Microfluidic droplet formation and manipulations for microdroplets used as miniaturized laboratories

Wang, Wei

2010

Wang, W. (2010). Microfluidic droplet formation and manipulations for microdroplets used as miniaturized laboratories. Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/47464>

<https://doi.org/10.32657/10356/47464>

Nanyang Technological University

Downloaded on 26 Apr 2023 17:25:28 SGT

MICROFLUIDIC DROPLET FORMATION AND MANIPULATIONS FOR MICRODROPLETS USED AS MINIATURIZED LABORATORIES

WANG WEI

School of Chemical and Biomedical Engineering

A thesis submitted to the Nanyang Technological University
in partial fulfillment of the requirement for the degree of
Doctor of Philosophy

2010

Acknowledgement

First I would like to express my sincere appreciation to my supervisor, Professor Li ChangMing, for his guidance, discussions, support and encouragement throughout my research at Nanyang Technological University. Many thanks to him for bringing me into this wonderful research field and his frequent advisory discussions with me about my research directions and technical details, which not only expanded my horizons, but also stimulated my creativity and imagination. The research abilities I learned from him are my life's treasure and will continue motivating me to face scientific challenges in the future. I would also like to express my deep thanks to Professor Charles Yang Chun for his kind help and warm encouragement through out this research.

Thanks also go to all my fellow graduate students at the Advanced Bio-Nano Center. Thank you very much for your friendship and I cherish the moments we shared together. The inspirational discussions with you and your assistance with experiments are greatly appreciated.

Most importantly, big thanks go to my lovely and pretty wife Pan MingMing for willing to endure four years of poverty. Without her love, dedication and constant support, this dissertation would not be possible. Finally, I would like to devote this dissertation to my mum, who is always in my heart. Her dream helped drive me throughout this study.

Table of contents

Acknowledgement	i
List of figures	v
List of schemes.....	viii
Summary	ix
Chapter 1 Introduction	1
1.1. Introduction	1
1.2. Motivations	3
1.3. Objective of study	4
1.3.1. Direct formation of compound droplet	4
1.3.2. Droplet Trapping and fusion for Droplet reaction assay on-chip	5
1.3.3. Static droplet array to facilitate real time monitoring of what occurred inside droplets	6
1.3.4. Droplet release integration into droplet reaction assay to facilitate further manipulation or analysis of post-reaction droplets	7
1.4. Organization.....	7
Chapter 2 Literature Review.....	10
2.1. Droplet microfluidics --- the second generation of microfluidics technology.....	10
2.2 Droplet control	12
2.2.1 Droplet generation	12
2.2.2 Droplet sorting.....	18
2.2.3 Droplet fusion.....	22
2.2.4 Droplet trapping, storage, and release	28
2.2.5 Light-induced droplet manipulation	33
2.2.6 Other manipulation techniques.....	34
2.3 Droplet detection	35
2.4 Droplet application	36
2.4.1 Cell encapsulation	37
2.4.2 Protein study.....	41
2.4.3 Emulsion polymerase chain reaction (ePCR)	43
2.4.4 Synthesis	45
2.4.5 High-throughput screening.....	49
2.4.6 Other applications of droplets	52
Chapter 3 Experimental Approaches	53
3.1. Methodology	53
3.1.1 Droplet microfluidic system: fabrication and system integration	53
3.1.1.1 Soft lithography	53
3.1.1.2 Oxygen plasma bonding and post-processing for water in oil emulsion formation	55
3.1.1.3 Obtaining microfluidic structures with high aspect ratio	56
3.1.1.4 Treatment of SU-8 mold for PDMS release	56
3.1.1.5 Electrode fabrication	57

3.1.1.6	Applying voltage and wire connection.....	58
3.1.1.7	Image extraction and data mining	58
3.1.2	Microfluidic droplet control	59
3.1.2.1	Surfactants.....	59
3.1.2.2	Droplet formation at T-junction	59
3.1.2.3	Droplet manipulation by DEP forces	62
3.1.2.4	Simulation of electrical field distribution.....	64
3.1.2.5	Droplet charging and manipulation by Coulomb force	64
3.1.2.6	Charge relaxation in oil phase	66
3.1.2.7	Fluid resistance	66
3.1.2.8	Stokes drag force.....	70
3.1.2.9	Surface tension.....	70
3.1.3	Characterization and applications of beads converted from droplets	72
3.1.3.1	Scanning electron microscope and energy dispersive spectrum.....	72
3.1.3.2	Raman spectroscopy	72
3.2.	Materials and equipments	73
3.2.1.	Reagents and chemicals	73
3.2.2.	Equipment	73
Chapter 4 Efficient On-demand Compound Droplet Formation: From Microfluidics to Microdroplets as Miniaturized Laboratories.....		75
4.1.	Introduction.....	75
4.2.	Platform development and experimental methods	76
4.3.	Formation of droplets in the absence of electrical field	79
4.4.	Formation of compound droplets and its principles	81
4.5.	Conclusions.....	85
Chapter 5 On-Demand Microfluidic Droplet Trapping and Fusion for On-Chip Static Droplet Assays		87
5.1.	Introduction.....	87
5.2.	On-demand droplet trapping and fusion.....	89
5.3.	Microfluidic structure design for droplet trapping, fusion and storage	95
5.4.	Static droplet array	98
5.5.	Conclusions.....	98
Chapter 6 On-demand Droplet Release for Droplet-Based Microfluidic System		100
6.1.	Introduction.....	100
6.2.	Integration of on-demand droplet trapping and fusion.....	103
6.3.	Droplet release by increasing flow of oil phase	104
6.4.	On-demand droplet release and its mechanism	105
6.5.	Conclusions.....	114
Chapter 7 Droplet Microfluidic Preparation of Au Nanoparticle-Coated Chitosan Microbeads for Flow-Through Surface-Enhanced Raman Scattering Detection		116
7.1.	Introduction	116

7.2.	Preparation and characterization.....	119
7.2.1	On-chip synthesis of chitosan microbeads	120
7.2.2	On-chip synthesis of Au nanoparticle-coated chitosan microbeads	122
7.2.3	Collection of on-chip generated microbeads	123
7.2.4	Characterization of on-chip prepared Au nanoparticle-coated chitosan microbeads...	124
7.2.5	On-chip single bead based SERS detection of R6G	124
7.2.6	Swelling test of Au nanoparticle-coated microbeads.....	124
7.3.	Results and discussion	125
7.4	Conclusions	135
Chapter 8 General Conclusion and Future Work		137
8.1.	General conclusion.....	137
8.2.	Future work	140
Abbreviate.....		142
Reference.....		144
Publications.....		156

List of figures

Figure 2.1	Schematic drawing of a piezo-driven droplet generator. (a) Location of actuator in the microfluidic device. (b) Cross-section view of the actuator position inside the PDMS device.....	15
Figure 2.2	Pneumatic valve assisted production of a droplet stream with a preset size order of 1×, 2×, 3×, 4×, 3×, 2×, 1×.....	16
Figure 2.3	Complex droplet patterns formed in channel assisted by a pneumatic valve integrated in microfluidic devices	16
Figure 2.4	Two aqueous phases are injected by the outer channels and synchronously emulsified by the central oil channel to form paired droplets. (a) Symmetric case. (b) Asymmetric case.....	17
Figure 2.5	Compound drops (a) comprise a gas bubble and aqueous drop are formed and flow through a continuous phase of silicone oil in a microchannel (b). (c) shows the schematic drawing of the device.....	18
Figure 2.6	Superposition of successive frames illustrating drop switching and sorting by laser actuation.....	20
Figure 2.7	Side view of electrocoalescence (1~9) and repelled motion of the merged droplet (10~15). Gray: 50 volume % ink + DI water solution. White: Pure DI water droplet.....	25
Figure 2.8	(a) The Dropspots device stores drops in a chamber array. (b) Brightfield image and schematic diagram illustrating the loading of the Dropspots device (scale = 100 μm).....	29
Figure 2.9	Droplet-trapping arrays. (a) Design of an individual trap. (b) Schematic flow profile for droplets approaching a trap. (c) Schematic flow profile when a droplet is residing in the trap. (d) Image of an array containing uniformly trapped aqueous droplets. (e)-(g) Brightfield images of droplet release over a 2-second time period.....	31
Figure 2.10	Schematic diagram of the droplet-based microfluidic device. It consists of two functional regions: a T-junction droplet generator and a droplet trap array. Droplets are continuously generated at the T-junction and immobilized in the droplet trap array. The inset is a magnified view of 10 traps with droplets.....	31
Figure 3.1	Sequential images showing the formation of water (in red)-in-oil emulsion droplet at a T-junction.....	60
Figure 3.2	Positive DEP force exerted on a polarized particle in a nonuniform electrical field.	62
Figure 3.3	Schematic drawing showing the customized cell for the verification of the droplet-charging process and the movement of a charged droplet in an electrical field.....	65
Figure 3.4	Channel structure and its detailed dimensions for controlling droplet movement in a branched network.....	68
Figure 3.5	Sequential images showing that droplets prefer to flow through the main channel path instead of the microwell path.	69
Figure 3.6	Equilibrium stage of a droplet aspirated into a micropipette.	71

