency excitation of SSAW at the varied power ratios was observed under the optical microscope (see Fig. 4.9). Similar to the numerical prediction three pressure nodes could be found at $0 \leq P_I \leq 90\%$, but only one pressure node was found at the center of the microchannel, at $P_I > 90\%$. In the experiment, some accumulated microparticles at the pressure node were not uniform, especially $P_I > 90\%$, which may be due to the formation of microparticle lumps at the high microparticle concentration. With the decrease of the power ratio, the percentages of microparticles at both the upper and lower nodes gradually increased, and the distributions of microparticles became uniform. The dependence of the location of the pressure node and microparticle concentration at each pressure node on the power ratio was determined experimentally (see Fig. 4.10). Numerical simulation and experiment results were compared with each other, and a great correlation was found between them ($R^2 = 0.85$ and 0.83 , respectively). The correlation coefficient represents the agreement of simulation results and experiment results. The percentages of microparticles at the side pressure nodes slightly increased with the decrease of power ratio (i.e., from $27.6 \pm 3.2\%$ to $28.0 \pm 2.8\%$ at the lower node and from $30.8 \pm 3.3\%$ to $36.2 \pm 2.4\%$ at the upper node at the power ratio from 85% to 75%) while that of microparticles at the centre decreased correspondingly (i.e., from $41.5 \pm 5.5\%$ to $35.8 \pm 4.1\%$).

Figure 4.9. The accumulation of 4- μm microspheres in a 300- μm microchannel at the pressure node by dual-frequency excitation at the varied power ratios of (a) $P_1 = 100\%$ (purely fundamental frequency), (b) $P_1 = 90\%$, (c) $P_1 = 85\%$, and (d) $P_1 = 0\%$ (purely third harmonic).

Figure 4.10. Comparison of simulation and experimental results of (a) the position of pressure node ($R^2 = 0.85$, $n = 37$) and (b) the microparticle concentration at each pressure node in the microchannel ($R^2 = 0.83$, $n = 31$) at the varied power ratios of P_1 .

Several vibration modes could be produced using the IDT patterned on LiNbO_3 (128° Y-cut) wafer. The frequency of 6.2 MHz in the measured S_{11} frequency response is the Rayleigh wave whose wave velocity is 3962 m/s in the substrate. Hence 17.7 MHz is its natural third harmonic frequency (f_3). A small split of the peak near 17.7 MHz may be the superposition of several components because of a slight difference of finger periods in the IDTs during the fabrication. However, the mode at the frequency of 13.5 MHz in the S_{11} spectrum has a much higher transmission efficiency (-12.3 dB) than both f_1 and f_3 . This mode may be the surface skimming bulk waves (SSBW), which are bulk acoustic waves propagating close to a planar crystal surface and having little attenuation by the surface because they satisfy the mechanical stress-free boundary conditions [171, 172]. It will be used in future study because of higher

frequency and acoustic pressure applied to the microparticles. The small discrepancies between the simulation and experiment results may be due to the difference in the working frequency of f_1 and f_3 [173-175]. In the simulation, f_3 (18.6 MHz) was set to be exactly three times of f_1 (6.2 MHz). However, f_1 and f_3 in the experiment were 6.1 MHz and 17.8 MHz, respectively. Another reason for discrepancy was from the 1D model used in predicting the accumulation positions. Although the calculation was simple, there was a slight difference with the 2D models. With the increase of the height and width of the microchannel, driving frequency, and acoustic power and the decrease of the microparticle size, the effect of acoustic streaming becomes more significant [142, 143]. As a result, the streaming rolls were spatial dependent across the microchannel and dominant closed to the microchannel walls. Dual-frequency excitation at SSAW proposed in this work was not limited to f_1 and f_3 . Other dual-frequency excitations, such as f_1 and f_5 , and f_3 and f_5 , are also applicable if their transmission coefficients are comparable. Although harmonics could also be produced in piezoceramics in the production of the bulk acoustic waves, the significant differences in their electrical impedances prevented the simultaneous excitation of dual-frequency mode. Our method may potentially be applied for microparticle/cell accumulation and sorting. For microparticle focusing by the dual-frequency excitation, much faster speed to the center of microchannel than that at f_1 (i.e., reduction of accumulation time by about 2-fold at $P_I = 95\%$) could be achieved. In addition, the position of pressure node and the microparticle concentration accumulated at the pressure node could be tuned by adjusting the power ratio. Although the microparticle sorting is possible by switching the acoustic field [166], that method required more equipment and high control complexity, such as two transducers, two power amplifiers, three function generators, and a relay. Moreover, it was found that if the percentage of the large microparticle is higher than 25%, the sorting efficiency might reduce significantly. Dual-frequency SSAW also has a potential for microparticle separation. For example, all microparticles could be firstly focused

at $y = 61 \text{ }\mu\text{m}$ by the dual-frequency SSAW at $P_I = 80\%$ (see Fig. 4.8a). Then the power ratio could be changed to 95% for a while long enough for large microparticles moving across $y = 89 \text{ }\mu\text{m}$. After that, the power ratio could be switched back to $P_I = 80\%$. Consequently, the large microparticles could move toward the central pressure node ($y = 150 \text{ }\mu\text{m}$) while the small microparticles could move towards the lower node ($y = 61 \text{ }\mu\text{m}$). Production of an arbitrary waveform is available in most commercial waveform generator and adjusting the excitation waveform is much easier than changing the hardware component. A feedback control loop by real-time monitoring the outcome and varying the excitation is also possible in order to enhance the throughput. Applications of dual-frequency SSAW in the microparticle accumulation and separation will be investigated further in the near future.

4.5 Summary

In conclusion, we have investigated the microparticle motion in the microchannel by SSAWs at dual-frequency excitation and developed an effectively tunable patterning technique by varying the power ratio of f_1 to the total acoustic power applied to a pair of IDTs. The number and position of pressure nodes for microparticle accumulation and the percentages of microparticles at each pressure node can be adjusted dynamically without changing any on-chip or off-chip parts. When the power ratio is higher than 90%, there was only one pressure node, same as that produced by f_1 only. In comparison, three pressure nodes could be produced with varying positions and microparticle concentration at the power ratio no greater than this threshold. Such a critical power ratio was found independent of the driving frequency, power input, and the diameter of microparticles. Experimental data were in good agreement with the theoretical prediction. This acoustically tunable technique is inherently non-invasive and provides a new excitation strategy in the investigation of microparticle manipulation for more

potential applications in the microarray, cell biology, regenerative medicine, tissue engineering, microparticle manipulation and colloidal studies.

Chapter 5 Acoustic manipulation of microparticle in a cylindrical tube for 3D printing

The capability of microparticle/objects patterning in the 3D printed structure could improve its performance and functionalities. In this chapter, a novel method to accumulate the microparticles in the cylindrical tube during the 3D printing process is proposed by acoustically exciting the structural vibration of the cylindrical tube at a specific frequency and subsequently focusing the microparticles at the produced pressure node towards the center of the tube by the acoustic radiation force. In the experimental setup, a piezoceramic plate was glued to the outside wall of a cylindrical glass tube with tapered nozzle. The accumulation of microparticles in the tube and printed structure was monitored microscopically, and the accumulation time and width were quantitatively evaluated. The measured vibration mode and the excitation frequency of the cylindrical glass tube (172 kHz) agreed quite well with our numerical simulation (168 kHz). Acoustic excitation could effectively and consistently accumulate the microparticles. It is found that the accumulation time and width of microparticles in the tube increase with the concentration of sodium alginate and microparticles in the ink. Lastly, the accumulation area of microparticles at the nozzle constriction region and outflow discharge that quantitatively present the degree of nozzle clogging were monitored over time without and with acoustic excitation.

5.1 Introduction

Nowadays, three-dimensional printing (3D printing) or additive manufacturing (AM) is broadly used in many industries [176, 177]. With less constraint of part fabrication, scalability of part dimension, and minimum tool cost make AM have advantage over other conventional manufacturing techniques [3]. Most of the printers utilize nozzle as a main component to delivery printing ink to the desired position to form the printed part. The printing

ink consists of solid microparticles/cells suspended in the liquid medium. Recent studies reported that spatial manipulation or patterning of microparticles/cells/objects in the AM may improve the performance and functionalities of printed structure. For instance, proper alignment and orientation of the fibers in a polymer matrix can transfer the loads away from critical locations for improved performance [4]. Another example of patterning is to fabricate hierarchically ordered materials at the microscale level that exploit material composition and capabilities at a variety of length scales. In tissue engineering, the ability to print 3D scaffolds with a controlled hierarchical structure could enhance the mechanical strength, which is desirable for load-bearing bone defect repair and regeneration [5]. Decorating the surface of carbon nanotubes with particular antibodies enables the detection of specific antigens as functional materials [6]. Application of multifunctional nano-composites with respective printing media may have common limitations, such as nozzle clogging [7]. Focusing the particles in the microchannel could delay the accumulation on the channel wall [170].

Recently, a few studies attempted to apply the external forces in assisting the printing process. For instance, magnetic force [6, 178, 179] could align the microparticles orientation at the interface [180]. But this method requires the use of microparticles/objects with specific electromagnetic properties or labelling the cells/proteins with magnetic nanoparticles, which is usually time-consuming and may cause some toxicity to organisms [181]. Similarly, using the electricity to manipulate the conductive and/or dielectric microparticles also requires certain electrical charge property [4, 182, 183]. However, the high electric field may induce heating, which may affect the viability of mammalian cells. Acoustic manipulation has also been applied for various applications, such as microparticle patterning [29, 184, 185], focusing [186, 187], and cell sorting [188, 189]. This approach relies on the relative density, compressibility, and size of the microparticles. Some attempted to use the acoustic waves to pattern the microparticles during or after the printing [190, 191]. However, the use of a rectangular nozzle,

which is uncommon and needs complicated manufacturing at a high cost, and manipulation of microparticles after the printing in a rectangular container may limit the practice of such technology. The acoustic focusing of microparticles at the center of the cylindrical tube was possible [32], but the effect on the printing structure was not shown and only fundamental vibration mode was explored. Importantly, the effects of experiment parameters (e.g., the concentration of microparticles and hydrogel) for printing (e.g., accumulation time and width) have not been investigated to completely evaluate the potential of this approach.

In this work, a low-frequency bipolar mode of the structural vibration of the cylindrical tube was studied and then utilized to concentrate the microparticles at the center of the tube and subsequently the printed structure. The effects of experimental parameters, such as the concentration of microparticles and alginate, on the printing were studied. The fluid viscosity of ink with included microparticles is an important factor for extrusion printing [192, 193]. The numerical simulation was first carried out to predict the excitation frequency, structural vibration, distribution of acoustic pressure in the cylindrical tube, and the corresponding accumulation of microparticles. The experimental excitation frequency of the structural vibration and accumulation of microparticles at the center of the cylindrical tube were similar to the simulation results. The time to accumulate microparticles to the center and their accumulation width in the tube were measured. The effect of concentration of sodium alginate and microparticles in the ink on the microparticle accumulation in the tube and the printed structure were studied. The printing capabilities without and with acoustic excitation were compared statistically. Furthermore, the ability of higher harmonics was also evaluated. Various patterns of microparticles in the printed structure could be controlled by adjusting the excitation frequencies.

5.2 Material and Methods

5.2.1 Numerical simulation

A 2D simulation model was established using finite element method (FEM) software (COMSOL 5.2, Stockholm, Sweden). A piezoceramic plate ($11 \times 11 \times 2 \text{ mm}^3$) was attached to the outer side of a cylindrical glass tube, whose inner and outer diameter were 6.4 mm and 7.0 mm, respectively (see Fig. 5.1). The electrical signals were supplied to the piezoelectric material to excite the longitudinal mechanical vibration which is perpendicular to the surface of the glass tube and then coupled into the liquid inside the glass tube. Triangle meshes were used in the FEM, and there were in total 7531 meshes in the domain of piezoceramic, glass tube, and fluid. The average mesh growth rate was 1.521. The smallest mesh size was $2 \text{ }\mu\text{m}$ at the interface between piezoceramic plate and glass cylinder. A total of 124 microparticles in the diameter of $50 \text{ }\mu\text{m}$ were distributed uniformly inside the tube initially. Numerical simulation was carried out using the modules of solid mechanics, electrostatic, acoustics, and particle tracing. Initially, eigenfrequency of the glass tube was calculated to determine the excitation frequency and stress-strain response (Eqs. 5.1 and 5.2). The outer boundaries of glass tube were freely bound. Then the electrical signal was applied to the piezoceramic plate in the frequency domain. With the piezoelectric effect, the electricity was converted to the stress and strain in the piezoceramic and then transferred to the glass which was described as the linear elastic material. At the interface between glass and fluid, the mechanical waves propagated into the fluid domain (Eq. 5.3). Trajectories of microparticles in the fluid were calculated (Eqs. 3.3 and 3.6) in the time domain at a step size of 1 ms. The primary acoustic radiation force applied to the microparticles pushes them towards the pressure node under the acoustic excitation. The material properties and parameters are illustrated in Table 5.1.

The linear behavior of the piezoelectric material is presented in the stress-charge and strain-charge forms and propagation of acoustic wave in the liquid (as described in Eqs 2.11 and 2.12)

Figure 5.1. Cross-section diagram of subdomains and boundary conditions in the FEM simulation.

Table 5.1. Material properties used in the numerical simulation

medium	parameter	value
water	density, ρ_w	997 kg/m ³
	speed of sound, c_w	1497 m/s
	viscosity, μ_w	0.890 mPa·s
	compressibility, κ_w	448 TPa ⁻¹
microparticle	density, ρ_p	1050 kg/m
	speed of sound, c_p	2350 m/s
	Poisson's ratio, ε_p	0.35
	compressibility, κ_p	249 TPa ⁻¹
glass tube	density, ρ	7600 kg/m ³
	Young's modulus, E	70 GPa
	Poisson's ratio, ν	0.23
piezoceramic	density, ρ	7600 kg/m ³
	speed of shear wave, v_T	2005 m/s
	speed of longitudinal wave, v_L	1700 m/s
	electromechanical coupling factors, k_{33} and k_{31}	0.68 and 0.33

5.2.2 Experimental setup

A piezoceramic plate (355, $11 \times 11 \times 2$ mm³, APC International, Mackeyville, PA, USA) was glued (Insta-Flex+, Bob Smith Industries, Atascadero, CA, USA) to a cylindrical glass tube (Glass Pasteur Pipet, Corning, NY, USA), whose inner and outer diameter were 6.38 mm and 7.04 mm, respectively at the center. A diagram of the experimental setup is illustrated in Fig. 5.2. The sinusoidal signal at a certain frequency was generated by a function generator (AFG3000, Tektronix, Beaverton, OR, USA) and then undergone a power amplifier (240L, ENI, Rochester, NY, USA). The power input to the device was 0.71 W. To maximize the electrical power transferred to the piezoceramic plate, a matching unit was built in the lab to adjust the output impedance to approximately 50 Ω (as shown in Fig. 5.2a), as measured by an impedance analyzer (R3272, Advantest Corp, Tokyo, Japan). The vibration pattern of the glass tube was measured using a laser Doppler vibrometer (PSV-500, Polytec GmbH, Waldbronn, Germany). Trajectories of the microparticle along the glass tube were observed by an industrial camera (55326, Edmund Industrial Optics, Barrington, NJ, USA) with a 25-mm focal length lens under the illumination of a LED light source (V-LSL666, Valore, Singapore). In addition, the cross-sectional images of microparticles in the cylindrical glass tube were captured using the light sheet. Fiber optic illuminator (MI-150, Edmund Optics, NJ, USA) and single branch light line guide (#53-986, Edmund Optics, NJ, USA) were used to produce the intensive flat light. Then, a cylindrical lens with a focal length of 25 mm (LOCPCXB22-25, Lighten Optics, Beijing, China) focused the light beam to the glass tube (as shown in Fig. 5.2b). The camera was then aligned vertically for photography. The temperatures of piezoceramic and glass tube were monitored noninvasively by a laser thermometer (AR320, Arco Science & Technology Ltd, Dongguan, Guangdong, China).

Figure 5.2. Schematic diagram of experimental setup to observe the motion of microparticles (a) along and (b) in the cross-section of the glass tube, and (c) representative photo of the accumulated microparticles in the glass tube under the acoustic activation.

