

The University of Adelaide

# **MICROFLUIDIC SYSTEM DEVELOPMENT FOR DRUG DELIVERY**

---

**School of Chemical Engineering**

**Mengjue Zhang**

26<sup>th</sup> August 2016

Supervisors: A/P Jingxiu Bi, Dr Hu Zhang, Prof. Mark Biggs

***For a thesis that does not contain work already in the public domain***

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signature

Date .....119116.....

## **Abstract**

Development and application of a microfluidic system for generating drug delivery carriers are investigated in this research. Various types of microfluidic devices are designed and fabricated for peptide nanotubes, liposome vesicles and double emulsions formation. The microfluidic system offers a better control over the formation process of all three drug delivery carriers. Comparing to traditional methods such as bulk mixing, the process efficiency, size and size distribution of the final products are significantly improved.

The results generated show that tuning the flow rate ratios between different reagents from the inlet streams successfully controls the sizes and size distributions of liposomes vesicles. The relationship between the flow rate ratio and the size of the resulting vesicles is established. Macrocyclic peptide (AP-169) that was found to self-assemble into an anti-parallel  $\beta$ -sheet nanotube with a triggering agent is successfully synthesized and purified for peptide nanotube self-assembling process. A microfluidic device is designed and fabricated to control the interaction between AP-169 and its self-assembling triggering agent, dimethyl sulfoxide.

Double emulsions with different radii are produced with the microfluidic system by adjusting the flow rate ratio between each phase of the solution, and changing the wetting properties of the microchannels. The stability of double emulsions is enhanced by introducing various surfactants. The sizes and size distributions of liposomes and double emulsions have been successfully controlled and optimized for drug delivery.

In conclusion, various drug delivery carriers have been successfully generated and optimized with a designed and modified microfluidic system. These products can be further applied in drug encapsulation, biomolecular screening and *in vitro* compartmentalization in the future.

## **Acknowledgement**

First and foremost, I would like to express my sincere gratitude to my supervisor Associate Professor Jingxiu Bi for her patience, motivation, and support throughout my bachelor and master's degrees. Besides my main supervisor, I would like to thank my co-supervisor: Dr Hu Zhang for his encouragement and effort on my project. I attribute the level of my Master degree to their encouragement and effort and without them this thesis, too, would not have been completed or written. One simply could not wish for better or friendlier supervisors.

For peptide synthesis and purification, I would like to thank Dr Ashok Pehere for his advice, support and encouragement. I would like to thank the Chemistry Department of the University of Adelaide for providing experimental materials and equipment to this project, and allowing me access to their laboratories. Dr Pehere has offered much advice and insight throughout my work on AP-169 synthesis. The experiments could not have been completed without his support.

Last but not the least, I would also thank my research group colleagues and my parents for supporting me throughout all my studies at the University of Adelaide.

## Table of Contents

Acknowledgement .....	2
Abstract .....	1
1. Chapter 1 Introduction .....	8
1.1 Background .....	8
1.2 Research scope and aims .....	8
1.2.1 Peptide synthesis, purification and self-assembling into nanotube structure .....	9
1.2.2 Liposome self-assembling formation in microfluidic system.....	9
1.2.3 Single/double emulsion formation in microfluidic system.....	10
1.3 Thesis structure .....	11
2. Chapter 2 Literature Review .....	12
2.1 Drug delivery .....	12
2.1.1 Drugs for delivery .....	13
2.1.2 Carriers for drug delivery.....	17
2.1.3 Challenges and gaps of drug delivery carrier formation with microfluidic system.	21
2.2 Peptides and self-assembly as drug carriers.....	23
2.2.1 Different application of peptide nanotubes.....	23
2.2.2 Peptide self-assembling control .....	24
2.2.3 Challenges and resolutions of peptide self-assembling control.....	25
2.3 Liposome and its applications in drug delivery .....	26
2.3.1 Liposome structure and formation .....	26
2.3.2 Liposome generation via microfluidic system.....	28
2.3.3 Challenges and potential solutions to liposome formation control.....	29
2.4 Double emulsion generation in a microfluidic chip.....	31
2.4.1 Double emulsions and generation methods .....	31
2.4.2 Double emulsions formation in microfluidic system.....	34
2.5 Microfluidic system applied in drug delivery .....	39
2.5.1 Design and applications of microfluidic systems .....	39
2.5.2 Fabrication and manufacturing of microfluidic system.....	42
3. Chapter 3 Peptide Synthesis and Self-assembly Control as Carriers of Drug Delivery .....	43
3.1 Introduction.....	43
3.2 Materials and methods .....	44
3.2.1 Material .....	44
3.2.2 Reaction and purification.....	45
3.2.3 Self-assembling rate control .....	48

3.3	Results and discussion .....	51
3.3.1	Reaction selection .....	51
3.3.2	Purification of product from each reaction .....	52
3.3.3	Characterisation .....	53
3.3.4	Self-assembly and control.....	59
3.4	Conclusions.....	60
4.	Chapter 4 Evaluation of Microfluidic System .....	61
4.1	Introduction.....	61
4.2	Material and method .....	62
4.2.1	Design, fabrication and modification on microfluidic chips .....	62
4.2.2	Liposome self-assembly by microfluidic system with controlling parameters .....	69
4.2.3	Single emulsions .....	70
4.2.4	Double emulsions.....	71
4.3	Results and discussion .....	71
4.3.1	Generation of liposomes .....	71
4.3.2	Single emulsion generation.....	76
4.3.3	Double emulsion generation (w/o/w).....	79
4.4	Conclusions.....	86
5.	Chapter 5 Conclusions and Future Work.....	87
5.1	Conclusions.....	87
5.2	Future work.....	88
	Reference .....	89