Figure 4.1	a) The layout of the microfluidic platform. (a: oil phase; b: Pt wire; c: water phase one; d: water phase two; e: Elongation of water thread; f: Compound droplet formed;). b) Schematic diagram of the channel and electrodes position [Channel width = 200 or 500 μm , Pt wire diameter = 25 μm (not in scale), channel depth = 40 μm].....	77
Figure 4.2	The formation of alternative droplets in the absence of electrical field	79
Figure 4.3	The coalescence of two water stream fronts and formation of two-solution-in-one compound droplets in the presence of electrical field; the voltage applied across Pt wire electrode and ITO electrode is 180 V). Scale bar – 200 μm	80
Figure 4.4	Numerical simulation of electrical field around the tip of the Pt-wire electrode. The white bar represents the Pt-wire electrode located in the center of the channel for the oil phase. Y-axis: center line of flow channel of oil phase; X axis: center line of flow channel of water phase.	84
Figure 5.1	a) Schematic drawing of the microfluidic platform showing the detailed structure of the channels, microwell, and electrodes; shaded areas represent electrodes embedded in the platform; b) With no electrical field applied, droplet moves along the main channel; c) With electrical field applied, droplet is pulled into the microwell and then trapped there; d) Voltage needed to implement droplet trapping for different flow conditions; e) Detail dimensions of electrodes and droplet trapping structure; Scale bar in b) and c) - - 300 μm	89
Figure 5.2	Sequential images showing the trapped droplet fused with a passing droplet (1–3). The fused droplet is trapped in the microwell again (4–5). After removing the electrical field, no more fusion occurs (6).....	93
Figure 5.3	a) Schematic drawing showing one droplet trapped in the microwell. b) Calculated minimum velocity of the main flow needed for the trapped droplet to escape from the narrow neck for droplets of various radii; as long as the flow velocity does not exceed this value for a given droplet, the trapped droplet will be safely stored in the microwell.	94
Figure 5.4	An array of microwells for droplet fusion and thus for individual monitoring and analyzing of mixing or chemical reactions	98
Figure 6.1	a) Schematic drawing showing the structure of microfluidic platform and layout of microwell/electrodes. b) Sequential images showing the droplet-trapping process (scale bar is 300 μm). c) Sequential images showing the droplet fusion process (scale bar is 300 μm). d) Driving voltages needed for droplet trapping under different flow conditions (Q_{water} fixed at 10 $\mu\text{l/hour}$).....	102
Figure 6.2	Sequential images (extracted from video, time interval is estimated as 125 ms) showing release of droplet trapped in microwell by increased flow of continuous phase... ..	105
Figure 6.3	a) Sequential images showing release of small-sized droplet trapped in microwell.; b) Sequential images showing release of large-sized droplet trapped in microwell. c) Driving voltages needed for the release of droplets in different diameters.....	107
Figure 6.4	a) Simulated distribution of electrical field around droplet trapped in microwell. b) Oscillation movement of droplet between two plate electrodes. c) Droplet constrained by the orifice (r_0 in radius for protruding portion).....	108

Figure 6.5	Mercury ion detection through droplet reactions in arrayed microwells on chip. (Tests 1 and 2 resulted in fluorescence after the reaction between probe droplet [RB-immobilized Au nanoparticle solution] and target droplet [10 μ M mercury ion solution].) Scale bar is 500 μ m.	113
Figure 7.1	a) Schematic drawing of the microfluidic platform showing the detailed structure of the channels, microwells, and electrodes; yellow areas represent electrodes embedded in the platform;. (b) Sequential images showing droplet trapping and fusion processes to form a large-sized chitosan droplet in the microwell: (1) in the absence of an electrical field, droplets move along the main channel; (2) with an electrical field applied, the droplet passing the microwell is pulled into the microwell and then trapped there; (3 ~ 5) with the electrical field applied, droplet coalescence occurs to form a large-sized droplet; (6) the formed large droplet is still trapped in the microwell. After removing the applied electrical field, no more droplet coalescence occurred. Scale bar was 300 μ m, Q_{oil} = 220 μ l/hour, and Q_{water} = 20 μ l/hour.	120
Figure 7.2	Three-dimensional numerical simulation of the distribution of electrical field around the microwell using the finite element software (COMSOL MULTIPHYSICS; DC = 1,800 V applied across paired electrodes).	126
Figure 7.3	a) Diameter of chitosan droplet decreased with time due to WDTO. b) Au nanoparticle-coated chitosan microbead synthesized in the microwell (scale bar = 300 μ m).	128
Figure 7.4	EDS pattern of the Au nanoparticle-coated chitosan microbeads	129
Figure 7.5	Density and size of Au nanoparticles formed on chitosan microbead. a) On microbead 1 prepared by 1,000 s UV irradiation for photoreduction. b) On microbead 3 prepared by UV irradiation for 2,000 s during synthesis. c) On microbead 2 prepared by UV irradiation for 1,500 s during synthesis. d) Au nanoparticle-coated chitosan microbeads observed by SEM (scale bar [white] = 20 μ m).	131
Figure 7.6	SERS of R6G enhanced by Au nanoparticle-coated chitosan microbead 2 and SERS obtained from R6G solution only as a reference (No Au NP).	131

List of schemes

Scheme 3.1 Microfluidic device fabrication through PDMS replication and oxygen plasma bonding.....55

Summary

As an emerging technique, Droplet Microfluidics has attracted great research and development interest for its capability to form micron sized droplets for applications in chemistry and biology. Microfluidic droplets are the basic cells in droplet microfluidic system and can be seen as isolated reactors compartmentalizing reactions and molecular processes. In order for the droplet microfluidic system to be configured according to functionality requirements, it is very essential to implement independent control of individual droplet. In this PhD thesis project, a series of droplet control techniques are developed and the manipulation mechanisms are investigated. With the developed techniques, compound droplets could be formed; targeted droplet could be trapped in microwell and droplet fusion could be on-demand triggered; trapped droplets could be on-demand released to microchannel for further analysis. The effective droplet manipulation schemes developed were expected to greatly facilitate the wide usage of droplets as naturally well-defined miniaturized laboratories.