5.2.3 Printing evaluation

In the bioprinting application, 1%–4% of sodium alginate, a common hydrogel in the biological studies, deionized (DI) water is widely used to construct the three-dimensional structure for cells [194, 195]. The addition of sodium alginate increases the viscosity of the medium from 2.54 cPs to 37.5 cPs as measured by a rheometer (DHR-2, TA Instruments, New Castle, DE, USA), which is similar to the previously reported value [196]. Various concentrations (e.g., 0.25%, 0.5%, 1.0%, 1.5%, and 2.0% w/w) of polystyrene microparticles (50 μm in diameter, Phosphorex, Hopkinton, MA, USA) suspended in the alginate solution (180947, Sigma-Aldrich, St. Louis, MO, USA) at the concentration of 1%, 2%, 3%, and 4% w/w were used as the printing medium (ink). Prior to each printing, the solution was spun by vortex (Maxi Mix III, Barnstead/Thermolyne, Dubuque, IA, USA) and degassed in a vacuum chamber (3608-1CE, Thermo Scientific, Waltham, MA, USA). The suspension was printed through an extrusion-based bioprinter (TechnoDigm, Singapore) on a petri dish (4", Corning, Sigma-Aldrich). The distribution of microparticles in the printed structures was observed under a light microscope (CKX-41, Olympus, Tokyo, Japan) with 4 $\times$ magnification, and then the captured images were quantitatively analyzed using digital processing software (ImageJ,

National Institute of Health, Bethesda, MD, USA) and calculation software (Matlab, MathWorks, Natick, MA, USA).

Distribution of microparticles in the glass tube was recorded, and the light intensity across the tube was used to analyze and quantify the characteristics of microparticle accumulation under the acoustic excitation. The change of measured peak light intensity, which is calculated from the obtained RGB color image as $0.299R + 0.587G + 0.114B$, in the course of the acoustic excitation is shown in Fig. 5.4e. When the variation is within $\pm 1\%$ of the maximum value, the microparticle accumulation is assumed to reach its stabilization. The corresponding time is defined as the accumulation time of microparticles. The full width at half maximum (FWHM) of the light intensity distribution at the stabilized stage was used to determine the accumulation width of microparticles (see Fig. 5.4d).

In the printed structures, the histogram of deposited microparticles was calculated from the captured images after determining the edge of all microparticles and then fitted using the Gaussian function.

$$f(x) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad (5.4)$$

where μ is the mean value, σ is the standard deviation. The corresponding FWHW in the Gaussian curve is given by

$$FWHW = 2\sigma \cdot \sqrt{2 \ln 2} = 2.355 \cdot \sigma \quad (5.5)$$

FWHW is used to evaluate the microparticle distribution and compare the performance of acoustic excitation in the printing process. It's well known that 95% of the microparticles are within 2 standard deviations ($\mu-2\sigma$, $\mu+2\sigma$) in the Gaussian distribution curve.

5.2.4 Statistical analysis

Student's t -test, which is method of testing hypotheses about the mean of a small sample drawn from a normally distributed population, was carried out to determine the statistical significances (95% confidence interval or p -value below 0.05) between different experimental conditions using SigmaPlot (Systat Software, San Jose, CA, USA). In each group, at least 6 data were included for the analysis.

5.3 Results

5.3.1 Vibration modes

Acoustic excitation aims to accumulate the microparticles during the printing process. In this work, the vibration direction is perpendicular to the glass tube. The use of fundamental mode which gathers microparticles to the center of the tube was first investigated (see Fig. 5.3). The predicted frequency in the numerical simulation is 168 kHz. The vibration on the surface of the glass tube was measured by the laser Doppler vibrometer and compared to the simulation results. From the scanned contour, there were two regions with high positive vibration velocity was observed at 83.2° (0.070 mm/s) and 277.8° (0.061 mm/s), close to the piezoceramic and its opposite side, which is similar to the previous studies [32]. However, a slight difference of the excitation frequency was observed (168 kHz in the simulation and 172 kHz in the experiment), which may be due to the discrepancies of material properties and inconsistent thickness of the glass tube. In addition, the microparticles assembled due to the secondary Bjerknes force (attractive inter-particle force), gradually grew to lumps, and then moved towards the pressure node in the cross-section of the glass tube [197, 198]. Overall, there are good agreements between the simulation and measurement.

Figure 5.3. (a) The simulated radial stress of glass tube at the excitation frequency of 168 kHz in kPa, (b) comparison of simulated (172 kHz) and measured (168 kHz) normalized vibration velocity in the polar plot, (c) time-average acoustic pressure in kPa at 168 kHz, (d) the locations of 50- μm microparticles after 0.2 seconds of excitation in the simulation, and cross-sectional image of microparticles (e) without and (f) with the acoustic excitation.

5.3.2 Accumulation of microparticles in the glass tube

Initially as the microparticles were located randomly in the tube, the light intensity distribution across the tube was quite uniform, whose profile may be associated with the laminar flow for extrusion. Under the acoustic excitation, most of the microparticles gradually moved toward the pressure node at the center of the tube so that the light intensity distribution had a sharp peak (see Fig. 5.4). However, some microparticles may attach to the inner wall of the glass tube due to the surface tension. During the microparticle accumulation, the peak light intensity (mostly at the center of the glass tube) exponentially rose to its maximum value. With the increase of microparticle concentration, the peak light intensity in the steady state increased correspondingly but at a longer microparticle accumulation time.

Figure 5.4. The representative photos of microparticles in the glass tube with 1% sodium alginate and 0.25% microparticle (a) before and (b) after the acoustic excitation, and the corresponding distributions of the normalized light intensity in (c) and (d), and (e) the change of the peak light intensity during the microparticle accumulation in the fluid with 1% alginate and varied microparticle concentrations.

The concentration of alginate and microparticles in the fluid plays a significant role in the hydrodynamics of microparticles, which subsequently determines the efficiency and effectiveness of microparticle accumulation (see Fig. 5.5). The microparticle accumulation width increased in 3.3-fold from 0.19 ± 0.07 to 0.64 ± 0.18 mm at the microparticle concentration from 0.25% to 2% and 1% alginate in the fluid. The corresponding increase was 2.4-fold from 0.30 ± 0.07 mm to 0.73 ± 0.12 mm with the increase of alginate from 1% to 4% and 0.5% microparticles in the fluid. The accumulation time increased almost linearly in 4.1-fold from 29.3 ± 3.5 s to 121.2 ± 16.1 s with the increase of alginate from 1% to 4%. However, there are fewer influences on the accumulation time by the concentration of microparticles than that of alginate. The corresponding value increased slightly in 1.3-fold from 30.3 ± 3.6 s to 38.1 ± 5.2 s with the increase of microparticle concentration from 0.25% to 2.0%. Overall, the accumulation time is more sensitive to the concentration of alginate than that of the microparticle, which may be due to the fluid viscosity.

Figure 5.5. Accumulation time and width of microparticles in the solution with (a) 1%, 2%, 3%, and 4% sodium alginate and 0.25% microparticles and (b) 0.25%, 0.5%, 1.0%, 1.5%, and 2.0% microparticles and 1% sodium alginate ($n = 6$ for each condition).

5.3.3 Microparticle distribution in the printed structure

The printed structure by the extrusion-based bioprinter was straight lines on the petri dish. The distribution of microparticles inside the printed structure was observed under the light microscope. It is found that microparticles distributed quite uniformly without an acoustic activation, but mostly at the center after the printing with the acoustic excitation due to the in prior accumulation in the glass tube (see Fig. 5.6).

Figure 5.6. (a) The printed structures with 2% sodium alginate and 0.5% microparticle on the petri dish, and zoomed photos illustrating the distribution of microparticle distribution inside them (b) without and (c) with an acoustic excitation during printing.

The microparticle distribution in the printed structure was represented in the histogram quantitatively and then fitted by the Gaussian curve. The accumulated microparticle widths (see Figs. 5.7a, b) from different alginate concentrations were compared. The acoustic excitation could accumulate the microparticles mostly at the center. The percentages in the three central bins of the histogram were $46.5 \pm 3.7\%$, $41.8 \pm 4.1\%$, $43.4 \pm 4.9\%$ and $32.8 \pm 5.2\%$ at the alginate concentration of 1% and the microparticle concentration of 0.25%, 0.5%, 1% and 2%, respectively. In comparison, the percentage of accumulated microparticles in the three central bins fairly dropped from $41.8 \pm 4.1\%$ to $35.6 \pm 5.7\%$ with the increase of alginate concentration from 1% to 4% at the microparticle concentration of 0.5%. In comparison to the conventional printing without the acoustic excitation, the values of FWHM were always larger than those of microparticles in alginate excited by acoustics at all experimental conditions ($p\text{-value} < 0.05$). The FWHM value increased with the concentrations of alginate and microparticles with the acoustic excitation, from 0.31 ± 0.13 mm to 1.13 ± 0.17 mm (3.7 fold) for

0.25% and 2% microparticle concentration and 1% alginate concentration. The FWHM value increased from 0.73 ± 0.11 mm to 1.39 ± 0.22 mm (1.9 fold) for 1% and 4% alginate concentration and 0.5% microparticle concentration, respectively (see Fig. 5.7c, d). The discrepancy between the width of the accumulated microparticle in the glass tube and printed structure is due to deflection of streamlines through the nozzle.

Figure 5.7. Histogram (solid line) and fitted Gaussian curve (dashed line) of microparticle distribution in the printing structure using the ink with 1% sodium alginate and 0.5% microparticle concentrations (a) without and (b) with the acoustic excitation. Comparison of the distributed microparticle width (c) at the various sodium alginate concentrations from 1% to 4% and microparticle concentration of 0.5% and (d) at the sodium alginate concentration of 1% and various microparticle concentrations from 0.25% to 2%. * shown in the figure represents statistical differences between the experimental results of a group without and with the acoustic excitation ($p < 0.05$).

5.3.4 High orders of structural vibration

The higher orders of structural vibration were also investigated here (see Fig. 5.8). In the numerical simulation, the driving frequencies of two high orders were found to be 393 kHz and 563 kHz. The microparticles accumulated at the pressure nodes (between the acoustic peaks). At 393 kHz, there were four symmetric beam patterns distributed evenly in the polar coordinate of the average acoustic field (see Fig. 5.8a on the left column) and subsequently four accumulation regions in the cross-section of the glass tube. In comparison, there were six symmetric acoustic beams at 563 kHz (see Fig. 5.8a on the right column). At 563 kHz, the microparticle at the center was more densely accumulated than that of 393 kHz. The resonant frequencies of high orders found in the experiment were 385 kHz (see Figs 5.8a, b on the left column) and 657 kHz (see Figs. 5.8a, b on the right column), which were slightly different from the simulation as the fundamental mode. The patterns of accumulated microparticles in the cross-section at these two frequencies were found similar to that of simulation. There were two accumulated microparticle streamlines along the glass tube (~ 1.8 mm away from each other with the microparticle accumulation width of 0.70 ± 0.24 mm, see Fig. 5.8c, left). In comparison, three main streamlines were observed (one at the center with the width of 0.25 ± 0.02 mm, and the other two ~ 1.3 mm away from the center with the width of 0.46 ± 0.12 mm, see Fig. 5.8c, right) at 657 kHz. The accumulation times are 24.67 ± 4.2 s and 6.88 ± 1.54 s at 385 kHz and 657 kHz, respectively, with the statistical difference ($p < 0.05$). After the printing, the histogram of microparticles distribution could be fitted by different Gaussian curves at the accumulation positions. The accumulation widths were 1.08 ± 0.34 mm at 385 kHz (see Fig. 5.8f, left), and 0.70 ± 0.21 mm (at the side streamlines) and 0.37 ± 0.09 mm (at the central streamline) at 657 kHz (see Fig. 5.8f, right).

Figure 5.8. Comparison of the (a) simulated acoustic pressure field in kPa, and (b) location of accumulated microparticles in the cross-section at 393 kHz (left column) and 563 kHz (right column), representative photos of accumulated microparticles (c) in the cross-section, (d) along the glass tube, (e) in the printed structure, (f) the histogram and fitted Gaussian curves for each accumulation lines under the acoustic excitation at 385 kHz (left column) and 657 kHz (right column).

5.3.5 Progress of microparticle accumulation in the nozzle

In this study, a smaller tube with an inner diameter of 0.8 mm and the excitation frequency of 899 kHz was used. From the cylindrical tube, the fluid and microparticles moved to the nozzle region. With the acoustic excitation, the location of microparticles still remained on the central streamline (see Fig. 5.9d). At the connection between the glass tube and nozzle, gradual change of inner diameter is recommended to minimize the deflection of fluid streamline.

Figure 5.9. Microparticle distribution in the cylindrical tube (a) without acoustic excitation, (b) at 2 seconds of acoustic excitation at 899 kHz and in the nozzle (c) without acoustic excitation, (d) with acoustic excitation.

Accumulation of microparticles in the nozzle was monitored over time. The accumulation is a quite unstable phenomenon and may appear visually at the slightly different timing. In this case, initially the flow stabilized (see Fig. 5.10a). Then, the accumulation was visually observed at 7 minutes and 30 seconds (see Fig. 5.10b). The accumulation began in the region near an opening of the constriction. At this stage, the accumulation increased slowly (see Figs. 5.11a, c, e) but there was no significant reduction of outflow discharge observed from the nozzle (see Figs. 5.11b, d, f). Thus, fluid flow remained, and the accumulation region expanded. Later on, the accumulation area grew rapidly (see Figs. 5.10c-e). This observed finding conformed to the quantitative analysis of accumulation area over time (see Fig. 5.11a at 8 minutes onwards). However, it took a few minutes for the outflow from the nozzle to reduce significantly (see Fig. 5.11b at 10 minutes onwards).

Figure 5.10. The progress of accumulation of microparticle on the nozzle over time (a) 7:00 min, (b) 7:28 min, (c) 7:36 min, (d) 8:01 min, (e) 10:27 min, and (f) 14:00 min.

5.3.6 Reduction of microparticle accumulation by acoustic excitation

As the progressive clogging is caused by a consecutive accumulation of microparticles. It slowly obstructs the inner wall of microchannel. Microparticles which travelled close to the wall had a high chance of irreversible accumulation. The acoustic excitation focused microparticle towards the center of the tube and subsequently the center of the nozzle. Thus, lower number of microparticles could reach and accumulate on the surface of the inner wall. With acoustic excitation, the accumulation area at 15 minutes was reduced by 2.90, 2.37, and 2.04 fold for 1%, 2%, and 3% of sodium alginate concentration in the fluid, respectively (see Figs. 5.11a, c, e).

Figure 5.11. Progression of accumulation area from the nozzle with and without acoustic excitation at the alginate concentration of (a) 1%, (b) 2%, and (c) 3%.

5.3.7 Printing Structure and microparticle distribution

Acoustic excitation prolonged the printing duration, as it delayed the microparticle accumulation and maintained the outflow discharge. The printed structures with and without the acoustic excitation were compared for consistency of the printed structure. Color contrast and the absolute value of the printed structure were calculated. It was found that acoustic excitation has a significant effect on the contrast and absolute value ($p < 0.05$) of 1% and 2% sodium alginate medium (see Fig. 5.12g). With the acoustic excitation, the contrast of the color in the printed structure reduced by 31% (from 29.23 ± 5.74 to 20.26 ± 3.50), 21% (from 33.17 ± 4.54 to 26.28 ± 5.20) and absolute value increased by 11% (from 133.59 ± 8.17 to 148.02 ± 3.79) and 16% (from 145.48 ± 11.34 to 168.85 ± 6.53) for 1% and 2% sodium alginate medium, respectively (see Fig. 5.12g). In addition, focusing of microparticle at the center of the printed structure was observed with the acoustic excitation (see Fig. 5.12d). The histograms of microparticle distribution were illustrated in Figs. 5.12e and f.

(a) **Without Acoustic Excitation**

(b) **With Acoustic Excitation**

Figure 5.12. Printed structure of square shape (a) without, (b) with the acoustic excitation and particle distribution inside the printed structure (c) without, (d) with the acoustic excitation, and histogram of particle distribution along the width of printed structure (e) without, (f) with the acoustic excitation, and (g) color contrast analysis of the printed structure after crosslinked. * shown in the figure represents statistical differences between the experimental results of a group without and with the acoustic excitation ($p < 0.05$).