## List of Figures

Figure 1-1 Thesis Structure.....	11
Figure 2-1 Routes of administration of drugs .....	13
Figure 2-2 Different types of carriers used for biomacromolecule intracellular delivery (Gu et al, 2011) .....	17
Figure 2-3 Model for nanotube containing lipopeptide C12-KLVFFAE .....	18
Figure 2-4 Structure of double emulsion (w/o/w) and multi-emulsions.....	20
Figure 2-5 Different classes of cyclic peptides which can form nanotubescontaining.....	23
Figure 2-6 Model of the self-assembling process of a peptide nanotube .....	25
Figure 2-7 Structure of POPC and liposomes.....	26
Figure 2-8 Details of microfluidic chip .....	28
Figure 2-9 Size and Size distribution of liposomes with different FRRs. ....	29
Figure 2-10 Liposome self-assembling issues .....	31
Figure 2-11 Bulk stirring double emulsion generation .....	32
Figure 2-12 Pulsed jetting/Consecutive capillary microfluidic device.....	33
Figure 2-13 Flow impact on double emulsion generation in both T and Cross-junction .....	34
Figure 2-14 Schematic Design of Double Emulsion Formation Flow in a Microfluidic Chip	35
Figure 2-15 Microfluidic platforms for production of drug and gene carriers. ....	41
Figure 3-1 Starting macrocycle (AP-169) with propylcine, leucine and lysine labelled.....	43
Figure 3-2 Starting compound generation .....	45
Figure 3-3 Peptide synthesis procedures.....	46
Figure 3-4 Capillary system setup for peptide nanotube self-assembling .....	49
Figure 3-5 Click reaction for functional loop formation .....	51
Figure 3-6 TLC analysis on the starting compound.....	52
Figure 3-7HNMR spectrum of the starting material.....	53
Figure 3-8 NMR reading of AP-169 .....	54
Figure 3-9 FT-IR spectrum reading of AP-169 .....	55
Figure 3-10 Crystallisation of macrocycles under confocal microscope.....	56
Figure 3-11 TEM image of self-assembled or crystallised nanotubes.....	57
Figure 3-12 Nanotube structure formation rate .....	58
Figure 4-1 Microfluidic channel designs .....	63
Figure 4-2 Modified double emulsion template design .....	64
Figure 4-3 Modified T-junction design for double emulsion generation .....	65
Figure 4-4 PDMS mix curing reaction (crosslinking) .....	66

Figure 4-5 Dimension of Microfluidic Chip used for liposome self-assembling .....	69
Figure 4-6 Microfluidic channel for single emulsion generation .....	70
Figure 4-7 Cross junction and T-junction channels are designed for double emulsion formation.....	71
Figure 4-8 Size distributions of self-assembled liposomes formed from various initial lipid concentrations .....	72
Figure 4-9 Effect of FRR on average diameter of self-assembled liposomes formed by hydrodynamic focusing of POPC with PBS side streams .....	74
Figure 4-10 Liposome Size Distributions at FRR 40.....	74
Figure 4-11 Unilamellar morphology of POPC vesicles in TEM (stained with OsO4) .....	75
Figure 4-12 Single emulsion w/o formed in microchannels.....	76
Figure 4-13 FRR influence on droplet size for water-in-oil droplet generation .....	77
Figure 4-14 Morphology of oil-in-water single emulsion generated.....	78
Figure 4-15 Interaction between channel wall and the intermediate phase of w/o/w emulsion .....	79
Figure 4-16 Water-in-oil-in-water double emulsion generated with microfluidic system .....	82
Figure 4-17 Pressure change of each phase with increasing flow rate, the slope is fluid resistance.....	84
Figure 4-18 Relationship between droplet inner and outer diameter ratio and flow rate ratio	85



## List of Tables

Table 2-1 Nanoparticles drug delivery compositiona and their applications .....	14
Table 2-2 Summary of advantages and disadvantages of common liposome formation .....	27
Table 2-3 Advantages and disadvantages of pulsed jetting process by microfluidic system ..	33
Table 2-4 commonly used surfactants and their HLB .....	37
Table 2-5 Comparison of Slab-gel protein electrophoresis and microfluidic chip .....	40
Table 2-6 Comparison between PDMS and glass microchannels .....	42
Table 3-1 Organic solvents at different mixing ratio .....	48
Table 3-2 Comparison of ring closing metathesis (RCM) and click reaction .....	51
Table 3-3 Reaction rate at different initial macrocycle concentration .....	58
Table 4-1 PDMS channel binding with no plasma treatment but different A (base elastomer) to B (curing agent) ratio at 80°C .....	67
Table 4-2 Flow rate trials for double emulsion generation .....	81
Table 4-3 Surfactant used in each phase and their concentrations .....	83