Chapter 1 Introduction

1.1. Introduction

In recent years, miniaturization has been a trend in areas such as chemistry, biochemistry, and biomedicine in terms of sample handling. In a lab, flasks, beakers, test tubes, and 96-well plates are conventionally used as containers to handle defined volumes of samples and reagents on a macroscale. Microfluidics, developed in the last two decades, has facilitated the manipulation of fluids in channels with dimensions of tens of micrometers. Compared with conventional systems, microfluidic technologies enable the use of smaller amounts of reagents to conduct reactions or detections in a much smaller platform. However, as more and more reactions or detections are made on the cellular and even molecular level, there is growing need for better tools for further scaled-down reactions or detections. At the same time, there is need for a higher degree of flexibility and complexity in fluid manipulation for more complicated tasks, such as high-throughput screening and parallel tasking. Further scaling down of a channel is inherently difficult because the fluid flows are dependent on channel dimensions. Using microvalves for flexible liquid control is never a solution due to space limitation and the difficulty of integration. Recent advances in microfluidics technology, microdroplet-based technology, have attracted tremendous interest in the implementation of further miniaturization and multiplexing. Instead of addressing continuous liquid flow in a microchannel, droplet-based microfluidics

manipulates samples and reagents in discrete nanoliter droplets. Analogous to digital electronics in the integrated circuit (IC) industry, droplet-based microfluidics is referred to as *digital microfluidics*.

In contrast to conventional microfluidics, which is normally single-phase continuous-flow, droplet-based microfluidics involves multiphase flow with droplets of one phase formed in other immiscible phases. With reactions or detections within droplets, the main advantages are obvious: smaller size, increased throughput density, and isolation even in small volumes. From microfluidics to microdroplets, the boundary of small-volume sample manipulation has been pushed to the nanoliter and even further to the picoliter. This has facilitated conducting chemical reactions with less concern for the diffusion effect and has provided a cell-like environment for understanding and studying the effect of molecular confinement. The decreased volume of droplets also means increased density in throughput. One might imagine that 1 ml of samples could generate more than 10^5 identical droplets. Researchers benefit from such high-density arrays of parallelized droplets when dealing with high-throughput screening, combinatorial chemistry, and so on. Miniaturized assays may be performed with droplet arrays because all droplets are separated from each other, and the cell-like structure of the droplets eliminates concerns about contamination, which other assays normally face. In this way, each droplet is a unique reactor allowing discrete chemical or biological transformation to be conducted while a large population of homogeneous droplets allows efficient

parallel processing and experimentation with large data sets.

1.2. Motivations

Discrete microscaled droplets are expected to play more and more roles in the chemical, pharmaceutical, and healthcare fields. In these applications, droplets are used as microreactors with precisely controlled reactions in a system volume scale of picoliters. When samples and reagents are confined in droplets in immiscible fluid, problems associated with continuous-flow microfluidic systems, such as cross-contamination between samples, adsorption on channel surfaces, and dilution of samples due to concentration gradient, may be avoided. Additional advantages such as fast mixing and thermal transfer may be achieved due to the microscale length of the droplet system.

Droplet-based microfluidic devices have already been demonstrated to perform microreactions between two kinds of samples, and the resultant droplets are normally identical in content. As an emerging technology, droplet microfluidics was expected to perform complex biological and chemical reactions, but this was limited due to the lack of effective methods to bring droplets carrying different reagents together for reactions. The fast movement of droplets also poses a challenge to the real-time monitoring of reactions in droplets. Resolving these needs will definitely broaden the use of droplet microfluidic technology and make it an attractive next generation of microfluidics.

1.3. Objective of study

The objective of this research is to investigate and develop effective droplet generation and manipulation to facilitate the use of microfluidic droplets as miniaturized laboratories. Various methods and techniques for performing droplet-based detections or reactions need to be explored together with their integration into droplet-based microfluidic systems. Efficient droplet fusion techniques and, preferably, on-demand droplet fusion within a droplet library need to be developed. Additionally, easy monitoring of what occurs inside droplets should be facilitated with the integration of techniques developed for a relevant droplet microfluidic system. More specifically, the following challenges and needs are catered to in this research.

1.3.1. Direct formation of compound droplet

Droplet microfluidics is desirable in terms of allowing reactions or detections to be conducted in picoliter-volume droplets. To conduct such reactions or detections, independent control of individual droplets is necessary such that droplets carrying different samples or reagents can be brought together and fused with each other and the outcome of the result can be easily monitored. Due to small size, non-rigidity, and motion of droplets, it remains a great challenge to implement effective droplet manipulations for on-demand reactions or detections. Such necessary droplet manipulations include droplet formation, transportation, sorting, fusion, and detection.

Traditionally, researchers form droplet pairs at a double T-junction or two single T-junctions followed by different droplet fusion schemes for reaction or detection. However, such systems are highly dependent on the periodic generation of droplet pairs and are very sensitive to flow disturbance due to the intrinsic competition flow between two aqueous streams. An alternative way is to make use of laminar flow to incorporate one isolation medium between two aqueous streams before the formation of compound droplets at a T-junction. Although this method has fewer requirements for the precision control of fluidic flow, the droplet formation is not on-demand, which hinders its applications, especially when a solvent extraction scheme needs to be employed. It is desirable to have a simple but effective on-demand droplet generation scheme to directly form compound droplets. Such on-demand compound droplet formation could simply realize the jump from continuous-flow microfluidics to droplet-based microfluidics. The first part of this thesis is dedicated to such on-demand compound droplet formation.

1.3.2. Droplet Trapping and fusion for Droplet reaction assay on-chip

Direct formation of compound droplets allows the triggering of reactions between droplet pairs. However, it is of great importance to implement droplet reaction assays on chip so that multiplexed droplet-based reactions or detections can be conducted. For microfluidic droplet-based platforms, there has been no successful implementation of such multiplexed droplet reaction assays on chip. The challenge lies in independent control over individual droplets to make the

on-demand reaction true. With thousands of droplets generated on chip, it is impossible to trace the movement of each droplet and to move each around when needed. An unusual strategy is needed to achieve that.

A possible solution might be droplet trapping, which is to trap droplets in specifically designed microfluidic structures such that the droplet of interest is positioned at a fixed location. Then, droplet fusion may be performed to coalesce the trapped droplet with the droplet passing it. Implementation of such a concept needs breakthrough discoveries in droplet manipulation and the design of a microfluidic structure.

1.3.3. Static droplet array to facilitate real time monitoring of what occurred inside droplets

Capturing what occurs inside droplets is another concern for a droplet reaction assay on chip, as well as other droplet reaction systems. A high-speed capturing system is needed in order to capture what occurs inside droplets, because droplets always move around. For fluorescence-based detection, the long exposure time poses a challenge to the high-speed capturing system. Still, a possible solution might be droplet trapping, which is to trap droplets into specifically designed microfluidic structures such that the droplet of interest is positioned at a fixed location and subjected to time-lapse monitoring. Occurring on demand is the first requirement for such droplet trapping because only the droplet of interest should be trapped for monitoring. After droplet coalescence, the fused droplet should still be trapped for monitoring of reactions inside the droplet. Such droplet

manipulations are extremely attractive because the integration of droplet trapping, fusion, and storage makes on-demand droplet reactions true. Together with individual control of the droplet manipulations at each trapping location, a real-time-monitoring, on-demand droplet reaction assay may be formed on chip.

1.3.4. Droplet release integration into droplet reaction assay to facilitate further manipulation or analysis of post-reaction droplets

For a droplet reaction assay formed on chip, there is need for on-demand release of the trapped droplet. With such a function, the trapping structure may be reused and will always be ready for the next trapping. Most importantly, after the reaction, the droplets can be released into the main channel for further manipulation and analysis. The challenge and, yet, the most desirable action is to integrate all the droplet manipulations mentioned above within a single platform for the implementation of droplet reaction assays on chip.

1.4. Organization

This dissertation is devoted to the exploration of microfluidic droplet formation and manipulation to facilitate the use of microdroplets as miniaturized laboratories. The organization of this dissertation is as follows:

Chapter 1 introduces the study and provides an overview of the motivation behind and objective of this PhD research.

Chapter 2 reviews recent developments of droplet microfluidics, including droplet generation, sorting, fusion, trapping, and detection, as well as droplet

applications in cell encapsulation, protein study, polymer chain reaction (PCR), particle synthesis, and high-throughput screening.