5.4 Discussion

The performance of proposed acoustic excitation in assisting the printing process was evaluated both numerically and experimentally in this study. It was found that the structural vibration produced by a piezoceramic plate simply attached to the cylindrical glass tube at a specific frequency could generate the pressure node(s) in the cross-section to accumulate the microparticles. Such capability of microparticle accumulation could enhance the printing functionality and reduce the risk of clogging. The motion of microparticles is also dependent on the hydrodynamic properties of streamlines, such as the concentrations of microparticles and sodium alginate. Moreover, the proposed method has the potential in the biological applications. It is noteworthy that this acoustic method is non-invasive and has low heat accumulation, 24-26°C over 10-15 minutes of acoustic excitation at room temperature ($\approx 24^\circ\text{C}$), which may pave the way to the use of temperature sensitive biological samples in maintaining their morphologies and viabilities. In the near future, the investigation of the motion of cells, their distribution in the printed structure, viability, and proliferation after the printing is required before the practical 3D bioprinting. The cell density and spatial distribution are critical to the morphogenetic development of an engineered tissue, including proliferation, differentiation, and migration [199]. Because of the smaller size of mammalian cells (e.g., 15-30 μm for Hela) and lower stiffness in comparison to the microparticles used here (e.g., ~ 120 kPa for Hela and ~ 3 GPa for polystyrene) much slower motion speed from acoustic radiation force is expected. In addition, the optimum viscosity of bio-ink should be explored, hindering the motion of biological cells at the high medium viscosity while spreading abundantly at the low medium viscosity [200, 201].

Our numerical simulation of the excitation frequency and the location of microparticles in the cylindrical tube agreed quite well with the experiment results. When the tube is driven for a long time or at the high power, the accumulation of microparticles will break into discrete

nodes. This is mostly due to a weakly coupled standing wave along the cylinder central axis of the glass tube and the formation of vortices until the introduction of thermal convective currents and eventual fluid boiling by the heating of the transducer [202]. The distance between these nodes along the glass tube is moderately constant (~8 mm) which is closed to the acoustic wavelength (8.63 mm). The formation of these nodes is affected by the tube symmetry, length, and edge conditions. For example, when an O-ring is placed at the nodal position, several neighboring nodes partially disappear. In contrast, there are no changes when placing the O-ring at the anti-nodal position.

The motion of microparticles is governed by the acoustophoretic (the acoustic radiation force given by Eq. 3.3) [101, 102], Stokes drag (resistance of microparticles in the medium given by Eq. 3.6) [23], and hydrodynamic forces [115]. At the high excitation frequency, the higher acoustic radiation force (Eq. 3.3) could speed up the particle motion and reduce the accumulation width as shown by the high order vibration modes [31, 203]. In contrast, the effect of longitudinal convection streaming, and bubble cavitation becomes significant at the low excitation frequency. The medium viscosity increases with the microparticle concentration and subsequently decreases the mobility of microparticles in the fluid [204]. In the highly viscous medium, the increased Stokes drag force pushes the microparticles in the opposite direction of the acoustic radiation force so that following the acoustophoretic force across the fluid streamline or the focusing of microparticles is hindered [127, 170]. As a result, the accumulation is prolonged with the large accumulation width at the high concentrations of alginate and microparticle [192]. However, the microparticle distribution in the glass tube is not exactly same as that in the printed structure, which is due to several factors such as the size and shape of the nozzle tip, extrusion pressure, scanning speed of the printer, and the medium viscosity of the ink. Thus, extensive work is required to achieve the desired microparticle accumulation width in the printed structure at each experimental condition. The design of

nozzle tip could be optimized. A short convergent constriction could bend the fluid streamline inward suddenly, which might shift the microparticle accumulation towards the center. This streamline bending effect is subsided by using a symmetric long convergent constriction and orifice slightly smaller than the tube [205].

The use of acoustic excitation in the nozzle delayed the accumulation of microparticles at the constriction area. The accumulation area at 15 minutes was reduced by 2.90, 2.37, and 2.04 fold for 1%, 2%, and 3% of sodium alginate concentration in the fluid, respectively. The constriction cross-sectional area was narrower over time [40, 170]. This gradual accumulation limited the quantity of the flow to pass through the nozzle [128, 206, 207]. With the acoustic excitation, the blockage of flow path was delayed. The experiment results from the outflow discharge rate were also agreeable with the accumulation area as discharge rate with the acoustic excitation was higher by 3.88, 3.56 and 3.68-fold for 1%, 2% and 3% of sodium alginate concentration, respectively than those without the acoustic excitation. The behavior of the microparticle accumulation at the constriction of the nozzle is quite similar to the microparticle accumulation in the microchannel discussed in the Chapter 3. Initially, it was found that the microparticle deposition begins at isolated locations on the wall of nozzle, followed by the accumulation of more microparticles and the coalescence of multiple accumulation sites nearby. For the separated sites, eventually, accumulation region will and with the other sites to form larger accumulation region. The progressive growth of the accumulation area increased with the concentration of alginate. The accumulation area of microparticles and alginate increased over time, but there were several stages during the process. At the initial stage, microparticles occasionally and randomly deposited on the microchannel wall due to the attractive force from the solid boundary depending on the value zeta potential of the microparticles. In this stage, the accumulation occurred slowly. But once the attractive force became larger with more deposited microparticles, and the accumulation

area increased moderately. The accumulation area increased exponentially which may be due to the obstruction of fluid flow path. The fluid is squeezed through the narrower path causing greater chance of microparticle to be trapped and deposited. Lastly, the aggregate or accumulation area expanded in the nozzle constriction. The microparticles accumulated dramatically towards the inlet, and the density of accumulation increased correspondingly owing to the compressed inter-particle space.

To improve the focusing efficiency and reduce the accumulation time, several strategies are suggested. Firstly, higher excitation power applied to the piezoceramic plate could increase the acoustic pressure and subsequently acoustic radiation force to the microparticles. Nevertheless, the risk of overheating is increased without appropriate thermal diffusion or cooling. Secondly, it is to increase the energy transmission efficiency from the piezoceramic plate to the glass tube. Using the piezoceramic plate whose poling direction is perpendicular to the glass tube surface or new piezo-composite materials with larger mechanical quality factor and lower dissipation factors may be the solutions. Finally, other vibration modes, such as thickness, thickness shear, longitudinal, cross-sectional shear, and torsional waves will be explored in the future. Flexural modes are the most important in terms of pressure variation inside the fluid because it enables relatively high normal velocities. By utilizing harmonic flexural vibration of the capillary, subharmonic acoustic pressure standing waves in the fluid can be generated inside the cylindrical tube [208].

5.5 Summary

We demonstrated a practical application of acoustic manipulation to assist the additive manufacturing (extrusion-based printing). The structural vibration of a cylindrical tube with tapered tip was produced, and the acoustic wave was coupled into the ink to accumulate the microparticles to the position of induced pressure node(s). The prediction of the excitation frequency and location of microparticles inside the glass tube in the numerical simulation

agrees quite well with the experimental results. Acoustic excitation has the statistically significant effect on the microparticle accumulation in the glass tube. The time and width of microparticle accumulation under the acoustic excitation increase with the concentration of alginate and microparticles in the ink. Although the microparticle concentration has a slightly more significant effect on the accumulation width than the alginate concentration, its effect on the accumulation time is much less. In the printed structure, the distribution of microparticles could be fitted well in a Gaussian curve, whose FWHM is usually larger than that in the glass tube during the printing process through the tapered tip. However, the dependence of microparticle accumulation in the printed structure on the microparticle and alginate concentrations is similar to that in the tube. The high orders of structural vibration could reduce the microparticle accumulation time and produce more complicated accumulation patterns. Besides, acoustic excitation in the nozzle could delay the accumulation area of microparticles at the nozzle constriction region and maintain the outflow discharge rate. Overall, this acoustic excitation technology could improve the patterning of microparticles in the AM and may be applied in the future 3D bioprinting.

Chapter 6 Cell Alignment and accumulation using acoustic nozzle for 3D printing

In this chapter, acoustic manipulation in the cylindrical glass nozzle by a structural vibration at the specific resonant frequency was further evaluated on biological cells. It is found that C2C12 cells (muscle cell line) were accumulated at the center of the nozzle and consequently on the printed construct at the fundamental frequency of 871 kHz. The distribution of cells fits quite well with a Gaussian distribution. The growth, morphology, and differentiation of the C2C12 cells were monitored for 7 days. Overall, the proposed acoustic approach is able to accumulate/pattern biological cells in the printed construct at a low cost, easy configuration, low power, and high biocompatibility.

6.1 Introduction

In the printing ink, cells are suspended randomly in the fluid. A spatial manipulation (e.g. patterning/arrangement) of cells may improve the performance and functionalities of the printed construct for various applications, such as alignment and orientation of fibers [4] and hierarchical arrangement of materials in microscale. The capability of patterning/arrangement biological cells in biomaterials would enhance the cell proliferation and differentiation. For instance, the ability to print 3D scaffolds with a controlled hierarchical structure could enhance the mechanical strength, which is desirable for load-bearing bone defect repair and regeneration [5]. Another example is decorating the surface of carbon nanotubes with particular antibodies enabling the detection of specific antigens as functional materials [6]. For cell scaffold, the quantity of biomaterials in the use could be minimized [209]. Besides, in comparison to uniformly suspended cells, accumulation of cells tends to enhance the growth in the same

condition and reduce the potential biodegradation which may release the toxic or unnatural byproducts [210].

Application of multifunctional nano-composites with respective printing media may have common limitations, such as nozzle clogging [7]. This clogging issue limits the nozzle of the bioprinter from printing cells in the high concentration and to the accurate position. However, the capability of patterning/accumulating cells at the center of the nozzle allows dense cells to be printed on the controlled position in the printed construct which could extend the capability of bioprinting when fabricating micro-organ, such as pancreas islet [211], micro-liver [212], vessel [213] and innervated skeletal muscle [214].

Skeletal muscle cells are commonly found throughout the body as the effector organ mediated by somatic nerves and reflex arcs. The key function of the skeletal muscles in association with the appendicular bones is to move the body in various directions. Basically, skeletal myocytes have an ability to regenerate after acute injuries depending on types and magnitudes of injuries. However, their regenerative ability may be impaired when they have gotten irreversible injuries such as severe acute trauma and irradiation, which finally results in a mass loss of skeletal muscles [215]. An engineered muscle structure to imitate the function of native muscle tissue is in great demand [216]. C2C12 cells are a skeletal myoblast cell line of *Mus musculus* [217], and their differentiation is compulsorily undergoing in the direction of striated myocyte development after a specific activation. Modulators of skeletal muscle adaptation and regeneration process are biochemical stimulator and cell activities. Importantly, the control of cell activity is a key modulator during this process [218, 219]. Closed distance between cells supports cell-cell interaction and cell activity [220, 221]. Histologically, the fully-differentiated skeletal muscles are patterned as the syncytia of striated myocyte fascicles by which the myocytes align themselves parallel to each another. Accordingly, adjacent myocytes must be kept within their critical displacements in order to form the fascicles

completely along their histogenesis. Hence, if 3D-bioprinter is able to pattern and print the cells at high concentration, this would support the development of myocyte fascicles and influence the cells to elongate and orientate towards one direction (along their major axis). To achieve patterning of cells, an external force applied to the cells is a promising approach.

There are couple of studies attempted to use external forces in assisting the printing process. For instance, magnetic force [6, 178, 179] could align the microparticles orientation at the interface [180]. But this method requires the use of microparticles/objects with specific electromagnetic properties or labelling the cells/proteins with magnetic nanoparticles, which is usually time-consuming and may cause some toxicity to the organisms [181]. Similarly, using the electricity to manipulate conductive and/or dielectric microparticles also requires certain electrical charge property [4, 182, 183]. However, high electric field may induce heating, which may affect the viability of the mammalian cells. Acoustic manipulation has already been applied for various applications, such as microparticle patterning [29, 156, 185], focusing [186, 187], and cell sorting [188, 189]. This approach relies on the relative density, compressibility, and size of the microparticles. The acoustic focusing of microparticles at the center of the cylindrical tube is achievable as shown in Chapter 5 [32], but it has not been applied to bioprinting application yet especially with biological cells. In addition, the effects of printing parameters (e.g. concentration of the cells and hydrogel) on the proliferation, differentiation and orientation of cells have not been investigated for the potential of this approach in 3D bioprinting.

The objective of this work is to utilize acoustic manipulation to pattern and accumulate the cells in the nozzle during 3D-bioprinting. This cell pattern subsequently appears in the printed construct. A structural vibration of cylindrical tube and patterning of cell accumulation were studied numerically and experimentally. Firstly, the resonant frequency was numerically predicted and validated with experiment. Subsequently, the distribution of biological cells

inside the printed hydrogel construct was investigated. Lastly, the growth of biological cells undergone the acoustic excitation was monitored for up to 7 days. The distribution and morphology of the cells without and with the acoustic excitation were compared.

6.2 Materials and Methods

6.2.1 Numerical Simulation Model

The details of numerical simulation setup were mentioned in the Section 5.2.1. Briefly, the model consisted of a glass tube, a piezoelectric plate, biological cells, and printed medium. Nevertheless, in this work, the small cylindrical glass tube was used. Its inner and outer diameter was 0.8 mm and 1.0 mm, respectively. Therefore, small piezoceramic plate in a dimension was of $2.0 \times 1.0 \times 0.5 \text{ mm}^3$ was attached to the glass tube. The properties of the materials could be referred to Table 5.1, with a cell compressibility of $3.78 \times 10^{-10} \text{ Pa}^{-1}$.

6.2.2 Cell culture, harvest, and differentiation

C2C12 cells, an immortalized mouse skeletal myoblast cell line (CRL-1772™, ATCC®, Manassas, VA, USA), were cultured in HyClone™ Dulbecco's modified eagle's medium (DMEM, GE Healthcare Life Sciences, HyClone Laboratories, Logan, UT, USA). It contained 10% fetal bovine serum (FBS, Gibco, Waltham, MA, USA) and 1% antibiotic-antimycotic solution, including 10,000 units/mL of penicillin, 10,000 µg/mL of streptomycin, and 25 µg/mL of amphotericin B (Gibco), in a cell culture flask.

At the confluence of 80-90%, the C2C12 cells were harvested with the standard trypsinization. Briefly, the cells were incubated with 0.25% trypsin-1mM EDTA solution (Lonza, Basel, Switzerland) at 37°C for 3 minutes. The reaction of trypsin was terminated with cell culture medium for 5 minutes at room temperature (25°C). The cells were then washed with PBS (phosphate buffered saline, Sigma-Aldrich) and centrifuged at 1,000 RPM for 5 minutes. The initial cell concentration was enumerated using the standard hemocytometry

(79001-00, Cole-Parmer, USA). The cells were finally embedded in 2 ml of 5% GelMA (gelatin methacrylate) at the concentration of 2.0×10^6 cells/mL for printing, respectively.

To activate cell differentiation, the printed cells were incubated with 10% horse serum (H0146, Sigma Aldrich, USA) in DMEM at 37°C in a humidified incubator (Heracell 150i, ThermoFisher Scientific, USA) with 5% CO₂. The culture medium was changed every three days.

6.2.3 GelMA preparation

In this study, 5% GelMA was prepared using the protocol [222]. A freeze-dried foamy GelMA was dissolved in Dulbecco's modified Eagle medium (DMEM, HyClone Laboratories, GE Healthcare Life Sciences, Logan, UT, USA). The GelMA solution was mixed with 0.2 g of a photoinitiator (Irgacure 2959, Sigma-Aldrich, USA) and then kept in a dark chamber at 37°C till use.

6.2.4 Experimental setup and evaluation of biological cell distribution

The piezoceramic plate (APC International, PA, USA) with a dimension of $2.0 \times 1.0 \times 0.5$ mm³ was glued to the glass tube. Similarly, the sinusoidal signal generated from a function generator (AFG3000, Tektronix, USA) at the resonance frequency of 877 kHz. The schematic diagram of the experiment setup was illustrated in Chapter 5 (see Fig 5.1). Subsequently, the mixture of C2C12 cells and GelMA was printed on a 4-inch petri dish using an extrusion-based printer (TechnoDigm, Singapore).

The distribution of C2C12 cells in the printed construct was observed under a light microscope (CKX-41, Olympus, Tokyo, Japan), and then the captured images were quantitatively analyzed using digital image processing software (ImageJ, National Institute of Health, USA) and computational software (Matlab, MathWorks, USA). Quantitative parameters evaluated in this work were spatial cell concentration, width of cell microconstruct,

coverage area, cell circularity and cell nuclei orientation. For spatial concentration, the similar evaluation method was applied to the microparticles previously in the Section 5.2.3. The width of cell microconstruct was measured from the distribution of printed cells in GelMA from one edge to another edge. The edge of cell microconstruct was using a quantitative criterion of $\approx 25\%$ change of grey scale value over 100 μm and qualitative observation from a light microscope. However, this criterion could be varied depending on contrast, brightness, and light microscopy imaging (e.g. bright-field, dark-field and phase-contrast). For orientation, the orientation of cell morphology and nuclei (observed from DAPI) was measured using NIH ImageJ software to quantitatively evaluate overall cell elongation and alignment.