Chapter 3 describes in detail the experimental approaches, materials, and instruments used in this study.

Chapter 4 demonstrates an on-demand microfluidic method to produce compound microdroplets used as microreactors. Detailed mechanisms are provided for the on-demand coalescence of two water stream fronts at a double T-junction with the aid of an applied electrical field. It is highlighted that this method achieves one-step droplet formation and fusion, and each compound droplet generated contains two or more different solution contents for reaction or probing. The *in situ* fusion of different streams and on-demand production of compound droplets fills a void to facilitate the efficient use of droplet-based microreactors.

Chapter 5 presents on-demand droplet trapping and fusion techniques to form a static droplet reaction assay on chip. The detailed design of the microfluidic structure for droplet trapping and fusion is discussed, and guidelines for the design structure to ensure only targeted droplets are trapped or fused are provided. It is shown that droplet fusion control allows the adding of samples or reagents into the trapped droplet, when needed, to trigger the reaction, and droplet trapping or storage after fusion allows for *in situ* and real-time monitoring of chemical reactions inside a droplet. It is highlighted that the methods and techniques presented in this study may greatly facilitate the wide application of droplet-based microfluidics as a powerful tool to study and optimize biological and chemical

reactions (e.g., enzyme kinetic study, protein folding, crystallization, etc.).

Chapter 6 introduces an effective technique to achieve on-demand droplet release on chip. It is shown that, with this approach, released droplets can join the droplet stream in the main channel with all of their contents preserved for further analysis. It is highlighted that such a method is well integrated into the droplet reaction assay previously developed by the author, which made all the necessary droplet manipulations for the formation of droplet reaction on chip available on a single microfluidic platform.

In chapter 7, an on-chip droplet method to *in situ* prepare Au nanoparticle-coated chitosan microbeads for highly sensitive surface-enhanced Raman scattering (SERS) detections is described. It is revealed that without manual loading of beads into the channel, the microfluidic droplet technique allows *in situ* conversion of polymer droplets to solid polymer microbeads. Followed by further chemical processing, the polymer beads may be converted to functional beads embedded in the channel. It is highlighted that such on-chip conversion of droplets to localized functional microbeads provides a universal approach to *in situ* fabrication of novel functional beads for bead-based microfluidic molecular detections.

Finally, in chapter 8, a general conclusion is given, and some perspectives on future research are summarized and proposed.

Chapter 2 Literature Review

This chapter mainly provides an overview of the background information about droplet microfluidics, the latest droplet-control mechanisms, and applications in the fields of chemistry and biology. For non-recent developments in droplet microfluidics, there are a number of excellent reviews for reference.¹⁻⁸.

2.1. Droplet microfluidics --- the second generation of microfluidics technology

Microfluidics is a promising technology for dealing with tiny volumes of fluids at the microscale, and it has great applications in both chemical and biological areas, such as genomics study, pharmaceutical development, proteomic analysis, combinatorial synthesis, clinical diagnostics, and so on. The first generation of microfluidics was based on transporting and manipulating continuously flowing fluid through microchannels on chips. Such a closed-channel continuous-flow system works well for most chemical and biological applications, but it is not adequate for complex tasks that require more flexible and complex fluid manipulations. The channel network architecture is inherently difficult to design for massive parallel detection or analysis because the change of flow parameters (e.g., flow resistance, pressure, concentration, etc.) at any flow path will affect the entire system.

With the need for multiple and concurrent assays on chip, the second generation of microfluidics, so-called droplet microfluidics, emerged. In droplet

microfluidics, discrete nanoliter or microliter droplets are manipulated, and the discrete droplets form a two-dimensional microfluidic array of identical basic cells for multiple and concurrent processing. Such manipulation of vastly reduced volume of fluids brings many advantages compared with continuous-flow microfluidics, as listed here:⁹

- 1) Tiny droplets create homogeneous, controlled conditions.
- 2) Very high surface-to-volume ratio of tiny droplets grants fast thermal transfer.
- 3) Each droplet moves as an independent nanoliter batch reactor.
- 4) Internal recirculation within droplets permits fast and efficient mixing.
- 5) Tiny droplets mean small reaction volumes, which significantly increase the effective concentration of the reaction reagents.
- 6) Ability to follow reactions in time.
- 7) Extraordinarily high potential throughput, as approximately 10^5 droplets are formed within 1 ml of emulsion.¹⁰
- 8) Emulsified tiny droplets form cell-like compartments.

In general, microfluidic droplets could be regarded as miniaturized laboratories and are the basic cells in droplet microfluidic system. Following the analogy of digital systems, researchers are putting tremendous efforts to implement independent control of each droplet so that the droplet microfluidic system could always be configured according to functionality requirements.

2.2 Droplet control

2.2.1 Droplet generation

The most common microfluidic droplet generation methods are through the use of T-junction¹¹⁻¹⁴ and flow-focusing¹⁵⁻¹⁹ geometries. However, in the past, research on droplet formation has mainly focused on the droplet size, size distribution, and formation rate; recently more efforts have been made toward independent control of those parameters, on-demand droplet generation, and more flexible controls over droplet composition. This is driven by the needs of combinatorial chemistry, high-throughput screening, and enzymatic assays. Traditionally, the tuning of droplet size during droplet generation occurs through varying flow conditions (i.e., decreased flow rate of the continuous phase leads to increase of droplet size). However, such tuning also leads to the change of droplet generation frequency and droplet velocity, which might not be desired for some analytical applications. Then, more flexible and decoupled control over droplet volume and droplet throughput is needed.

On the other hand, on-demand droplet generation is the extreme case of independent control of droplet sizes and droplet generation frequencies. Such extreme control is valuable for droplet coalescence, droplet synchronization, and precise droplet metering. For example, through on-demand droplet generation, droplets released into the main stream of continuous phase may have different compositions, different volumes, and different spacing from each other, which will

greatly facilitate reaction screening and kinetics study. With further control over droplet formation, droplet-droplet and droplet-bubble pairs may be formed, which could provide a route toward chemical synthesis, molecular separations, and screening.

- **Independent control of droplet size, formation frequency, and spacing**

Normally, the dynamic control of the droplet size during droplet formation is achieved by applying different flow conditions. A decreased flow rate of the continuous phase typically increases the size of the droplets, and the velocity of the drops also decreases. Different strategies are employed to decouple the drop size from the drop velocity.

A pneumatically actuated membrane valve provides a simple way to control the dimensions of the droplet size and frequency in real time over a wide range with fast response. For flow focusing, the membrane valve may be localized either at the focusing orifice or the flow-focus side channel. Changing the dimensions of the droplet formation orifice can effectively vary the droplet size, and changing the dimensions of the side channel can change the droplet generation frequency^{20, 21}. A similar technique is applied to the T-junction microchannel, with either channel wall or the cross-section²² deformed by the applied air pressure and the droplets fine tuned without adjusting the flow rate of the continuous/disperse phase.

An even more complicated liquid-chopping technique is coupled with the flow-focusing platform such that a pre-focused stream can be dynamically chopped into droplets by utilizing the active pneumatic choppers²³. At each fixed

flow rate ratio of the continuous and disperse phase, droplet size is determined by the chopping frequency. The slow response of the poly(dimethylsiloxane) PDMS membrane limits the chopping frequency, and the smallest size of the droplet generated is 3.42 μm , as reported²³.

Instead of the channel or orifice dimensions being mechanically varied, both electrowetting and temperature-based control are used to directly tackle the properties of the continuous phase and disperse phase. It was reported that the viscosity of the continuous phase changed with temperature, and this could be used as an additional droplet size control in fixed-flow conditions²⁴. Based on these findings, M. George et al. combined the temperature adjustment with the flow rate change of the continuous phase to obtain an expanded control range of droplet volumes and velocities. The main drawback of such temperature-based control is the droplet heating effect, which is not preferred for certain chemical or biological sample processes.

Compared with temperature-based control, electrowetting has less impact on the droplet heating effect with relatively low AC voltage applied to the continuous flow of the continuous phase. The applied AC voltage induces a change of contact angle of the disperse phase on the channel bottom surface where the electrode is located²⁵. Combined with the variation of the inlet pressure of the disperse phase in the flow-focusing device, a continuous range of droplet sizes can be rapidly tuned without change in inlet pressure of the continuous phase.

- **On-demand droplet generation with feedback control**

Figure 2.1 Schematic drawing of a piezo-driven droplet generator. (a) Location of actuator in the microfluidic device. (b) Cross-section view of the actuator position inside the PDMS device²⁶.

Piezoelectric actuation is one option for integration into a T-junction or flow-focusing microfluidic platform for on-demand droplet formation as shown in Figure 2.1^{26, 27}. By contacting with the PDMS membrane sealing the channel, the piezoelectric actuator converts electrical signals into mechanical translation, which then precisely determines the volume of the droplet generated. With this technique, a wide range of droplet sizes may be obtained, and droplet size is less affected by the orifice geometry, scale, and flow resistance of the downstream. With multiple inlets and multiple actuators, synchronous droplet dispensing is achieved and droplet merging is successfully demonstrated.

The integration of an active valve into a microfluidic platform is another effective way to achieve on-demand droplet formation. In a combination of a T-junction and an active switching valve connecting the microfluidic channel to a sample reservoir, on-demand, and even complex, droplet patterns along the channel may be obtained as shown in Figures 2.2 and 2.3²⁸⁻³⁰.

Figure 2.2 Pneumatic valve assisted production of a droplet stream with a preset size order of $1\times$, $2\times$, $3\times$, $4\times$, $3\times$, $2\times$, $1\times$ ²⁸.

Figure 2.3 Complex droplet patterns formed in channel assisted by a pneumatic valve integrated in microfluidic devices [a) and b) from references 29 and 30, respectively].

Precise control of the volume of the droplet dispensed is an additional requirement for on-demand droplet formation for certain applications. To enable this, close loop control of droplet dispensing is integrated into both DEP droplet dispensing and electrowetting on dielectric (EWOD) based droplet formation systems^{31, 32}. The volume of the dispensed droplet is measured through either capacitive sensing or optical sensing through distributed optical fibers and then fed back to the control module. Compared with systems without real-time control of droplet dispensing³³, the feedback control system can improve the dispensing reliability by self-compensation.

● Production of droplet pairs

Figure 2.4 Two aqueous phases are injected by the outer channels and synchronously emulsified by the central oil channel to form paired droplets. (a) Symmetric case. (b) Asymmetric case³⁴.

Formation of stable droplet pairs is very attractive, as paired droplets carrying different reagents can be coalesced downstream for initiating reactions. Instead of using a double T-junction, a microfluidic structure with two spatially separated nozzles is recommended for highly stable generation of droplet pairs at certain flow conditions, as shown in Figure 2.4³⁴. It is shown that in the pairing regime, a power law behavior is related to the droplet production frequencies and volumes.

In step emulsification, droplets pinch off from the main stream due to the Rayleigh–Plateau instability caused when the liquid jet encounters a sudden decrease of channel aspect ratio (height/width). Chokkalingam et al. made use of a double step emulsification device to produce droplet pairs³⁵. Such double step emulsification resulted in strictly alternating droplet formation, even when there was limited flow rate difference between the two aqueous phases. With further increment of the flow rate difference, triplets instead of droplet pairs may be formed in a stable way.

In addition to liquid–liquid droplet pairs formed in the continuous phase, liquid–bubble pairs can also be produced in a very stable way, as shown in Figure

2.5, which may find potential applications in chemical synthesis, molecular separations, and screening^{36, 37}.

Figure 2.5 Compound drops (a) comprise a gas bubble and aqueous drop are formed and flow through a continuous phase of silicone oil in a microchannel (b). (c) shows the schematic drawing of the device.³⁶

2.2.2 Droplet sorting

Droplet sorting is a critical process for droplet applications because most times, droplets are generated in an array format, and there is a need to sort specific droplets out of the population for purification, further processing, or analysis. Sorting may be categorized into passive and active, where passive sorting is dependent on the difference between the to-be-sorted droplet species and the rest of the droplets (e.g., their size), and active sorting can be realized by applying external forces on any targeted droplet. Whereas, in the past, most sorting, such as by electrostatic charge and DEP-actuated sorting, was dependent on the specific properties of the droplets to be sorted compared to the bulk liquid-like charge or dielectric constant, the latest, further-developed methods such as thermal, laser, and surface acoustic wave (SAW)-activated sorting are independent of the properties of droplets, which, thus, tenders more flexibility during screening process. Besides those sorting approaches,

magnetically activated droplet manipulation, including sorting, has also been demonstrated and finds application in areas such as polymer chain reactions (PCRs).

- **Thermally actuated droplet sorting**

During droplet generation, heating of the continuous phase will reduce the viscosity of the continuous phase and then lead to the change of droplet dimension. Similarly, Nguyen et al. demonstrated that by placing a microheater at a bifurcation and applying controlled heating, droplets may be entirely switched into a desired branch due to the induced thermocapillarity and reduction of flow resistance³⁸. The sorted droplets tend to flow into the branch with thermal heating. The good thing is that the operation temperature is 38°C which might be acceptable for most cell or enzyme-based applications.

- **Laser switching and sorting**

Laser-activated droplet sorting, as shown in Figure 2.6, has been continuously studied by Delville et al.³⁹ and the sorting is based on the thermocapillary flow induced by the localized heating from the laser. When the laser is shined on the droplet, interfacial tension gradients are generated and lead, in turn, to a thermocapillary force on the drop to cause the trajectory change. The use of near infrared radiation (IR) excitation in the design makes the optical absorption of water negligible and amenable to biological applications. With the large thermocapillary force generated, a 100% sorting efficiency for droplet velocities to the cm/s may be achieved.

Figure 2.6 Superposition of successive frames illustrating drop switching and sorting by laser actuation³⁹.

● Surface acoustic wave actuated sorting

The principle of SAW-activated sorting is that the local fluid transferring momentum may be altered when an elliptically oscillating wave is generated on the surface of a piezoelectric substrate (e.g., LiNbO_3) by an alternating RF frequency. For droplet sorting, the piezoelectric substrate-formed interdigital transducer (IDT) is normally placed on one side of the channel before a Y-branch flow, and the sorting of either aqueous droplets or polymer droplets can be accomplished by simply turning the RF signal to the IDT on and off⁴⁰. As mentioned, the advantage of SAW sorting is its independence of droplet properties over the bulk carrier liquid. However, the sorting speed is not clearly indicated and high-speed single-droplet sorting might be underdeveloped.

● Magnetically activated sorting

Superparamagnetic nanoparticles may be integrated into droplets so that the droplets may be manipulated when subjected to an external magnetic field.

Superparamagnetic nanoparticles are preferred because they have no magnetic memory, so continuous manipulation is possible. In the presence of a magnetic field, the force exerted on droplets is proportional to the gradient of the magnetic field and concentration of magnetic nanoparticles⁴¹. Luo et al. demonstrated that magnetic force could be used to controllably direct droplets into three distinct channels. Compared with other droplet-sorting approaches, magnetically activated sorting offers a long range and large sorting force. There is also less concern for the weak interaction between the magnetic field and the aqueous droplet. Because the force comes from magnetic particles placed inside droplets, there is no requirement on the properties of the droplet media, and even ionic samples can be manipulated in this way. In terms of system design, both thin-film microcoils and external magnetic bars can be used for the application of a magnetic field. Besides droplet sorting in channel, movement of a superparamagnetic droplet in an open chamber with controlled positioning of a magnetic bar is also demonstrated for the application of droplet-based PCR^{42, 43}.