6.2.5 MHC-immunofluorescence of aligned C2C12 in printed construct

To increase the permeation of printed GelMA to the antibody, the constructs were incubated with cool IFPerm III[®] at 4°C for 2 days. Furthermore, they were washed twice with PBS and desiccated. They were then incubated with anti-human myosin heavy chain (MHC) antibody (Clone MF 20, Developmental Studies Hybridoma Bank (DSHB), 1:10) at 4°C for 2 days and washed twice with PBS prior to the secondary antibody incubation. The constructs were later incubated with anti-human immunoglobulin-G antibody labelled with FIT-C (Fluorescein-5-isothiocyanate, Sigma-Aldrich, 1:50) at 4°C for 1 day. They were washed twice with PBS and counterstained with DAPI (4',6-diamidino-2-phenylindole, D9542, Sigma-Aldrich). Data acquisition was performed under fluorescent microscopy.

6.3 Results

6.3.1 Accumulation of biological cells by acoustic excitation

The resonant frequency of structural vibration of the cylindrical glass tube was numerically and experimentally determined as 871 kHz and 877 kHz, respectively. The corresponding time-averaged acoustic pressure and cell accumulation in the fluid from the top view were illustrated (see Figs 6.1a and 6.1b). In the experiment, the similar behavior was

found as cells suspended in the fluid were accumulated towards the center of the glass tube with acoustic excitation. It is noteworthy that the timing of microparticle and cell to reach the pressure node is different. Acoustic manipulation microparticle and cells is the accumulation speed. The accumulation of 6- μm microparticles towards the center of the cylindrical tube took about 0.73 sec. However, it took 1.66 sec for 6- μm C2C12 cells to reach the center of the cylindrical tube. It is due to the fact that the compressibility of microparticle is half of compressibility of the cell. Hence, acoustic radiation force acting on the cells is lower in magnitude.

Figure 6.1. Numerical simulation of (a) time-averaged acoustic pressure field in kPa and (b) cell distribution in the cylindrical nozzle at 871 kHz.

Figure 6.2. Representative micrograph of C2C12 cells in 3D printed construct of 5% GelMA (a) without and (b) with the acoustic excitation, and cell distribution fitted Gaussian curve in dashed line (c) without and (d) with the acoustic excitation, also (e) plot of mean values and standard deviations from fitted Gaussian curve, $n=6$.

Subsequently, during the acoustic excitation, the cells in 5% GelMA were printed on the 4-inch petri dish. The width of the printed construct is about 1.0-1.3 mm. After printing, the distribution of cells in the printed construct was analyzed. It was found that the cell spreading was fairly random and incoherent (see Fig. 6.2a) for the cells without the acoustic excitation. Meanwhile, the cells undergone acoustic excitation spread densely at the center, but sparsely near the edge of the printed construct (see Fig 6.2b). The distribution of cells located in the printed construct had relatively low concentration (below 0.8) on the left (below 0.1 mm in the printing width) and the right sides (above 0.11 mm) of the printed GelMA construct, and the cell concentration increased and remained quite constant within 0.37-0.92 mm (see Fig 6.2c). The cell distribution without acoustic excitation was fitted with the Gaussian curve with a mean value ($\mu \pm \text{sd}_\mu$) and standard deviation ($\sigma \pm \text{sd}_\sigma$) of 0.66 ± 0.17 and 0.42 ± 0.12 mm, respectively. While the distribution of cell undergone the acoustic excitation had a high value of normalized cell distribution (above 0.9) at the center (within 0.45-0.65 mm) (see Fig 6.2d). Besides, the fitted distribution has $\mu \pm \text{sd}_\mu$ and $\sigma \pm \text{sd}_\sigma$ of 0.62 ± 0.23 and 0.27 ± 0.07 mm, respectively.

6.3.2 Accumulation and growth of cells undergone acoustic excitation

The performance of acoustic nozzle in the 3D bioprinting was further evaluated using C2C12 muscle cells. Without the acoustic excitation, cell distribution was quite random after printing. On the day 4, the cells grew individually throughout the whole printed construct, sprouted out, and connected with the sprouting cells nearby. On the day 7, the cells grew further and showed a compaction of the structure due to C2C12 differentiation and myotube formation (see Figs. 6.3a-c). Meanwhile, the cells undergone the acoustic excitation showed a distinct dense cell distribution at the center of the printed construct. On the day 4, the dense cell structure was observed as the cells grew and connected to the adjacent cells. Some cells could even sprout outward from the central line where the cells accumulate densely. On the day 7, the compaction of the structure was observed which squeezed the cell structure outline inward (see Figs. 6.3d, 6.3e, and 6.3f).

Figure 6.3. Morphology and distribution of the C2C12 cells in 5% GelMA without the acoustic excitation on the (a) day 1, (b) day 4, (c) day 7, and with the acoustic excitation on the (d) day 1, (e) day 4, (f) day 7.

6.3.3 Width of cell microconstruct

Figure 6.4. Cell density in the printed GelMA construct on day 1, 4 and 7 (a) without, (b) with acoustic excitation and (c) width cell microconstruct without and with the acoustic excitation over period (days) of cell culture in GelMA, and (d) live/dead staining of C2C12 cells.

The distributions of cells without and without acoustic excitation were monitored for 7 days. For cell without acoustic excitation, the cells initially spread thoroughly the whole printed construct. Subsequently, the cells grew individually without a significant connection between cells. The width of cell boundary or microconstruct expanded over time (Fig 6.4a) within a

range of 0.76 and 0.98 mm. Similarly, the width of cell microconstruct with acoustic excitation focused at the center from 0.38 mm on day 1 and expanded over time to reach 0.57 mm on day 7 (Fig.6.4b). But the width of cell microconstruct with acoustic excitation is statistically significant lower than width of cell microconstruct without acoustic excitation ($p < 0.001$, see Fig 6.4c). Also, its mean value was reduced by 2.04-fold and 1.74-fold on day 1 and 7. Cell viability of C2C12 with and without excitation are $95.1 \pm 3.6\%$ and $95.9 \pm 3.8\%$, respectively. There is no significant differences ($p > 0.05$) in comparison to the control group suggest that acoustic excitation has little effect on the cell viabilities.

6.3.4 Orientation of C2C12 cells undergone acoustic excitation

The C2C12 without the acoustic excitation spread thoroughly in the GelMA construct, and the cell density was visually equal. The cells grew and filled completely in GelMA construct within 5 days of cell culture. It was found that the cell without the acoustic excitation sprout out in random directions, but with great values of standard deviation (see Figure 6.5a). Meanwhile, the cells with the acoustic excitation grew and exhibited high cell density at the center of the printed construct. Thence, the cells undergone acoustic excitation showed distinct aligned pattern along the printing direction. The orientation plot showed a strong tendency of alignment, the majority of the cells being aligned from -30° to 30° (see Figure 6.5d). The highest peak of the cell orientation was found at 0° which is defined as a major axis. Moreover, it suggests that most of the acoustically activated cells were aligned parallel to each another resulting in the reduced deviation. In addition, the phase-contrast microscopy also revealed the partial formation of muscular cell syncytia in the 3D constructs indicated by the cell fusion and the formation of short muscle cell bundles (see Figure 6.5d).

Figure 6.5. Elongation and alignment of cells in the GelMA construct (a) without and (b) with the acoustic excitation, and a normalized number of cell in each orientation angle (c) without and (d) with the acoustic excitation, each value was represented with mean $\pm$ std.

6.3.5 Immunofluorescent staining

(a)

(b)

(c)

(d)

(e)

(f)

Figure 6.6. Immunofluorescence (IF) against myosin heavy chain (green color) and cell nuclei counterstained by DAPI (blue color) of the cell construct (a) without, (b) with acoustic excitation, (c) histology of skeletal muscle tissue, (d) zoomed-in area obtained from the white window, and normalized number of cell nuclei in each orientation angle (e) without, (f) with acoustic excitation, each value was represented with mean $\pm$ std, and (g) standard deviation of fitted Gaussian curve and cell nuclei circularity index.

After printing, without the acoustic excitation, the cells were stochastically distributed throughout the printed construct. The cell-to-cell syncytium was clearly unobserved. The myosin heavy chain (MHC)-immunofluorescence and DAPI of the differentiated C2C12 cells in the printed constructs are shown in Fig 6.6a. Meanwhile, most of the cells undergone the acoustic excitation aligned parallel to form the tandems of muscular fascicles like their natural counterparts in the skeletal muscles. Their combined MHC and DAPI stain is illustrated in Fig 6.6b. The higher magnification of the area (obtained from the white window in Fig 6.6b) is represented in Fig 6.6c [223] which unveils the similarity of the orientation of the C2C12 muscle fibers to the natural skeletal muscle fibers (Fig 6.6d). In addition, most of the C2C12 cells were overtly positive for MHC-IF; however, the intensity of the fluorescent signals has seemingly increased in case of the excited cells, which implies a more differentiation of those cells when compared to the non-activated cells.

C2C12 cells were maintained in GelMA construct during the whole process (acoustic excitation, printing, UV curing and IF staining). Fluorescent micrographs show cell nuclei

(blue color), and myosin heavy chain (green color) for the cells without (see Fig 6.6a) and with acoustic excitation (see Fig.6.6b). Cell nuclei were counterstained with DAPI. With fluorescent signal, the orientation of cell nuclei was analyzed. The normalized number of cell orientation was plotted versus orientation angle of cell nuclei ranging from -90° to $+90^{\circ}$. Firstly, the quantitative analysis within the same plot and the group of cells (without or with excitation) was evaluated. It was found that the cell without the acoustic excitation has no significant pattern (see Fig. 6.6e) and its standard deviation is relatively high. There is no statistical difference of cell nuclei orientation angle for the cells without acoustic excitation. On the other hand, the number of cell nuclei orientation with the acoustic excitation shows a sharp Gaussian curve pattern (see Fig. 6.6f). Also, a prominently high number of cells from -30° to $+30^{\circ}$ orientation angle was found. Its value peaks at 0° and shows a horizontal baseline from -100° to -40° and 40° to 100° orientation angle (see the blue horizontal dotted line in Fig 6.6f). Even though the standard deviation is high, the plot of average $\pm$ sd from -10° to 10° does not overlap the baseline, which implies a distinct statistical difference ($p < 0.01$).

The difference across the groups of cells without and with acoustic excitation was further evaluated. The curve in Figs. 6.6e and 6.6f were fitted with the Gaussian curve. Afterwards, the standard deviation of the fitted curve was compared between the groups. A huge significantly lower value of standard deviation of the fitted Gaussian curve was found (1.91-fold, $p < 0.01$, grey bars in Fig. 6.6b). In addition, the circularity of cell nuclei was measured and compared between cells without and with acoustic excitation. It was found that the cells with acoustic excitation have a statistically significant lower value of cell nuclei circularity as compared to the cells without acoustic excitation ($p < 0.01$, white bars in Fig. 6.6g).

6.4 Discussion

The patterning of the cells using the acoustic excitation was demonstrated in this work. There is a good agreement between numerical simulation and experimental validation on the excitation frequency and location of cell accumulation in the cylindrical tube and the nozzle. The resonant frequency of structural vibration of the cylindrical glass tube was numerically and experimentally determined as 871 kHz and 877 kHz, respectively. Also, in this work, the error of predicted resonant frequency from simulation and actual resonant frequency from the experiment is in a similar range ($\approx 0.7\%$) in comparison to previous studies [32, 98, 202]. The slight difference in the excitation frequency might be due to the inconsistency of the tube diameter along the longitudinal axis and material properties. Cylindrical tube with quite similar inner diameter but different in material properties have different resonant frequency [32]. In addition, the timing of microparticle and cell to reach pressure node is different (0.73 sec and 1.66 sec for microparticle and cells, respectively). This is due to the fact that acoustic radiation force is dependent on diameter, compressibility, and density of the cells. Specifically, the compressibility of the microparticle is half of surrounding medium. On the contrary, the cell has a quite similar compressibility with the surrounding medium. As a result, cells were pushed with a significant lower magnification of acoustic radiation force as described in Eq.3.3.

Evaluation of cell distribution after printing indicated a significantly lower value of standard deviation (0.27 ± 0.07 mm) of cell undergone acoustic excitation while distribution of cells without excitation has a standard deviation of 0.42 ± 0.12 mm. This implies that acoustic waves well accumulate cells towards the center (p -value < 0.05) which suggested that acoustic excitation in the cylindrical tube could accumulate cells in the nozzle and in the printed GelMA construct. This is similar to the previous studies which microparticles were accumulated in the capillary tube [32, 224] and cylindrical tube in the flow cytometer [33]. Additionally, both cell groups have slight shift of the central line of cell distribution (represented by mean value) away

from the center of the printed GelMA construct (≈ 0.06 mm away from the center), which may be due to the imbalance of the printing stage and the mass-loaded effect of the piezoceramic plate.

Subsequently, the cells have been monitored for 7 days for their growth, morphological changes, and differentiation. The width of accumulated C1C12 cells throughout the printed construct showed a slight shrinkage which is usually found in the differentiation of the cells in the confined space [225-227]. With the acoustic excitation, the cells were densely packed at the center of the nozzle and printed construct (see Fig. 6.3d), while cells in the control group were scattered (see Fig. 6.3a). The orientation of the cell elongation showed that most of the cells elongated along the major axis. The acoustic manipulation of cells shows a significant dense cell structure before and even after cell differentiation, while cells in the control group without acoustic excitation were distributed more scattered. Both orientation of cell morphology (see Fig. 6.5d) and cell nuclei (see Fig. 6.6f) represented significant cell orientation along the major axis. This strengthens the evidence of improved C2C12 cell orientation by using acoustic excitation. Moreover, C2C12 cells are the skeletal myoblast cell line and differentiate to form the skeletal muscles. Accordingly, they must densely pack together to form the muscle fascicles. Therefore, the closely tight aggregation of these cells might expedite the cell-to-cell interaction causing a completion of skeletal muscle morphogenesis [228-230]. In addition, the low value of cell nuclei circularity suggested that the cell morphology is fastigiate along one direction (the major axis). Implicitly, this represents better elongation and proliferation of C2C12 cells undergone acoustic excitation. Also, the significantly lower value of cell nuclei circularity of C2C12 cells with acoustic excitation suggested that acoustic excitation is able to promote the elongation and orientation [231, 232].

To optimize the orientation of the cells, the increase of acoustic power may be one of the solutions. In the nozzle, the cells move from their original position towards the center by

acoustic radiation force. This force could be increased directly with the increase of power input to the piezoelectric transducer which increases the vibration magnitude. Higher radiation force causes the microparticle/cells to be accumulated densely [192] with less timing [156, 233]. Dense cell density in narrow confinement could promote the cell elongation and orientation. C2C12 cells showed a better cell orientation in the narrower confinement (e.g. PDMS, MPC polymer microchannel) [226, 234, 235] representing a sharper cell orientation plot.

This acoustic excitation at the nozzle could be applied to other types of cells due to the fact most of the biological cells required closed contact between cells to grow. Most of the biological cells require contact with nearby cells to support its proliferation and differentiation. The primary cells derived from the human organs (e.g. liver, heart, and skin) could be also further tested. Accumulation of cells may provide a suitable environment for them to attach with nearby cells.

Another advantage of this acoustic excitation is to selectively accumulate microparticle/cells by the diameter of the cells. The magnitude of acoustic radiation force acting on the large cells is higher as it is proportional to the cube of cell's diameter. Hence, this technique is able to accumulate only large cells into a densely packed single or multiple lines while leaving small cells scattered randomly in the printed construct. This is to imitate the allocation of co-culture system in nature. For example, a human blood vessel in the dermis grown from endothelial cells (mostly HUVEC) surrounded by groups of fibroblasts, pericyte, and muscle cells. The proposed method to imitate the environment of the vessel is to apply acoustic excitation to co-culture of HUVECs (larger cells, $\approx 10\ \mu\text{m}$ dia.) and fibroblasts (smaller cells, $\approx 4\ \mu\text{m}$ dia.). Due to the size difference (≈ 2.5 times), under the same acoustic field, the fibroblasts receive ≈ 15 times lower in magnitude. Under the acoustic excitation which is enough for HUVEC to move within a couple of seconds, while the fibroblasts virtually remain at the original location.