● **Size-dependent sorting**

Passive, droplet size-based sorting is successfully implemented through laminar flow focusing and spreading in a pinched microchannel. The principle of such sorting lies in the amplification of the droplet-to-droplet laminar distance when all droplets move from a narrow channel to a wide channel. In a pinched segment, different-sized droplets are pushed against the channel side wall by the introduction of a buffer stream. When the droplet exits the pinched segment and

enters the broadened segment of the channel, the distance from the droplet center to the channel wall is amplified, and the droplets may be sorted according to size. The main advantage of this pinching and spreading-based sorting is that continuous separation can be achieved without complex platform design and operations. It has been demonstrated that multiple outlets can be coupled to the sorting process, and for sure, multiple pinching and spreading structures can also be integrated into a single platform for obtaining droplets with narrow size distribution. Experiments show that the length of the pinched segment is critical for precise sorting of droplets. Thus, Kristensen et al.⁴⁴ optimized the pinching geometry, resulting in an improvement of separation.

2.2.3 Droplet fusion

One of the advantages associated with a microfluidic droplet system is that it realizes adding reagents to droplets by controlled droplet fusion, which is extremely difficult to implement in bulk emulsion. In order for droplet fusion, droplets need to be brought together followed by film drainage, rupturing, and then coalescence. In the latest development in droplet fusion, some new structures such as pillar, microwell, and electrode array have been designed to assist in bringing a defined number of droplets together for initiating coalescence⁴⁵⁻⁴⁷. For most passive droplet fusion approaches, the concentration of surfactants around droplets is a concern⁴⁸ and may not even be allowed for the presence of surfactants⁴⁹; there is such a need to study the effect of surfactant concentration on effective

droplet coalescence. For active droplet fusion, electrocoalescence still plays the main role, while charge-based droplet fusion has also been demonstrated⁵⁰.

Whereas, in the past, droplet fusion was limited to droplet–droplet coalescence, droplet merging with the fluid stream emerged as an effective approach to return the droplet to the aqueous phase for further analysis⁵¹⁻⁵³. By doing this, some highly sensitive and already well-developed detection approaches for the continuous flow microfluidic system, such as mass spectrometry (MS) and capillary electrophoresis (CE), can be coupled to the droplet-based high-throughput screening platform⁵¹⁻⁵³.

● Droplet-droplet fusion

Anna et al. studied droplet collisions at a T-junction, characterized the response for different droplet sizes and speeds, and obtained the necessary conditions for droplet coalescence⁴⁹. While this kind of droplet coalescence involved no surfactants, Griffiths et al. varied the concentration of surfactants before and after passive droplet coalescence at a zig-zag microfluidic structure⁴⁸. Re-injecting droplets into the channel with the oil phase at low concentration of the surfactant allows 99% above droplet pairwise coalescence to be achieved; whereas, in the droplet incubation process, a higher concentration of surfactant in the oil phase (e.g., 4%) is needed in order to obtain fully stabilized droplets. Since there are different requirements for the stability of droplets for different droplet manipulation processes, such re-injection of droplets into the oil phase with a different concentration of surfactant provides a simple but effective approach to

obtaining the optimized stability of droplets.

When droplets are fully stabilized by high concentration of surfactant, specific strategies are needed to bring droplets together to trigger fusion by inducing instability at their interfaces. Niu *et al.* designed a droplet-merging chamber formed by rows of pillars in a channel, where droplets entering the chamber stop and wait for coming droplets to collide with them, which is followed by fusion^{45, 46}. The fusion process continues until the enlarged droplet is pushed out of the chamber due to the step-by-step increased hydrodynamic force. The droplet-merging process may be either passive or active through electrocoalescence, and a defined number of droplets may be fused every time through the careful control of droplet size and flow conditions. In another work by Zagnoni *et al.*, electrocoalescence of multiple droplets was also demonstrated by applying a pulsed DC waveform at a square-shaped electrode array embedded in the channel⁴⁷.

Droplet movement and fusion may also be accomplished by exploiting a Coulombic force acting on a conductive droplet immersed in oil phase. Jung *et al.* made use of an electrode dot array on a chip substrate to charge droplets, move droplets together, and then fuse droplets carrying opposite charges, as shown in Figure 2.7⁵⁰.

Figure 2.7 Side view of electrocoalescence (1~9) and repelled motion of the merged droplet (10~15). Gray: 50 volume % ink + DI water solution. White: Pure DI water droplet.⁵⁰

Another trend in droplet–droplet fusion is to make this process on-demand; thus, selective fusion of targeted droplets carrying different reagents may be made whenever needed. Such work is demonstrated by Wang *et al.* with the assistance of a specifically designed microwell coupled with a droplet-trapping function⁵⁴.

● Droplet-fluid fusion

Droplet-droplet fusion is necessary for conducting droplet based chemical reactions. However, accessing the chemical information post reaction is even more critical. In particular, highly sensitive detection of droplet contents is of great interest for monitoring chemical reactions or biochemical processes. Most times optical approaches such as fluorescence, are employed for droplet detection, although this has a disadvantage in terms of the fluorescent labels used. Since MS can be well integrated into microfluidics and has been found to have broad applications, the integration of such label-free, highly sensitive detection is, then,

extremely meaningful. Besides the integration of MS, CE is another interesting detection technique to be coupled with droplet microfluidics. Through novel designs of microfluidic structures, both the compartmentalization of CE-separated bands into droplets and loading droplets into CE separations may be performed. Thus, enhanced analytical resolution and broad applications in proteomics, genomics, metabolomics, and so on are expected.

One approach to bringing droplets encapsulated in the carrier phase into aqueous fluid is to flow an aqueous stream parallel to the carrier phase and then to send the droplet into the fluid stream through EHD force induced by the applied voltage pulse^{51, 52}. With the removing of hydrophobic carrier fluid in this way, droplets can be extracted into the aqueous stream flow through a conventional electrospray source for the conduction of electrospray ionization (ESI) MS. Such droplet-fluid fusion is fast and simple to trigger, and the fusion can be triggered by fluorescent dyes carried by droplets and excited by laser light from optical fiber embedded in the channel. Broad application of this droplet-fluid fusion technique in directed evolution, cell assays, and protein screening might be expected. Besides the droplet-fluid fusion by polarization, Kelly et al. initiated a passive droplet-fluid fusion method through an array of cylindrical posts positioned between the carrier fluid and the aqueous stream⁵⁵. Due to the interfacial tension between the two immiscible fluids, the 3 μm apertures formed by the cylindrical posts prevented bulk transfer, while only droplets passing by the aperture rapidly coalesced with the aqueous stream upon contacting the posts. With the integrated design of the

fusion and the electrospray emitter, a shorter distance from the fusion region to the ESI emitter could be obtained, and thus, much higher detection sensitivity was claimed over that previously reported^{51, 52}.

To couple CE with a droplet microfluidic system, Niu et al. and Roman et al. employed different microstructures^{53, 56}. Niu et al. made use of a passive pillar array just before a T-junction to filter out oil and to let only the droplets merge with the continuous CE buffer. The filtered oil was aspirated out from the outlet of the channel connected to the pillar array. Since there was no surfactant added into the oil phase, the coalescence occurred easily and automatically, and it was reported that there was no oil being introduced into the CE channel. On the other hand, a K-shaped microfluidic structure was utilized by Roman et al. for CE coupling, and the surfaces of different channels were selectively modified into hydrophobic or hydrophilic, such that a stable parallel flow of oil and CE buffer could be formed at the cross-point of the K channel. When droplet passed by the cross-point, coalescence between the droplet and the CE buffer occurred and part of the droplet was then extracted into the CE buffer, which was subjected to further separation processing.

So far for all approaches of droplet-fluid fusion, the active droplet-fusion through polarization has the advantage of high throughput, while the other passive methods are simple to implement and avoid the need for synchronized electrical pulses.