6.5 Summary

Patterning of biological cells in the 3D printed construct using the structural vibration was evaluated in this work. The accumulation of cells at the center of the cylindrical tube was investigated both numerically and experimentally. The results of numerical simulation and experiment are agreeable on excitation frequency and location of cells. In the experiment, cells were accumulated at the center of the nozzle and consequently at the printed construct, the cell distribution with acoustic excitation has a significantly lower value of standard deviation (0.27 ± 0.07 mm) than the control without the acoustic excitation (0.42 ± 0.12 mm). Furthermore, the acoustic excitation could also be used for patterning C2C12 cells in the 3D printed construct. After printing, the distribution of cells is found to be dense at the center of the printed construct. Subsequently, it is found that the acoustically-excited cells establish cellular connections and elongate towards the printing direction. Also, immunofluorescent staining indicates a greater alignment/orientation of cell nuclei and myosin heavy chain produced from differentiation of C2C12. Lastly, acoustically-excited C2C12 cells represent a significantly improved orientation of cell nuclei with a high number of oriented cells along the major axis in comparison to the control. The zoomed-in figure unveils the similarity of the orientation of the acoustically-excited C2C12 muscle fibers to the natural skeletal muscle fibers. This acoustic excitation is a convenient, cost-effective and biocompatible method for patterning and accumulation of cells. There are several advantages such as allowing high cell density printing and patterning of cells without nozzle clogging issue. Importantly, it increases the number of orientated cells along the major axis and enhances cell elongation and differentiation.

Chapter 7 Conclusion and Future work

7.1 Conclusion

The use of acoustic excitation with PDMS microchannel and nozzle of 3D printer was demonstrated in this research work. Acoustic technique is capable of patterning the microparticles/cells during 3D printing process and suppresses the nozzle clogging. Prior to the study with the nozzle of 3D printer, SAW was applied to the constriction area to investigate the reduction of microparticle accumulation in PDMS microchannel in Chapter 3. Initially, the progressive growth of the accumulation area without SAW excitation was studied. The accumulation area increases with the concentration of alginate and the constriction angle. Also, the accumulation area can be fitted by an exponential curve ($R^2 > 0.9$). Then, SSAW was applied continuously to the microchannel in order to reduce the microparticle accumulation and suppress clogging. Significant reduction in the accumulation area was found (2.0-3.7 folds) regardless of the constriction angle but decreasing with a concentration of alginate or the fluid viscosity. It implies that acoustic technique has a significant effect on decreasing the accumulation area and suppressing the nozzle clogging.

Afterwards, in Chapter 4, SAW dual-frequency excitation was utilized to enhance the tunability and efficiency on microparticles/cells manipulation by SAW. This technique varies the power ratio of fundamental to the third harmonic applied to a pair of IDTs. The number and position of pressure nodes for particle accumulation and the percentages of particles at each pressure node can be adjusted dynamically without changing any on-chip or off-chip parts. Once the power ratio is above 90%, there is only one pressure node, same as that produced by the fundamental frequency only. In comparison, three pressure nodes could be produced with varying positions and particle concentration at the power ratio below 90%. Such

a critical power ratio is independent of the driving frequency, power input, and the diameter of particles.

In Chapter 5, the use of acoustic manipulation to directly assist the additive manufacturing (extrusion-based printing) was demonstrated. The structural vibration of a cylindrical tube with tapered tip was produced, and the acoustic wave was coupled into the ink to accumulate the microparticles to the position of induced pressure node(s). The prediction of the excitation frequency and location of microparticles inside the glass tube in the numerical simulation agrees quite well with the experimental results. Acoustic excitation has the statistically significant effect on the microparticle accumulation in the glass tube. The time and width of microparticle accumulation under the acoustic excitation increase with the concentration of alginate and microparticles in the ink. Although the microparticle concentration has a slightly more significant effect on the accumulation width than the alginate concentration, its effect on the accumulation time is significantly less. In the printed structure, the distribution of microparticles could be fitted well in a Gaussian curve, whose FWHM is usually larger than that in the glass tube during the printing process through the tapered tip. However, the dependence of microparticle accumulation in the printed structure on the microparticle and alginate concentrations is similar to that in the tube. The high orders of structural vibration could reduce the microparticle accumulation time and produce more complicated accumulation patterns. Besides, acoustic excitation in the nozzle could delay the accumulation area of microparticles at the nozzle constriction region and maintain the outflow discharge rate.

In Chapter 6, biological cells (muscle cell lines, C2C12) were patterned in the 3D printed construct using the acoustic nozzle. The accumulation of cells at the center of the cylindrical tube was investigated both numerically and experimentally. The results of the numerical simulation and experiment are agreeable on excitation frequency and location of

cells. In the experiment, cells were accumulated at the center of the nozzle and consequently at the printed construct. The cell distribution with acoustic excitation has a significantly lower value of standard deviation (0.27 ± 0.07 mm) than the cell without the acoustic excitation (0.42 ± 0.12 mm). Furthermore, the acoustic excitation could also be used for patterning C2C12 cells in the 3D printed construct. After printing, the distribution of cells is accumulated densely at the center of the printed construct. Subsequently, it is found that the acoustically-excited cells establish cellular connections and elongate towards the printing direction. Also, immunofluorescent staining indicates a greater alignment/orientation of cell nuclei and myosin heavy chain produced from differentiation of C2C12. Lastly, acoustically-excited C2C12 cells represent a significantly improved orientation of cell nuclei with a high number of oriented cells along the major axis in comparison to the cells without the acoustic excitation. The zoomed-in figure unveils the similarity of the orientation of the acoustically-excited C2C12 muscle fibers to the natural skeletal muscle fibers. This acoustic excitation is a convenient, cost-effective and biocompatible method for patterning and accumulation of cells. Also, there are several advantages such as allowing high cell density printing, patterning of cells without nozzle clogging issue. Importantly, it increases the number of orientated cells along the major axis and enhances cell elongation and differentiation.

In summary, the results of this research work achieve the objectives of the study. This acoustic manipulation method is incorporated with the nozzle of the 3D printer to allow patterning and accumulation of microparticle/biological cells as well as suppress clogging of the nozzle.

7.2 Future work

The results from this research work suggest that acoustic excitation could pattern/accumulate the microobjects suspended in printing ink and suppress of nozzle clogging. The future work is discussed, but not limited to the following points.

For patterning/accumulation of cells, it is widely known that most of the biological cells require contact with nearby cells to support its proliferation and differentiation. Accumulation by the acoustic waves could gather cells together and enhance the biological activities of cells. In the future, this technology could be applied to other types of cells. The primary cells derived from the human organs (e.g. liver, heart, and skin) could be also further tested. Accumulation of cells may provide a suitable environment for cells to attach with nearby cells.

The accumulation pattern of microparticles with the power input is a crucial experiment condition to be studied. In the future, these phenomena could be further investigated especially at the transition between regimes. Basically, there are three main regimes proposed in this research work depending on the accumulation pattern of microparticles in the cylindrical glass tube. Firstly, with an optimum power input (which is in 0.28-0.42 W) applied to the piezoelectric plate, dipole mode is formed in the tube. This cause microparticles to form a single line at the center of the tube by acoustic radiation force (See Fig.7.1a). Secondly, with slightly higher power (≈ 0.63 W), the microparticles further form several nodes along longitudinal direction while maintain at the central location of the cylindrical tube (See Fig.7.1b). Thirdly, however, at the high-power input (over 1.2 W), vortexes could be formed and rotate microparticles away from the central location. Above all, the exact value of power input in each regime depends on experiment condition such as material, thickness of cylindrical tube and piezoelectric material, polarization direction, excitation frequency, coupling efficiency and piezoelectricity modulus.

Figure 7.1. Accumulation of microparticles in cylindrical glass tube at (a) 0.28 W and, (b) 0.63 W [202].

Moreover, there is a potential to apply this technology to aerosol printing. This technique is able to manipulate microparticles/cells or water droplet in the air. Currently, the nozzle of the aerosol printer consists of high-pressure airflow and liquid stream at the center to deliver the printing ink. The high-pressure air flows through the nozzle and squeezes the stream of printing ink at the center. The stream of printing ink is injected onto the substrate and the high-pressure air is released to the environment. This aerosol jet is usually used for printing electronic parts. The printing ink consists of solvent and metallic powder which is a main component of the circuit. However, to achieve high electrical performance and conductivity, the printed metallic powder should gather densely enough and be deposited on the desired area as accurately as possible [236]. With the current air flow, the liquid stream could be a squeeze, but it is not enough to pack the metallic microparticles/powder together. Also, during printing very high air pressure could not be used as it affects the printing efficiency. The acoustic method could potentially be applied together with the air pressure method to accumulate the metallic powder/microparticles gather and control the liquid stream toward the center. In practice, the resonance frequency of the transducer is lower by three times as the speed of sound in air is one-third of the speed of sound in water. Also, the density of air is much lower than that of water which increases acoustic contrast factor significantly. Importantly, in the air, the drag force is much lower in magnitude as the viscosity of air ($18 \mu\text{Pa}\cdot\text{s}$) is lower than that of water ($0.8 \text{ mPa}\cdot\text{s}$) by about 50 times. Finally, the microparticles/cells move theoretically faster in the air without a consideration of vibration propagation efficiency from solid to air or water.

To commercialize this technology, the individual user could install acoustic nozzle kit with their syringe (for the extrusion-based printer). The first step is to simplify and downsize the electrical supply devices (signal generator and power amplifier). The signal generator and power amplifier in the lab are large as they are designed for wide range of frequency (e.g. 100 kHz- 25 MHz range). However, there is only one resonance frequency for the specific inner diameter of the nozzle. The device could be designed as a clip to attach around the nozzle of any printers. Lastly, the guideline of excitation frequency and power level should be given to the user to install and operate the acoustic device incorporated with the nozzle.

References

- [1] D. Rotman, "Microscale 3-D Printing. (cover story)," *Technology Review*, vol. 117, pp. 38-41, 05//May/Jun2014 2014.
- [2] C. Schubert, M. C. van Langeveld, and L. A. Donoso, "Innovations in 3D printing: a 3D overview from optics to organs," *BRITISH JOURNAL OF OPHTHALMOLOGY*, vol. 98, pp. 159-161, 2014.
- [3] A. M. R. G. a. L. University. (13 April). *The 7 Categories of Additive Manufacturing*. Available: <http://www.lboro.ac.uk/research/amrg/about/>
- [4] L. R. Holmes and J. C. Riddick, "Research summary of an additive manufacturing technology for the fabrication of 3D composites with tailored internal structure," *JOM*, vol. 66, pp. 270-274, 2014.
- [5] Q. Fu, E. Saiz, and A. P. Tomsia, "Direct ink writing of highly porous and strong glass scaffolds for load-bearing bone defects repair and regeneration," *Acta biomaterialia*, vol. 7, pp. 3547-3554, 2011.
- [6] A. R. A. Fattah, A. M. Abdalla, S. Mishriki, E. Meleca, F. Geng, S. Ghosh, *et al.*, "Magnetic Printing of a Biosensor: Inexpensive Rapid Sensing To Detect Picomolar Amounts of Antigen with Antibody-Functionalized Carbon Nanotubes," *ACS applied materials & interfaces*, vol. 9, pp. 11790-11797, 2017.
- [7] T. A. Campbell and O. S. Ivanova, "3D printing of multifunctional nanocomposites," *Nano Today*, vol. 8, pp. 119-120, 2013.
- [8] B. Dersoir, M. R. de Saint Vincent, M. Abkarian, and H. Tabuteau, "Clogging of a single pore by colloidal particles," *Microfluidics and Nanofluidics*, vol. 19, pp. 953-961, 2015.
- [9] C. Kim, D. Espalin, A. Cuaron, M. A. Perez, E. MacDonald, and R. B. Wicker, "A study to detect a material deposition status in fused deposition modeling technology," in *Advanced Intelligent Mechatronics (AIM), 2015 IEEE International Conference on*, 2015, pp. 779-783.
- [10] T. Billiet, M. Vandenhaute, J. Schelfhout, S. Van Vlierberghe, and P. Dubruel, "A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering," *Biomaterials*, vol. 33, pp. 6020-6041, 2012.
- [11] J. M. Meacham, A. O'Rourke, Y. Yang, A. G. Fedorov, F. L. Degertekin, and D. W. Rosen, "Micromachined Ultrasonic Print-Head for Deposition of High-Viscosity Materials," *Journal of Manufacturing Science and Engineering*, vol. 132, pp. 030905-030905, 2010.
- [12] V. Mironov, T. Boland, T. Trusk, G. Forgacs, and R. R. Markwald, "Organ printing: computer-aided jet-based 3D tissue engineering," *TRENDS in Biotechnology*, vol. 21, pp. 157-161, 2003.
- [13] S. Parsa, M. Gupta, F. Loizeau, and K. C. Cheung, "Effects of surfactant and gentle agitation on inkjet dispensing of living cells," *Biofabrication*, vol. 2, p. 025003, 2010.
- [14] M. E. Pepper, V. Seshadri, T. Burg, B. W. Booth, K. J. Burg, and R. E. Groff, "Cell settling effects on a thermal inkjet bioprinter," in *Engineering in Medicine and Biology Society, EMBC, 2011 Annual International Conference of the IEEE*, 2011, pp. 3609-3612.
- [15] R. R. Jose, M. J. Rodriguez, T. A. Dixon, F. Omenetto, and D. L. Kaplan, "Evolution of Bioinks and Additive Manufacturing Technologies for 3D Bioprinting," *ACS Biomaterials Science & Engineering*, 2016/03/25 2016.
- [16] B. G. Thomas and H. Bai, "Tundish nozzle clogging-application of computational models," in *Steelmaking Conference Proceedings*, 2001, pp. 895-912.

- [17] K. Rackers and B. Thomas, "Clogging in continuous casting nozzles," *Urbana*, vol. 51, p. 61801, 1995.
- [18] Y.-f. CHEN, J.-s. LI, L.-n. AN, L.-y. SUN, and R.-z. LIU, "Physical Simulation on the Mold Flow Field Impacted by Nozzle Clogging [J]," *Industrial Heating*, vol. 5, p. 017, 2012.
- [19] T. Wang, C. C. Cook, S. Serban, T. Ali, G. Drago, and B. Derby, "Fabrication of glucose biosensors by inkjet printing," *arXiv preprint arXiv:1207.1190*, 2012.
- [20] S.-P. Chen, H.-L. Chiu, P.-H. Wang, and Y.-C. Liao, "Inkjet Printed Conductive Tracks for Printed Electronics," *ECS Journal of Solid State Science and Technology*, vol. 4, pp. P3026-P3033, 2015.
- [21] M. J. Rosen and J. T. Kunjappu, *Surfactants and interfacial phenomena*: John Wiley & Sons, 2012.
- [22] S. S. Chadwick, "Ullmann's Encyclopedia of Industrial Chemistry," *Reference Services Review*, vol. 16, pp. 31-34, 1988.
- [23] G. K. Batchelor, *An introduction to fluid dynamics*: Cambridge university press, 2000.
- [24] A. Lee, K. Sudau, K. H. Ahn, S. J. Lee, and N. Willenbacher, "Optimization of experimental parameters to suppress nozzle clogging in inkjet printing," *Industrial & Engineering Chemistry Research*, vol. 51, pp. 13195-13204, 2012.
- [25] A. Janda, D. Maza, A. Garcimartín, E. Kolb, J. Lanuza, and E. Clément, "Unjamming a granular hopper by vibration," *EPL (Europhysics Letters)*, vol. 87, p. 24002, 2009.
- [26] I. Zuriguel, D. R. Parisi, R. C. Hidalgo, C. Lozano, A. Janda, P. A. Gago, *et al.*, "Clogging transition of many-particle systems flowing through bottlenecks," *Scientific reports*, vol. 4, 2014.
- [27] F. Guo, Y. Xie, S. Li, J. Lata, L. Ren, Z. Mao, *et al.*, "Reusable acoustic tweezers for disposable devices," *Lab on a Chip*, 2015.
- [28] Y. Chen, S. Li, Y. Gu, P. Li, X. Ding, L. Wang, *et al.*, "Continuous enrichment of low-abundance cell samples using standing surface acoustic waves (SSAW)," *Lab on a Chip*, vol. 14, pp. 924-930, 2014.
- [29] J. Shi, D. Ahmed, X. Mao, S.-C. S. Lin, A. Lawit, and T. J. Huang, "Acoustic tweezers: patterning cells and microparticles using standing surface acoustic waves (SSAW)," *Lab on a Chip*, vol. 9, pp. 2890-2895, 2009.
- [30] M. C. Jo and R. Guldiken, "Particle manipulation by phase-shifting of surface acoustic waves," *Sensors and Actuators A: Physical*, vol. 207, pp. 39-42, 2014.
- [31] J. Shi, X. Mao, D. Ahmed, A. Colletti, and T. J. Huang, "Focusing microparticles in a microfluidic channel with standing surface acoustic waves (SSAW)," *Lab on a Chip*, vol. 8, pp. 221-223, 2008.
- [32] G. Goddard and G. Kaduchak, "Ultrasonic particle concentration in a line-driven cylindrical tube," *The Journal of the Acoustical Society of America*, vol. 117, pp. 3440-3447, 2005.
- [33] G. Goddard, J. C. Martin, S. W. Graves, and G. Kaduchak, "Ultrasonic particle-concentration for sheathless focusing of particles for analysis in a flow cytometer," *cytometry part A*, vol. 69, pp. 66-74, 2006.
- [34] S. V. Murphy and A. Atala, "3D bioprinting of tissues and organs," *Nat Biotech*, vol. 32, pp. 773-785, 08/print 2014.
- [35] M. S. Mannoer, Z. Jiang, T. James, Y. L. Kong, K. A. Malatesta, W. O. Soboyejo, *et al.*, "3D Printed Bionic Ears," *Nano Letters*, vol. 13, pp. 2634-2639, 2013/06/12 2013.
- [36] J. W. Gooch, *Encyclopedic Dictionary of Polymers*, 2007.
- [37] P. Ghosh, "DLVO Theory and Non-DLVO Forces " in *NPTEL - Chemical Engineering - Interfacial Engineering* ed, 2012.

- [38] H. Hamaker, "The London—van der Waals attraction between spherical particles," *physica*, vol. 4, pp. 1058-1072, 1937.
- [39] R. Winterton, "Van der Waals forces," *Contemporary Physics*, vol. 11, pp. 559-574, 1970.
- [40] B. Dersoir, "Clogging in micro-channels: from colloidal particle to clog," Université Rennes 1, 2015.
- [41] W. B. Russel, D. A. Saville, and W. R. Schowalter, *Colloidal dispersions*: Cambridge university press, 1992.
- [42] A. Glendinning and W. Russel, "The electrostatic repulsion between charged spheres from exact solutions to the linearized Poisson-Boltzmann equation," *Journal of colloid and interface science*, vol. 93, pp. 95-104, 1983.
- [43] D. Y. Chan, R. M. Pashley, and L. R. White, "A simple algorithm for the calculation of the electrostatic repulsion between identical charged surfaces in electrolyte," *Journal of Colloid and Interface Science*, vol. 77, pp. 283-285, 1980.
- [44] V. Ramachandran, R. Venkatesan, G. Tryggvason, and H. S. Fogler, "Low Reynolds number interactions between colloidal particles near the entrance to a cylindrical pore," *Journal of colloid and interface science*, vol. 229, pp. 311-322, 2000.
- [45] C. Zhang, "Hydration Force," in *Encyclopedia of Tribology*, Q. J. Wang and Y.-W. Chung, Eds., ed Boston, MA: Springer US, 2013, pp. 1704-1708.
- [46] P. Atkins and J. De Paula, *Physical chemistry for the life sciences*: Oxford University Press, USA, 2011.
- [47] R. Chang, *Physical chemistry for the biosciences*: University Science Books, 2005.
- [48] H. M. Wyss, D. L. Blair, J. F. Morris, H. A. Stone, and D. A. Weitz, "Mechanism for clogging of microchannels," *PHYSICAL REVIEW-SERIES E-*, vol. 74, p. 061402, 2006.
- [49] V. Ramachandran and H. S. Fogler, "Plugging by hydrodynamic bridging during flow of stable colloidal particles within cylindrical pores," *Journal of Fluid Mechanics*, vol. 385, pp. 129-156, 1999.
- [50] K. Sharp and R. Adrian, "On flow-blocking particle structures in microtubes," *Microfluidics and Nanofluidics*, vol. 1, pp. 376-380, 2005.
- [51] M. Auset and A. A. Keller, "Pore-scale visualization of colloid straining and filtration in saturated porous media using micromodels," *Water resources research*, vol. 42, 2006.
- [52] B. Mustin and B. Stoeber, "Deposition of particles from polydisperse suspensions in microfluidic systems," *Microfluidics and nanofluidics*, vol. 9, pp. 905-913, 2010.
- [53] A. Sauret, E. C. Barney, A. Perro, E. Villermaux, H. A. Stone, and E. Dresseire, "Clogging by sieving in microchannels: Application to the detection of contaminants in colloidal suspensions," *Applied Physics Letters*, vol. 105, p. 074101, 2014.
- [54] M. Alaskar, K. Li, and R. Horne, "Influence of particle size on its transport in discrete fractures: pore-scale visualization using micromodels," in *38th Stanford Geothermal Workshop*, Stanford, CA, 2013.
- [55] D. Gassara, P. Schmitz, A. Ayadi, and M. Prat, "Modelling the effect of particle size in microfiltration," *Separation Science and Technology*, vol. 43, pp. 1754-1770, 2008.
- [56] I. B. Hassan, C. Lafforgue, A. Ayadi, and P. Schmitz, "In situ 3D characterization of monodispersed spherical particle deposition on microsieve using confocal laser scanning microscopy," *Journal of Membrane Science*, vol. 454, pp. 283-297, 2014.
- [57] G. Brans, A. van Dinther, B. Odum, C. Schroën, and R. Boom, "Transmission and fractionation of micro-sized particle suspensions," *Journal of membrane science*, vol. 290, pp. 230-240, 2007.

- [58] V. Ramachandran and H. S. Fogler, "Multilayer deposition of stable colloidal particles during flow within cylindrical pores," *Langmuir*, vol. 14, pp. 4435-4444, 1998.
- [59] G. C. Agbanga, É. Climent, and P. Bacchin, "Experimental investigation of pore clogging by microparticles: Evidence for a critical flux density of particle yielding arches and deposits," *Separation and Purification Technology*, vol. 101, pp. 42-48, 2012.
- [60] P. Bacchin, A. Marty, P. Duru, M. Meireles, and P. Aimar, "Colloidal surface interactions and membrane fouling: investigations at pore scale," *Advances in colloid and interface science*, vol. 164, pp. 2-11, 2011.
- [61] J. Marshall and J. Kitchener, "The deposition of colloidal particles on smooth solids," *Journal of Colloid and Interface Science*, vol. 22, pp. 342-351, 1966.
- [62] N. Ryde, N. Kallay, and E. Matijević, "Particle adhesion in model systems. Part 14.—Experimental evaluation of multilayer deposition," *J. Chem. Soc., Faraday Trans.*, vol. 87, pp. 1377-1381, 1991.
- [63] C.-H. Ko and M. Elimelech, "The "shadow effect" in colloid transport and deposition dynamics in granular porous media: measurements and mechanisms," *Environmental science & technology*, vol. 34, pp. 3681-3689, 2000.
- [64] Z. Adamczyk, B. Siwek, M. Zembala, and P. Belouschek, "Kinetics of localized adsorption of colloid particles," *Advances in Colloid and Interface Science*, vol. 48, pp. 151-280, 1994.
- [65] P. R. Johnson and M. Elimelech, "Dynamics of colloid deposition in porous media: Blocking based on random sequential adsorption," *Langmuir*, vol. 11, pp. 801-812, 1995.
- [66] J. F. Brady and G. Bossis, "Stokesian dynamics," *Annual review of fluid mechanics*, vol. 20, pp. 111-157, 1988.
- [67] M. Whittle, B. S. Murray, J. Chen, and E. Dickinson, "Simulation and experiments on colloidal particle capture in a shear field," *Langmuir*, vol. 16, pp. 9784-9791, 2000.
- [68] B. Zhao, Z. Zhang, and X. Li, "Numerical study of the transport of droplets or particles generated by respiratory system indoors," *Building and Environment*, vol. 40, pp. 1032-1039, 2005.
- [69] B. Zhao, Z. Zhang, X. Li, and D. Huang, "Comparison of Diffusion Characteristics of Aerosol Particles in Different Ventilated Rooms by Numerical Method," *ASHRAE Transactions*, vol. 110, 2004.
- [70] S. Murakami, S. Kato, S. Nagano, and Y. Tanaka, "Diffusion characteristics of airborne particles with gravitational settling in a convection-dominant indoor flow field," in *ASHRAE Winter Meeting*, 1992, pp. 82-97.
- [71] S. Friedlander and H. Johnstone, "Deposition of suspended particles from turbulent gas streams," *Industrial & Engineering Chemistry*, vol. 49, pp. 1151-1156, 1957.
- [72] C. N. Davies, *Aerosol science* vol. 1102: Academic Press London, 1966.
- [73] A. Dehbi, "A CFD model for particle dispersion in turbulent boundary layer flows," *Nuclear engineering and design*, vol. 238, pp. 707-715, 2008.
- [74] A. Guha, "Transport and deposition of particles in turbulent and laminar flow," 2008.
- [75] H. El-Batsh and H. Haselbacher, "Numerical investigation of the effect of ash particle deposition on the flow field through turbine cascades," in *ASME Turbo Expo 2002: Power for Land, Sea, and Air*, 2002, pp. 1035-1043.
- [76] R. M. Brach and P. F. Dunn, "A mathematical model of the impact and adhesion of microspheres," *Aerosol Science and Technology*, vol. 16, pp. 51-64, 1992.
- [77] H.C.Wang, *Aerosol Sci.*, vol. 13, pp. 386-396, 1990.

- [78] M. Soltani and G. Ahmadi, "On particle adhesion and removal mechanisms in turbulent flows," *Journal of Adhesion Science and Technology*, vol. 8, pp. 763-785, 1994.
- [79] G. Destgeer and H. J. Sung, "Recent advances in microfluidic actuation and micro-object manipulation via surface acoustic waves," *Lab on a Chip*, 2015.
- [80] Y. Ai, C. K. Sanders, and B. L. Marrone, "Separation of Escherichia coli bacteria from peripheral blood mononuclear cells using standing surface acoustic waves," *Analytical chemistry*, vol. 85, pp. 9126-9134, 2013.
- [81] P. Bacchin, Q. Derckx, D. Veyret, K. Glucina, and P. Moulin, "Clogging of microporous channels networks: role of connectivity and tortuosity," *Microfluidics and nanofluidics*, vol. 17, pp. 85-96, 2014.
- [82] S. Li, F. Guo, Y. Chen, X. Ding, P. Li, L. Wang, *et al.*, "Standing Surface Acoustic Wave Based Cell Coculture," *Analytical chemistry*, vol. 86, pp. 9853-9859, 2014.
- [83] M. Bumiller. (2015, 18/4). Dispersing Powders in Liquid. Available: http://www.horiba.com/fileadmin/uploads/Scientific/Documents/PSA/Webinar_Slides/TR016.pdf
- [84] F. Guo, P. Li, J. B. French, Z. Mao, H. Zhao, S. Li, *et al.*, "Controlling cell-cell interactions using surface acoustic waves," *Proceedings of the National Academy of Sciences*, vol. 112, pp. 43-48, 2015.
- [85] C. Witte, J. Reboud, R. Wilson, J. Cooper, and S. Neale, "Microfluidic resonant cavities enable acoustophoresis on a disposable superstrate," *Lab on a Chip*, vol. 14, pp. 4277-4283, 2014.
- [86] A. Kundt and O. Lehmann, "Ueber longitudinale Schwingungen und Klangfiguren in cylindrischen Flüssigkeitssäulen," *Annalen der Physik*, vol. 229, pp. 1-12, 1874.
- [87] P. Vainshtein, M. Fichman, K. Shuster, and C. Gutfinger, "The effect of centreline particle concentration in a wave tube," *Journal of Fluid Mechanics*, vol. 306, pp. 31-42, 1996.
- [88] A. Goldshtein, K. Shuster, P. Vainshtein, M. Fichman, and C. Gutfinger, "Particle motion in resonance tubes," *Journal of Fluid Mechanics*, vol. 360, pp. 1-20, 1998.
- [89] W. Coakley, J. Hawkes, M. Sobanski, C. Cousins, and J. Spengler, "Analytical scale ultrasonic standing wave manipulation of cells and microparticles," *Ultrasonics*, vol. 38, pp. 638-641, 2000.
- [90] A. Haake and J. Dual, "Micro-manipulation of small particles by node position control of an ultrasonic standing wave," *Ultrasonics*, vol. 40, pp. 317-322, 2002.
- [91] M. Saito, K. Itagaki, K. Hayashi, and K. Tsubata, "Composite materials with ultrasonically induced layer or lattice structure," *Japanese journal of applied physics*, vol. 38, p. 3028, 1999.
- [92] S. Yamahira, S.-i. Hatanaka, M. Kuwabara, and S. Asai, "Orientation of fibers in liquid by ultrasonic standing waves," *Japanese Journal of Applied Physics*, vol. 39, p. 3683, 2000.
- [93] S. Gupta, D. L. Feke, and I. Manas-Zloczower, "Fractionation of mixed particulate solids according to compressibility using ultrasonic standing wave fields," *Chemical Engineering Science*, vol. 50, pp. 3275-3284, 1995.
- [94] G. Whitworth, M. Grundy, and W. Coakley, "Transport and harvesting of suspended particles using modulated ultrasound," *Ultrasonics*, vol. 29, pp. 439-444, 1991.
- [95] Y. Yamakoshi and Y. Noguchi, "Micro particle trapping by opposite phases ultrasonic travelling waves," *Ultrasonics*, vol. 36, pp. 873-878, 1998.
- [96] M. Wiklund, S. Nilsson, and H. M. Hertz, "Ultrasonic trapping in capillaries for trace-amount biomedical analysis," *Journal of applied physics*, vol. 90, pp. 421-426, 2001.

- [97] M. Wiklund, P. Spégel, S. Nilsson, and H. M. Hertz, "Ultrasonic-trap-enhanced selectivity in capillary electrophoresis," *Ultrasonics*, vol. 41, pp. 329-333, 2003.
- [98] G. R. Goddard, C. K. Sanders, J. C. Martin, G. Kaduchak, and S. W. Graves, "Analytical performance of an ultrasonic particle focusing flow cytometer," *Analytical chemistry*, vol. 79, pp. 8740-8746, 2007.
- [99] G. V. Borgiotti and E. M. Rosen, "The state vector approach to the wave and power flow analysis of the forced vibrations of a cylindrical shell. Part I: Infinite cylinders in vacuum," *The Journal of the Acoustical Society of America*, vol. 91, pp. 911-925, 1992.
- [100] G. V. Borgiotti and E. M. Rosen, "The state vector approach to the wave analysis of the forced vibration of a cylindrical shell. Part II: Finite cylinders in vacuum," *The Journal of the Acoustical Society of America*, vol. 93, pp. 864-874, 1993.
- [101] P. Glynne-Jones and M. Hill, "Acoustofluidics 23: acoustic manipulation combined with other force fields," *Lab on a Chip*, vol. 13, pp. 1003-1010, 2013.
- [102] H. Bruus, "Acoustofluidics 7: The acoustic radiation force on small particles," *Lab on a Chip*, vol. 12, pp. 1014-1021, 2012.
- [103] M. Settnes and H. Bruus, "Forces acting on a small particle in an acoustical field in a viscous fluid," *Physical Review E*, vol. 85, p. 016327, 2012.
- [104] K. Gnanasekaran, T. Heijmans, S. van Bennekom, H. Woldhuis, S. Wijnia, and H. Friedrich, "3D printing of CNT-and graphene-based conductive polymer nanocomposites by fused deposition modeling," *Applied Materials Today*, vol. 9, pp. 21-28, 2017.
- [105] F. J. Kamphoefner, "Ink jet printing," *Electron Devices, IEEE Transactions on*, vol. 19, pp. 584-593, 1972.
- [106] B. Derby, "Inkjet printing of functional and structural materials: fluid property requirements, feature stability, and resolution," *Annual Review of Materials Research*, vol. 40, pp. 395-414, 2010.
- [107] H. Sirringhaus, T. Kawase, R. Friend, T. Shimoda, M. Inbasekaran, W. Wu, *et al.*, "High-resolution inkjet printing of all-polymer transistor circuits," *Science*, vol. 290, pp. 2123-2126, 2000.
- [108] G. MacBeath and S. L. Schreiber, "Printing proteins as microarrays for high-throughput function determination," *Science*, vol. 289, pp. 1760-1763, 2000.
- [109] J. Lavers and L. Kadar, "Application of electromagnetic forces to reduce tundish nozzle clogging," *Applied Mathematical Modelling*, vol. 28, pp. 29-45, 2004.
- [110] W. T. Coakley, "Ultrasonic separations in analytical biotechnology," *Trends in biotechnology*, vol. 15, pp. 506-511, 1997.
- [111] A. Lenshof, C. Magnusson, and T. Laurell, "Acoustofluidics 8: Applications of acoustophoresis in continuous flow microsystems," *Lab on a chip*, vol. 12, pp. 1210-1223, 2012.
- [112] D. J. Collins, T. Alan, K. Helmersen, and A. Neild, "Surface acoustic waves for on-demand production of picoliter droplets and particle encapsulation," *Lab on a Chip*, vol. 13, pp. 3225-3231, 2013.
- [113] F. Xu, T. D. Finley, M. Turkaydin, Y. Sung, U. A. Gurkan, A. S. Yavuz, *et al.*, "The assembly of cell-encapsulating microscale hydrogels using acoustic waves," *Biomaterials*, vol. 32, pp. 7847-7855, 2011.
- [114] P. B. Muller, R. Barnkob, M. J. H. Jensen, and H. Bruus, "A numerical study of microparticle acoustophoresis driven by acoustic radiation forces and streaming-induced drag forces," *Lab on a Chip*, vol. 12, pp. 4617-4627, 2012.
- [115] H. Bruus, "Acoustofluidics 1: Governing equations in microfluidics," *Lab on a Chip*, vol. 11, pp. 3742-3751, 2011.