2.2.4 Droplet trapping, storage, and release

Droplet trapping and storage is a newly developed technique through which individual droplets may be positioned at specific locations in the channel, and thus, a static droplet array may be formed on chip. Such formation of a static droplet array has many advantages for droplet microfluidic systems. First of all, droplets are positioned separately from each other, and there is no concern for unwanted droplet coalescence, even during the thermal cycling (i.e., PCR) process. Secondly, static positioning greatly facilitates the observation of any reaction change inside the droplets, and there is less dependence on the high-speed camera system. Even real-time monitoring for extended periods is no longer a problem. Thirdly, with on-demand droplet trapping, a multiplexed droplet array may be formed. Together with on-demand droplet fusion, on-demand multiple droplet-based reactions may be easily conducted and subjected to real-time monitoring. It may be said that droplet trapping is an essential first step toward more complex droplet manipulations.

Schmitz and Rowat et al. designed a Dropspots device, which incorporates an array of well-defined round chambers for droplet confinement, as shown in Figure 2.8⁵⁷. All round chambers are serially connected with narrow constrictions in between, such that when under pressure, droplets may squeeze through the constrictions and fill the round chamber one by one. Once the flow stops, droplet flow will also stop, and driven by the surface tension, each droplet will occupy one chamber only. Such serially connected, closely positioned chambers and a

high-density droplet array may, thus, be formed. This design enables time-lapse studies of individual droplets within a large number of statically positioned droplets. With droplets physically confined in chambers, on-chip incubation is also possible, without concern for droplet coalescence. An additional advantage is that, subsequently, all droplets in chambers may be recovered and reflowed downstream by injecting oil into the device and applying pressure. Similar structures have also been presented by the same group and have been utilized for cell assay and nucleation study^{58, 59}.

Figure 2.8 (a) The Dropspots device stores drops in a chamber array. (b) Brightfield image and schematic diagram illustrating the loading of the Dropspots device (scale = 100 μm)⁵⁷.

Other designs of microfluidic structures for passive droplet trapping and storage have also been demonstrated. Niu et al. utilized an array of U-shaped trapping chambers to trap individual droplets within each chamber, as shown in Figure 2.9⁶⁰. An exhaust channel is designed to associate with each U-shaped channel to allow oil to flow through it. When a droplet is trapped, the exhaust channel is blocked by the captured droplet, and this, then, prevents a second droplet from entering the chamber. With this design, random loading of large numbers of droplets into the U-shaped chambers is demonstrated. The loaded droplets may simply be released by the reverse flow of the carrier fluid. In another unique droplet trapping and storage device, Shi et al. carefully designed the channel dimensions such that the change of channel flow resistance before and after droplet trapping was utilized for passive droplet trapping⁶¹. As shown in Figure 2.10, microwells are attached to the main channel and the flow resistance is much smaller through the microwell than through the main channel. In this way, passing droplets prefer to enter the microwell instead of flowing along the main channel. Once the microwell is occupied by the droplet, the increased flow resistance through the microwell path prevents other droplets from entering and being trapped. The advantage of this system is its simplicity in design, fabrication, and operation. The droplet formation and droplet trapping/storage are well coupled together. However, the release of trapped droplets is not demonstrated.

Figure 2.9 Droplet-trapping arrays. (a) Design of an individual trap. (b) Schematic flow profile for droplets approaching a trap. (c) Schematic flow profile when a droplet is residing in the trap. (d) Image of an array containing uniformly trapped aqueous droplets. (e)-(g) Brightfield images of droplet release over a 2-second time period.⁶⁰

Figure 2.10 Schematic diagram of the droplet-based microfluidic device. It consists of two functional regions: a T-junction droplet generator and a droplet trap array. Droplets are continuously generated at the T-junction and immobilized in the droplet trap array. The inset is a magnified view of 10 traps with droplets.⁶¹

Boukellal et al. described a droplet-trapping device that also employs the

hydrodynamic resistance change before and after droplet trapping⁶². The trapping design has a trapping well together with a bypass channel. Droplets followed by the trapped droplet will be directed to the bypass channel due to the increased flow resistance through the trapping well. The group gave detailed analysis of the hydrodynamic resistance of the channel network at different flow conditions.

In contrast to the passive droplet-trapping approaches mentioned above, active droplet trapping relies on external power or electrical control to selectively move passing droplets into storage structures. Such on-demand trapping allows multiplex static droplet arrays to be formed on chip and is more useful in terms of conducting reaction and detection when combined with a droplet fusion process. Part of the research work in this thesis is on the developing of such on-demand trapping techniques. The author designed microstructures with microwells attached to the main channel, and the geometries of the microwell and the main channel were specifically designed such that the droplets flowing through the main channel encounter less hydrodynamic resistance than the droplets flowing through the microwell⁵⁴. To move targeted droplets into the microwell, voltage is applied across electrodes embedded around microwell to induce DEP force on the droplets. Once droplets move into the microwell, the electrical field is removed, and the trapped droplet is kept in the microwell because the hydrodynamic pressure is not large enough to deform the droplet and let it escape from the microwell. The interesting thing is that, similar to the application of an electrical field, trapped droplets may be selectively fused with passing droplets, and the fused droplet can

be trapped into the microwell again. This droplet trapping and fusion scheme opens a new route to conducting on-demand droplet-based reactions and renders real-time study of chemical reactions inside a droplet possible.

2.2.5 Light-induced droplet manipulation

Recent advances in light-induced droplet manipulation have provided a new and promising route toward the developing of a droplet manipulation platform. The main advantages to using light are that it may be introduced remotely and the focusing area may be easily measured down to the micrometer. Most of the time, in droplet manipulation, light is used to generate local thermocapillary stress on droplets such that droplets may be directed in flow, and even stopped^{39, 63, 64}. However, light may also be coupled with a photoconductor for droplet manipulation driven by electrowetting^{65, 66} or DEP force^{67, 68}. In either case, the principle lies in the photoconductive layer (e.g., amorphous silicon [a-Si], for which the conductivity can be significantly increased when subjected to optical illumination). When an a-Si layer, silicon dioxide (SiO₂) layer, and Teflon layer are sequentially coated on a glass substrate and an AC bias is applied across the droplet and those layers, the light illumination shifts the voltage drop from the a-Si layer to the dielectric SiO₂ and Teflon layers, causing the reduction of the contact angle and then a wetting force on the droplet. Such optoelectrowetting can induce translational speed on a droplet up to 3.6 mm/s, as reported⁶⁶. For light-induced DEP manipulation of droplets, a droplet sits in a PDMS chamber on top of the a-Si

layer coated on glass, and a lateral electrical field is generated across the PDMS chamber. With light illuminated near the edge of a droplet, a local electrical field gradient is generated due to the local photoconductivity drop of the a-Si layer, and this results in a net DEP force, which drives the droplet away from the light beam. Specifically, the DEP force exerted on a droplet is estimated as $\sim 10^{-8}$ N in scale⁶⁸. In order to transport droplets along both lateral directions, a pair of closely positioned diamond-shaped light beams is proposed and proved to generate strong electrical field gradients in both lateral directions for droplet actuation⁶⁷.

2.2.6 Other manipulation techniques

In addition to the manipulation approaches mentioned above, some other techniques are also reported. For example, a single beam acoustic device was utilized by Lee et al. to demonstrate the trapping of a single droplet in a manner similar to an optical tweezers, while the special range of the traps was much wider than that of optical traps⁶⁹. An EWOD device was fabricated on flexible printed circuit board (PCB) paper by Abdelgawad et al., and the group demonstrated droplet manipulation across a wide range of geometries, such as inclined, declined, twisted, and even upside-down architectures⁷⁰. Gravity force was also utilized by Liu et al. for successful droplet manipulations, including droplet generation, transportation, and coalescence⁷¹. An integration of EWOD and optoelectronic tweezer for droplet manipulation on an open microfluidic platform was reported to have combined benefits in terms of low actuation power and electrically

reprogrammable microfluidic operations of individual droplets. In addition, incubation of droplets in delay lines⁷², actuation of droplets by magnetic field^{73–75}, arrangement of droplets in different orders in channel⁷⁶, and passive coalescence of a defined number of droplets with an abacus channel⁷⁷ are reported respectively.