- [116] H. Bruus, "Acoustofluidics 2: Perturbation theory and ultrasound resonance modes," *Lab on a Chip*, vol. 12, pp. 20-28, 2012.
- [117] M. Settnes and H. Bruus, "Theoretical analysis of viscous corrections to the acoustic radiation force on cells in microchannel a-coustophoresis," *Proc. 15th MicroTAS*, pp. 160-162.
- [118] J. Dinesh, "Modelling and Simulation of a Single Particle in Laminar Flow Regime of a Newtonian Liquid," in *Proceedings of the COMSOL Conference, Bangalore*, 2009.
- [119] J. C. Loudet, P. Hanusse, and P. Poulin, "Stokes Drag on a Sphere in a Nematic Liquid Crystal," *Science*, vol. 306, pp. 1525-1525, 2004-11-26 00:00:00 2004.
- [120] K. Chen, M. Wu, F. Guo, P. Li, C. Y. Chan, Z. Mao, *et al.*, "Rapid formation of size-controllable multicellular spheroids via 3D acoustic tweezers," *Lab on a Chip*, vol. 16, pp. 2636-2643, 2016.
- [121] S. Khalil and W. Sun, "Bioprinting endothelial cells with alginate for 3D tissue constructs," *Journal of biomechanical engineering*, vol. 131, p. 111002, 2009.
- [122] S. Wüst, M. E. Godla, R. Müller, and S. Hofmann, "Tunable hydrogel composite with two-step processing in combination with innovative hardware upgrade for cell-based three-dimensional bioprinting," *Acta biomaterialia*, vol. 10, pp. 630-640, 2014.
- [123] D. L. Cohen, W. Lo, A. Tsavaris, D. Peng, H. Lipson, and L. J. Bonassar, "Increased mixing improves hydrogel homogeneity and quality of three-dimensional printed constructs," *Tissue Engineering Part C: Methods*, vol. 17, pp. 239-248, 2010.
- [124] W. Schuurman, V. Khristov, M. Pot, P. Van Weeren, W. Dhert, and J. Malda, "Bioprinting of hybrid tissue constructs with tailorable mechanical properties," *Biofabrication*, vol. 3, p. 021001, 2011.
- [125] S.-J. Song, J. Choi, Y.-D. Park, S. Hong, J. J. Lee, C. B. Ahn, *et al.*, "Sodium Alginate Hydrogel-Based Bioprinting Using a Novel Multinozzle Bioprinting System," *Artificial Organs*, vol. 35, pp. 1132-1136, 2011.
- [126] L. Y. Yeo and J. R. Friend, "Surface acoustic wave microfluidics," *Annual Review of Fluid Mechanics*, vol. 46, pp. 379-406, 2014.
- [127] Y. Sriphutkiat and Y. Zhou, "Particle accumulation in microchannel and its reduction by surface acoustic wave (SAW)," 2016.
- [128] Z. B. Sendekie and P. Bacchin, "Colloidal jamming dynamics in microchannel bottlenecks," *Langmuir*, vol. 32, pp. 1478-1488, 2016.
- [129] M. Whittle, B. S. Murray, and E. Dickinson, "Simulation of colloidal particle scattering: Sensitivity to attractive forces," *Journal of Colloid and Interface Science*, vol. 225, pp. 367-377, 2000.
- [130] J. Bergendahl and D. Grasso, "Prediction of colloid detachment in a model porous media: hydrodynamics," *Chemical Engineering Science*, vol. 55, pp. 1523-1532, 2000.
- [131] B. Mustin and B. Stoeber, "Single layer deposition of polystyrene particles onto planar Polydimethylsiloxane substrates," *Langmuir*, vol. 32, pp. 88-101, 2015.
- [132] B. Mi and M. Elimelech, "Chemical and physical aspects of organic fouling of forward osmosis membranes," *Journal of Membrane Science*, vol. 320, pp. 292-302, 2008.
- [133] C. Colosi, S. R. Shin, V. Manoharan, S. Massa, M. Costantini, A. Barbetta, *et al.*, "Microfluidic Bioprinting of Heterogeneous 3D Tissue Constructs Using Low-Viscosity Bioink," *Advanced Materials*, vol. 28, pp. 677-684, 2016.
- [134] B. M. Gumbiner, "Cell adhesion: the molecular basis of tissue architecture and morphogenesis," *Cell*, vol. 84, pp. 345-357, 1996.
- [135] J. N. Israelachvili, *Intermolecular and surface forces: revised third edition*: Academic press, 2011.

- [136] Z. Adamczyk and P. Weroński, "Application of the DLVO theory for particle deposition problems," *Advances in Colloid and Interface Science*, vol. 83, pp. 137-226, 1999.
- [137] B. Ninham, "On progress in forces since the DLVO theory," *Advances in colloid and interface science*, vol. 83, pp. 1-17, 1999.
- [138] D. Ahmed, A. Ozcelik, N. Bojanala, N. Nama, A. Upadhyay, Y. Chen, *et al.*, "Rotational manipulation of single cells and organisms using acoustic waves," *Nat Commun*, vol. 7, 03/23/online 2016.
- [139] A. Anhäuser, R. Wunenburger, and E. Brasselet, "Acoustic rotational manipulation using orbital angular momentum transfer," *Physical review letters*, vol. 109, p. 034301, 2012.
- [140] C. R. Seager and T. G. Mason, "Slippery diffusion-limited aggregation," *Physical Review E*, vol. 75, p. 011406, 2007.
- [141] L. A. Kuznetsova and W. T. Coakley, "Applications of ultrasound streaming and radiation force in biosensors," *Biosensors and Bioelectronics*, vol. 22, pp. 1567-1577, 2007.
- [142] N. Nama, R. Barnkob, Z. Mao, C. J. Kähler, F. Costanzo, and T. J. Huang, "Numerical study of acoustophoretic motion of particles in a PDMS microchannel driven by surface acoustic waves," *Lab on a Chip*, vol. 15, pp. 2700-2709, 2015.
- [143] C. Devendran, T. Albrecht, J. Brenker, T. Alan, and A. Neild, "The importance of travelling wave components in standing surface acoustic wave (SSAW) systems," *Lab on a Chip*, vol. 16, pp. 3756-3766, 2016.
- [144] Y. Liu, Y. Li, A. M. el-Hady, C. Zhao, J. Du, Y. Liu, *et al.*, "Flexible and bendable acoustofluidics based on ZnO film coated aluminium foil," *Sensors and Actuators B: Chemical*, vol. 221, pp. 230-235, 2015.
- [145] L. Mengwei, L. Junhong, M. Jun, and W. Chenghao, "Design and fabrication of a MEMS Lamb wave device based on ZnO thin film," *Journal of Semiconductors*, vol. 32, p. 044006, 2011.
- [146] X. Zhou, C. Zhao, R. Hou, J. Zhang, K. Kirk, D. Hutson, *et al.*, "Sputtered ZnO film on aluminium foils for flexible ultrasonic transducers," *Ultrasonics*, vol. 54, pp. 1991-1998, 2014.
- [147] J. W. Jorgenson and K. D. Lukacs, "High-resolution separations based on electrophoresis and electroosmosis," *Journal of Chromatography A*, vol. 218, pp. 209-216, 1981.
- [148] P. R. Gascoyne and J. Vykoukal, "Particle separation by dielectrophoresis," *Electrophoresis*, vol. 23, p. 1973, 2002.
- [149] N. Xia, T. P. Hunt, B. T. Mayers, E. Alsberg, G. M. Whitesides, R. M. Westervelt, *et al.*, "Combined microfluidic-micromagnetic separation of living cells in continuous flow," *Biomedical microdevices*, vol. 8, pp. 299-308, 2006.
- [150] A. Ashkin, J. Dziedzic, and T. Yamane, "Optical trapping and manipulation of single cells using infrared laser beams," *Nature*, vol. 330, pp. 769-771, 1987.
- [151] P. Y. Chiou, A. T. Ohta, and M. C. Wu, "Massively parallel manipulation of single cells and microparticles using optical images," *Nature*, vol. 436, pp. 370-372, 2005.
- [152] M. Yamada and M. Seki, "Hydrodynamic filtration for on-chip particle concentration and classification utilizing microfluidics," *Lab on a chip*, vol. 5, pp. 1233-1239, 2005.
- [153] M. A. Burguillos, C. Magnusson, M. Nordin, A. Lenshof, P. Augustsson, M. J. Hansson, *et al.*, "Microchannel acoustophoresis does not impact survival or function of microglia, leukocytes or tumor cells," *PLoS One*, vol. 8, p. e64233, 2013.
- [154] S.-C. S. Lin, X. Mao, and T. J. Huang, "Surface acoustic wave (SAW) acoustophoresis: now and beyond," *Lab on a chip*, vol. 12, pp. 2766-2770, 2012.

- [155] F. Petersson, L. Åberg, A.-M. Swärd-Nilsson, and T. Laurell, "Free flow acoustophoresis: microfluidic-based mode of particle and cell separation," *Analytical chemistry*, vol. 79, pp. 5117-5123, 2007.
- [156] X. Ding, S.-C. S. Lin, B. Kiraly, H. Yue, S. Li, I.-K. Chiang, *et al.*, "On-chip manipulation of single microparticles, cells, and organisms using surface acoustic waves," *Proceedings of the National Academy of Sciences*, vol. 109, pp. 11105-11109, 2012.
- [157] F. Petersson, A. Nilsson, C. Holm, H. Jönsson, and T. Laurell, "Continuous separation of lipid particles from erythrocytes by means of laminar flow and acoustic standing wave forces," *Lab on a chip*, vol. 5, pp. 20-22, 2005.
- [158] J. Nilsson, M. Evander, B. Hammarström, and T. Laurell, "Review of cell and particle trapping in microfluidic systems," *Analytica chimica acta*, vol. 649, pp. 141-157, 2009.
- [159] J. Friend and L. Y. Yeo, "Microscale acoustofluidics: Microfluidics driven via acoustics and ultrasonics," *Reviews of Modern Physics*, vol. 83, p. 647, 2011.
- [160] G. Destgeer, K. H. Lee, J. H. Jung, A. Alazzam, and H. J. Sung, "Continuous separation of particles in a PDMS microfluidic channel via travelling surface acoustic waves (TSAW)," *Lab on a chip*, vol. 13, pp. 4210-4216, 2013.
- [161] Y. Chen, A. A. Nawaz, Y. Zhao, P.-H. Huang, J. P. McCoy, S. J. Levine, *et al.*, "Standing surface acoustic wave (SSAW)-based microfluidic cytometer," *Lab on a Chip*, vol. 14, pp. 916-923, 2014.
- [162] C. Ratier and M. Hoyos, "Acoustic programming in step-split-flow lateral-transport thin fractionation," *Analytical chemistry*, vol. 82, pp. 1318-1325, 2010.
- [163] Z. Mandralis, D. Feke, W. Bolek, W. Burger, and E. Benes, "Enhanced synchronized ultrasonic and flow-field fractionation of suspensions," *Ultrasonics*, vol. 32, pp. 113-122, 1994.
- [164] T. Laurell, F. Petersson, and A. Nilsson, "Chip integrated strategies for acoustic separation and manipulation of cells and particles," *Chemical Society Reviews*, vol. 36, pp. 492-506, 2007.
- [165] N. Harris, R. Boltryk, P. Glynne-Jones, and M. Hill, "A novel binary particle fractionation technique," *Physics Procedia*, vol. 3, pp. 277-281, 2010.
- [166] Y. Liu and K.-M. Lim, "Particle separation in microfluidics using a switching ultrasonic field," *Lab on a Chip*, vol. 11, pp. 3167-3173, 2011.
- [167] M. Brissaud, "Characterization of piezoceramics," *IEEE transactions on ultrasonics, ferroelectrics, and frequency control*, vol. 38, pp. 603-617, 1991.
- [168] X. Ding, Z. Peng, S.-C. S. Lin, M. Geri, S. Li, P. Li, *et al.*, "Cell separation using tilted-angle standing surface acoustic waves," *Proceedings of the National Academy of Sciences*, vol. 111, pp. 12992-12997, 2014.
- [169] K. Yosioka and Y. Kawasima, "Acoustic radiation pressure on a compressible sphere," *Acta Acustica united with Acustica*, vol. 5, pp. 167-173, 1955.
- [170] Y. Sriphutkiat and Y. Zhou, "Particle Accumulation in a Microchannel and Its Reduction by a Standing Surface Acoustic Wave (SSAW)," *Sensors*, vol. 17, p. 106, 2017.
- [171] P. Bloch, N. Doe, E. Paige, and M. Yamaguchi, "Observations on surface skimming bulk waves and other waves launched from an IDT on lithium niobate," in *1981 Ultrasonics Symposium*, 1981, pp. 268-273.
- [172] P. D. Bloch, E. G. S. Paige, and M. W. Wagg, "On the reflection of surface skimming bulk waves," *IEEE Transactions on Sonics and Ultrasonics*, vol. 29, pp. 104-111, 1982.

- [173] L. Le Brizoual, O. Elmazria, F. Sarry, M. El Hakiki, A. Talbi, and P. Alnot, "High frequency SAW devices based on third harmonic generation," *Ultrasonics*, vol. 45, pp. 100-103, 2006.
- [174] D.-T. Phan and G.-S. Chung, "High sensitivity SAW UV sensor by using third harmonic based on ZnO/Si structure," in *2010 IEEE Sensors*, ed.
- [175] D.-T. Phan and G.-S. Chung, "Characteristics of SAW UV sensors based on a ZnO/Si structure using third harmonic mode," *Current Applied Physics*, vol. 12, pp. 210-213, 2012.
- [176] K. V. Wong and A. Hernandez, "A review of additive manufacturing," *ISRN Mechanical Engineering*, vol. 2012, 2012.
- [177] M. Vaezi, H. Seitz, and S. Yang, "A review on 3D micro-additive manufacturing technologies," *The International Journal of Advanced Manufacturing Technology*, vol. 67, pp. 1721-1754, 2013.
- [178] D. Kokkinis, M. Schaffner, and A. R. Studart, "Multimaterial magnetically assisted 3D printing of composite materials," *Nature communications*, vol. 6, 2015.
- [179] T. Ahn, H.-J. Kim, J. Lee, D.-G. Choi, J.-Y. Jung, J.-H. Choi, *et al.*, "A facile patterning of silver nanowires using a magnetic printing method," *Nanotechnology*, vol. 26, p. 345301, 2015.
- [180] L. Wang, F. Li, M. Kuang, M. Gao, J. Wang, Y. Huang, *et al.*, "Interface Manipulation for Printing Three-Dimensional Microstructures Under Magnetic Guiding," *small*, vol. 11, pp. 1900-1904, 2015.
- [181] A. Nel, T. Xia, L. Mädler, and N. Li, "Toxic potential of materials at the nanolevel," *science*, vol. 311, pp. 622-627, 2006.
- [182] P. van der Asdonk, S. Kragt, and P. H. Kouwer, "Directing Soft Matter in Water Using Electric Fields," *ACS applied materials & interfaces*, vol. 8, pp. 16303-16309, 2016.
- [183] G. Kim, D. Moeller, and Y. Shkel, "Orthotropic polymeric composites with microstructure tailored by electric field," *Journal of composite materials*, vol. 38, pp. 1895-1909, 2004.
- [184] X. Ding, S.-C. S. Lin, M. I. Lapsley, S. Li, X. Guo, C. Y. Chan, *et al.*, "Standing surface acoustic wave (SSAW) based multichannel cell sorting," *Lab on a Chip*, vol. 12, pp. 4228-4231, 2012.
- [185] B. Raeymaekers, C. Pantea, and D. N. Sinha, "Manipulation of diamond nanoparticles using bulk acoustic waves," *Journal of Applied Physics*, vol. 109, p. 014317, 2011.
- [186] P. P. A. Suthanthiraraj, M. E. Piyasena, T. A. Woods, M. A. Naivar, G. P. López, and S. W. Graves, "One-dimensional acoustic standing waves in rectangular channels for flow cytometry," *Methods*, vol. 57, pp. 259-271, 2012.
- [187] M. E. Piyasena, P. P. Austin Suthanthiraraj, R. W. Applegate Jr, A. M. Goumas, T. A. Woods, G. P. López, *et al.*, "Multinode acoustic focusing for parallel flow cytometry," *Analytical chemistry*, vol. 84, pp. 1831-1839, 2012.
- [188] T. Franke, S. Braunmüller, L. Schmid, A. Wixforth, and D. Weitz, "Surface acoustic wave actuated cell sorting (SAWACS)," *Lab on a chip*, vol. 10, pp. 789-794, 2010.
- [189] X. Ding, J. Shi, S.-C. S. Lin, S. Yazdi, B. Kiraly, and T. J. Huang, "Tunable patterning of microparticles and cells using standing surface acoustic waves," *Lab on a chip*, vol. 12, pp. 2491-2497, 2012.
- [190] T. M. Llewellyn-Jones, B. W. Drinkwater, and R. S. Trask, "3D printed components with ultrasonically arranged microscale structure," *Smart Materials and Structures*, vol. 25, p. 02LT01, 2016.

- [191] L. Friedrich, R. Collino, T. Ray, and M. Begley, "Acoustic control of microstructures during direct ink writing of two-phase materials," *Sensors and Actuators A: Physical*, 2017.
- [192] M. W. Ley and H. Bruus, "Continuum modeling of hydrodynamic particle–particle interactions in microfluidic high-concentration suspensions," *Lab on a Chip*, vol. 16, pp. 1178-1188, 2016.
- [193] R. Crowson and M. Folkes, "Rheology of short glass fiber-reinforced thermoplastics and its application to injection molding. II. The effect of material parameters," *Polymer Engineering & Science*, vol. 20, pp. 934-940, 1980.
- [194] K. Y. Lee and D. J. Mooney, "Alginate: properties and biomedical applications," *Progress in polymer science*, vol. 37, pp. 106-126, 2012.
- [195] J. Sun and H. Tan, "Alginate-based biomaterials for regenerative medicine applications," *Materials*, vol. 6, pp. 1285-1309, 2013.
- [196] Sigma-Aldrich. *Product Specification - Alginic acid sodium salt from brown algae*. Available: <http://www.sigmaaldrich.com/catalog/product/sigma/a1112?lang=en®ion=SG>
- [197] A. Garcia-Sabaté, A. Castro, M. Hoyos, and R. González-Cinca, "Experimental study on inter-particle acoustic forces," *The Journal of the Acoustical Society of America*, vol. 135, pp. 1056-1063, 2014.
- [198] S. Sepehrihnama, K.-M. Lim, and F. S. Chau, "Numerical study of interparticle radiation force acting on rigid spheres in a standing wave," *The Journal of the Acoustical Society of America*, vol. 137, pp. 2614-2622, 2015.
- [199] Y. Li, T. Ma, D. A. Kniss, L. C. Lasky, and S. T. Yang, "Effects of filtration seeding on cell density, spatial distribution, and proliferation in nonwoven fibrous matrices," *Biotechnology progress*, vol. 17, pp. 935-944, 2001.
- [200] Š. Šikalo, H.-D. Wilhelm, I. Roisman, S. Jakirlić, and C. Tropea, "Dynamic contact angle of spreading droplets: Experiments and simulations," *Physics of Fluids*, vol. 17, p. 062103, 2005.
- [201] A. Yarin, "Drop impact dynamics: splashing, spreading, receding, bouncing...", *Annu. Rev. Fluid Mech.*, vol. 38, pp. 159-192, 2006.
- [202] G. R. Goddard, "Ultrasonic Concentration in a Line-Driven Cylindrical Tube," Los Alamos National Lab.(LANL), Los Alamos, NM (United States)2004.
- [203] J. N. Israelachvili, *Intermolecular and surface forces*: Academic press, 2015.
- [204] A. J. Ladd, "Hydrodynamic transport coefficients of random dispersions of hard spheres," *The Journal of chemical physics*, vol. 93, pp. 3484-3494, 1990.
- [205] J. C. Chow and K. Soda, "Laminar flow in tubes with constriction," *The Physics of Fluids*, vol. 15, pp. 1700-1706, 1972.
- [206] C. Henry, J.-P. Minier, and G. Lefèvre, "Towards a description of particulate fouling: From single particle deposition to clogging," *Advances in colloid and interface science*, vol. 185, pp. 34-76, 2012.
- [207] P. Bacchin, "Interfacially driven transport in narrow channels," *arXiv preprint arXiv:1802.07031*, 2018.
- [208] M. Araz, "Generation Of Sub-Wavelength Acoustic Stationary Waves In Microfluidic Platforms: Theory And Applications To The Control Of Micro-Nanoparticles And Biological Entities," 2010.
- [209] J. Lee, M. Sato, H. Kim, and J. Mochida, "Transplantation of scaffold-free spheroids composed of synovium-derived cells and chondrocytes for the treatment of cartilage defects of the knee," *Eur Cell Mater*, vol. 22, pp. 275-90, 2011.
- [210] I. T. Ozbolat and Y. Yu, "Bioprinting toward organ fabrication: challenges and future trends," *IEEE Transactions on Biomedical Engineering*, vol. 60, pp. 691-699, 2013.

- [211] J. Li, M. Chen, X. Fan, and H. Zhou, "Recent advances in bioprinting techniques: approaches, applications and future prospects," *Journal of translational medicine*, vol. 14, p. 271, 2016.
- [212] R. C. Chang, K. Emami, A. Jeevarajan, H. Wu, and W. Sun, "Microprinting of liver micro-organ for drug metabolism study," in *Biological Microarrays*, ed: Springer, 2011, pp. 219-238.
- [213] D. Richards, J. Jia, M. Yost, R. Markwald, and Y. Mei, "3D bioprinting for vascularized tissue fabrication," *Annals of biomedical engineering*, vol. 45, pp. 132-147, 2017.
- [214] H.-W. Kang, I. K. Ko, J. H. Kim, S. J. Lee, J. J. Yoo, and A. Atala, "3-D Bioprinting of Innervated Skeletal Muscle Tissue for Accelerated Restoration of Muscle Function."
- [215] L. Teodori, A. Costa, R. Marzio, B. Perniconi, D. Coletti, S. Adamo, *et al.*, "Native extracellular matrix: a new scaffolding platform for repair of damaged muscle," *Frontiers in physiology*, vol. 5, p. 218, 2014.
- [216] Y. J. Choi, T. G. Kim, J. Jeong, H. G. Yi, J. W. Park, W. Hwang, *et al.*, "3D Cell Printing of Functional Skeletal Muscle Constructs Using Skeletal Muscle-Derived Bioink," *Advanced healthcare materials*, vol. 5, pp. 2636-2645, 2016.
- [217] D. McMahon, P. Anderson, R. Nassar, J. Bunting, Z. Saba, A. Oakeley, *et al.*, "C2C12 cells: biophysical, biochemical, and immunocytochemical properties," *American Journal of Physiology-Cell Physiology*, vol. 266, pp. C1795-C1802, 1994.
- [218] P. Bajaj, B. Reddy, L. Millet, C. Wei, P. Zorlutuna, G. Bao, *et al.*, "Patterning the differentiation of C2C12 skeletal myoblasts," *Integrative Biology*, vol. 3, pp. 897-909, 2011.
- [219] P. Diel, D. Baadners, K. Schlüpmann, M. Velders, and J. Schwarz, "C2C12 myoblastoma cell differentiation and proliferation is stimulated by androgens and associated with a modulation of myostatin and Pax7 expression," *Journal of molecular endocrinology*, vol. 40, pp. 231-241, 2008.
- [220] L. A. KUNZ-SCHUGHART, M. Kreutz, and R. Knuechel, "Multicellular spheroids: a three-dimensional in vitro culture system to study tumour biology," *International journal of experimental pathology*, vol. 79, pp. 1-23, 1998.
- [221] C. Yuasa, Y. Tomita, M. Shono, K. Ishimura, and A. Ichihara, "Importance of cell aggregation for expression of liver functions and regeneration demonstrated with primary cultured hepatocytes," *Journal of cellular physiology*, vol. 156, pp. 522-530, 1993.
- [222] D. Loessner, C. Meinert, E. Kaemmerer, L. C. Martine, K. Yue, P. A. Levett, *et al.*, "Functionalization, preparation and use of cell-laden gelatin methacryloyl-based hydrogels as modular tissue culture platforms," *Nature protocols*, vol. 11, p. 727, 2016.
- [223] G. Stovall. (2013). Skeletal Muscle Histology. Available: <http://pulpbits.net/4-skeletal-muscle-tissue-slide/skeletal-muscle-histology/>
- [224] K. Yasuda, S.-i. Umemura, and K. Takeda, "Concentration and fractionation of small particles in liquid by ultrasound," *Japanese journal of applied physics*, vol. 34, p. 2715, 1995.
- [225] S. Ostrovidov, S. Ahadian, J. Ramon-Azcon, V. Hosseini, T. Fujie, S. P. Parthiban, *et al.*, "Three-dimensional co-culture of C2C12/PC12 cells improves skeletal muscle tissue formation and function," *Journal of tissue engineering and regenerative medicine*, vol. 11, pp. 582-595, 2017.
- [226] M. Costantini, S. Testa, E. Fornetti, A. Barbeta, M. Trombetta, S. M. Cannata, *et al.*, "engineering Muscle networks in 3D gelatin Methacryloyl hydrogels: influence of

- Mechanical stiffness and geometrical confinement," *Frontiers in bioengineering and biotechnology*, vol. 5, p. 22, 2017.
- [227] H. Aubin, J. W. Nichol, C. B. Hutson, H. Bae, A. L. Sieminski, D. M. Cropek, *et al.*, "Directed 3D cell alignment and elongation in microengineered hydrogels," *Biomaterials*, vol. 31, pp. 6941-6951, 2010.
 - [228] D. B. Kolesky, R. L. Truby, A. S. Gladman, T. A. Busbee, K. A. Homan, and J. A. Lewis, "3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs," *Advanced materials*, vol. 26, pp. 3124-3130, 2014.
 - [229] C. Norotte, F. S. Marga, L. E. Niklason, and G. Forgacs, "Scaffold-free vascular tissue engineering using bioprinting," *Biomaterials*, vol. 30, pp. 5910-5917, 2009.
 - [230] Y. Sriphutkiat, S. Kasetsirikul, and Y. Zhou, "Formation of cell spheroids using Standing Surface Acoustic Wave (SSAW)," *International Journal of Bioprinting*, vol. 4, 2018.
 - [231] S. Ahadian, J. Ramón-Azcón, S. Ostrovidov, G. Camci-Unal, V. Hosseini, H. Kaji, *et al.*, "Interdigitated array of Pt electrodes for electrical stimulation and engineering of aligned muscle tissue," *Lab on a Chip*, vol. 12, pp. 3491-3503, 2012.
 - [232] S. Ostrovidov, V. Hosseini, S. Ahadian, T. Fujie, S. P. Parthiban, M. Ramalingam, *et al.*, "Skeletal muscle tissue engineering: methods to form skeletal myotubes and their applications," *Tissue Engineering Part B: Reviews*, vol. 20, pp. 403-436, 2014.
 - [233] Y. Sriphutkiat and Y. Zhou, "Particle manipulation using standing acoustic waves in the microchannel at dual-frequency excitation: Effect of power ratio," *Sensors and Actuators A: Physical*, vol. 263, pp. 521-529, 2017.
 - [234] K. Nagamine, T. Kawashima, T. Ishibashi, H. Kaji, M. Kanzaki, and M. Nishizawa, "Micropatterning contractile C2C12 myotubes embedded in a fibrin gel," *Biotechnology and bioengineering*, vol. 105, pp. 1161-1167, 2010.
 - [235] D. Wang, W. Zheng, Y. Xie, P. Gong, F. Zhao, B. Yuan, *et al.*, "Tissue-specific mechanical and geometrical control of cell viability and actin cytoskeleton alignment," *Scientific reports*, vol. 4, p. 6160, 2014.
 - [236] C. Kim, D. Espalin, M. Liang, H. Xin, A. Cuaron, I. Varela, *et al.*, "3D Printed Electronics With High Performance, Multi-Layered Electrical Interconnect," *IEEE Access*, vol. 5, pp. 25286-25294, 2017.

List of Publications, Patents and Awards

Awards

- Best Paper Award, the 11th Regional Conference on Chemical and Biomedical Engineering 2018
- Best Teaching Assistant Award (out of 110 TAs), Nanyang Technological University

Provisional Patent (PCT Awaiting)

- **Yannapol Sriphutkiat** and Yufeng Zhou, Acoustic Manipulation Of Microparticles/Cells And Suppression Of Nozzle Clogging For Additive Manufacturing, *Provisional Patent Application No. 10201709717Q*

Journal Publications

- **Yannapol Sriphutkiat**, and Yufeng Zhou, Particle manipulation using standing acoustic waves in the microchannel at dual-frequency excitation: effect of power ratio, *Sensors & Actuators: A. Physical*, **263**, 521-529, 2017.
- **Yannapol Sriphutkiat** and Yufeng Zhou, Particle accumulation in a microchannel and its reduction by a standing surface acoustic wave (SSAW), *Sensors*, **17(1)**, 106, 2017.
- **Yannapol Sriphutkiat**, Surasak Kasetsirikul and Yufeng Zhou, Formation of cell spheroids using Standing Surface Acoustic Wave (SSAW), *International Journal of Bioprinting*, **4(1)**, 2018,
- Yufeng Zhou and **Yannapol Sriphutkiat**, Microparticle Manipulation by Standing Surface Acoustic Waves with the Dual-Frequency Excitations, *Journal of Visualized Experiments*, 138, e58085, 2018.

- **Yannapol Sriphutkiat** and Yufeng Zhou, Acoustic manipulation of microparticle in a cylindrical tube for 3D printing, *Under-revision*
- **Yannapol Sriphutkiat** and Yufeng Zhou, Cell Alignment and accumulation using acoustic nozzle for 3D printing, *Under-revision*

Conference Proceedings

- **Yannapol Sriphutkiat** and Yufeng Zhou, Accumulation of microparticle in 3D printed construct using acoustic nozzle, *Proceedings of the 3rd International Conference on Progress in Additive Manufacturing (Pro-AM 2018)*, Singapore
- **Yannapol Sriphutkiat** and Yufeng Zhou, Patterning of microparticles/cells through the acoustic-assisted nozzle for 3D printer, Regional Conference on Electrical and Electronics Engineering (RCEEE 2018), Penang, Malaysia
- **Yannapol Sriphutkiat**, Surasak Kasetsirikul and Yufeng Zhou, Study of cell spheroid formation using low and high frequency standing surface acoustic wave (SSAW), *International Conference on Biofabrication 2017*, Beijing, China.
- **Yannapol Sriphutkiat** and Yufeng Zhou, Accumulation of microparticles along radial axis of cylindrical tube using low and high frequency acoustic wave, *Regional Conference on Environmental Engineering 2017*, Hanoi, Vietnam.
- **Yannapol Sriphutkiat** and Yufeng Zhou, Particle Manipulation using dual-frequency excitation of standing surface acoustic wave, *EAC Lab-on-a-chip Conference A*Star 2016*, Singapore.
- **Yannapol Sriphutkiat** and Yufeng Zhou, Particle Accumulation in Microchannel and Its Reduction by Surface Acoustic Wave (SAW), *Proceedings of the 2nd International Conference on Progress in Additive Manufacturing (Pro-AM 2016)*, Singapore