Droplet heating is also a necessary manipulation for certain applications (e.g., PCR and laser-assisted heating have applications in such areas^{78, 79}). On the other hand, compared with oil, glass, and PDMS, water has a large dielectric loss at a GHz-varied electrical field and, thus, absorbs more power when subjected to microwave dielectric heating. This was well utilized by Issadore et al. to generate local heating of droplets in channel, and a rapid heating is achieved with the droplet temperature increased as much as 30°C in only 15 ms⁸⁰.

2.3 Droplet detection

Droplet detection is critical because it allows the monitoring of what occurred inside the droplets, and then, further manipulation or control over reaction may be achieved. Traditional droplet detection based on fluorescence is still widely used by preloading fluorescence labels into droplets^{81, 82}. In addition, some other droplet-probing approaches have been explored and reported, including electrochemical, MS, CE, SERs, and surface plasmon resonance imaging (SPRi). Electrochemical measurement was employed for the detection and characterization of droplet formation, droplet volume, and flow behavior^{83, 84}. Han et al. also utilized electrochemical detection for measuring rapid enzyme kinetics with reaction time smartly controlled by pneumatic valves integrated in the droplet

platform⁸⁵. Integration of MS^{51, 52, 55} or CE^{53, 56} into a droplet microfluidic system was reported by several groups, as mentioned in the droplet–liquid fusion section of this review. While most approaches to MS integration involved droplet-liquid fusion, Pei et al. found that in ESI-MS, oil that segregates droplets could be directly sprayed together with droplets and similar detection results could be obtained⁸⁶. In this way, without transferring droplets into the aqueous stream, both detection limit and throughput could be increased.

SERs detection provides another fast and sensitive detection when integrating into the droplet microfluidic system. In a demonstration of mercury (II) ions detection in droplets, SERs-sensitive molecule rhodamine B (RB) was adsorbed on gold nanoparticles. In the presence of mercury (II) ions, the strong affinity between mercury (II) ions and gold nanoparticles caused the release of RB, which was detected by the reduced SERs signal. Compared with fluorescence-based detection, one order of magnitude enhancement of detection sensitivity could be achieved with SERs-based detection. In another work, SPRi was coupled to a specially designed EWOD platform for in situ monitoring of reactions occurring at the gold surface film⁸⁷. Such integration avoided sample loading or array spotting because EWOD droplet transporting was employed. Multiple reactions without the use of labels could be conducted and detected with the multi-channel platform design.

2.4 Droplet application

Droplets can be utilized for cell encapsulation, protein study, emulsion polymerase chain reaction, synthesis, high-throughput screening, and so on.

2.4.1 Cell encapsulation

The cell is the basic unit of biological systems. It is crucial to obtain statistical information over large populations of cells for diagnostics or study of cellular function. Tiny droplets may be employed as miniaturized vessels for living cells, as well as live organisms, because the dimensions of the droplets match those of the cells and organisms^{88, 89}. With cells compartmentalized in droplets, a number of manipulations and processes may be carried out, including cell cultures, enzymatic reaction study, secrete detection, cytotoxicity or drug candidate screening, and so on. Most importantly, microfluidic droplets can be uniformly generated in a high-throughput manner. Compared with cellular study through flow cytometry, the droplet method confines cells, or even a single cell, in an isolated microenvironment; thus, cell assays based on analysis or manipulations may be performed. On the other hand, with cells residing within each individual droplet and chemically isolated from all other droplets, cell-secreted molecules can rapidly attain detectable levels. Thus, quick and sensitive cell assays may be performed.

Loading of cells into droplets can be either a random process or a controlled process. In a purely random process, the distribution of a number of cells per droplet follows the Poisson distribution, and in order to obtain more droplets containing only a single cell, most droplets generated contain no cells. To obtain precise information about the distribution of heterogeneous biological processes within cell populations, single cell encapsulation in droplets is preferred, and such controlled cell encapsulations have been studied recently. Chabert et al. developed

a hydrodynamic method for encapsulation of single cells into droplets and spontaneous self-sorting of these droplets to obtain only single cell-loaded droplets⁹⁰. In flow-focusing droplet generation, the flow rate difference between the two focusing oil phases induces a drift of the cell-containing stream position relative to the focusing channel axis, and a drift of droplets is generated. Large-sized droplets containing cells are then subjected to a large force, quickly recovering them into the channel axis line and sorting them at a Y-junction. Although this approach achieves a high single-cell encapsulation rate, there are still a large number of empty and unusable droplets generated, which is inefficient. In another smart single-cell encapsulation process developed by Edd and Carlo et al., high-density cells are evenly spaced by flowing rapidly through a high-aspect ratio channel⁹¹. Then, the flow of oil phase is adjusted and single cell encapsulation is achieved when the cells arrive at the droplet generator with the frequency of droplet formation. Not limited to single-cell or particle loading into droplets, Abate et al. demonstrated the encapsulation of a controllable number of particles per droplet with a device consisting of two flow-focusing junctions arranged in series⁹². The strategy was to separately control the size of the droplets and spacing between particles with two flow-focusing processes. When closely packed particles reached the first junction, aqueous phases were introduced to space particles. At the second junction, the oil phase flow was adjusted to encapsulate the desired number of particles into each droplet formed. Although the deformable gel particles were used in the experiment, it is expected that such

encapsulation could also be applied to cell encapsulation.

Kinetics of enzymes expressed by cells may be obtained through quantitative cell-based enzyme assays in droplets, as developed by Huebner and Olguin et al⁹³. It was shown that the enzyme activities measured were in good agreement with large-scale assays. However, with cell and substrate confined in droplets, the local, rapid build-up of enzyme concentration may be quickly monitored by the assay within a minute.

A major trend in cell-based assays is a preference for integration of cell encapsulation, incubation, analysis, and even cell cultivation and transfection. One approach is to assign different functions to individual modules, and droplets are transferred from module to module for different processes. Köster et al. utilized such a module-based system to encapsulate, incubate, and manipulate cells in droplets for the study of cell survival, secretion, and proliferation after cell recovery from emulsion⁵⁸. In another integrated system, Hufnagel and Huebner et al. seeded and cultured mammalian CHO-K1 cells followed by on-chip cell transfection with a gene encoding green fluorescence protein (GFP)⁹⁴. After that, the detached and collected cells were delivered into droplets by a droplet-making module for subsequent analysis. Similarly, in a modular microfluidic platform for cytotoxicity screening, Brouzes et al. integrated cell encapsulation, incubation, and even cell growth on chip followed by on-chip coalescence of cell-containing droplets with drug-containing droplets, incubation for cell treatment, and subsequent fluorescence-based cytotoxicity screening⁹⁵. The utilization of an

optical coding scheme by Brouzes et al. for the generation of a drug library enabled the identification of each drug member during the subsequent screening, and such optically encoding of droplet libraries is versatile for performing all high-throughput droplet-based screening.

By encapsulating cells in droplets, molecular tools like electroporation may be performed more efficiently than usual. Zhan et al. demonstrated the delivery of enhanced green fluorescent protein (EGFP) plasmid into Chinese hamster ovary (CHO) cells by simply flowing the cell-containing droplets through a pair of electrodes in channel⁹⁶. Such delivery of genes into host cells within confined droplets paved the way to conduct functional genomics studies by taking advantage of the high throughput of droplet microfluidics. In addition to cell compartments in water droplets, cell encapsulation in gel^{97, 98} or microcapsules⁹⁹ has also been reported. Morimoto et al. prepared anchored alginate-PLL microcapsules for cell confinement and continuous observation⁹⁹. Cells inside the semi-permeable microcapsules were found to be successfully proliferated. By coupling pH-sensitive dye to *E. coli*-encapsulated polymer microspheres, Funfak et al. investigated the pH change during the metabolic activity of *E. coli*, and thus, information about the cell cultivation inside nanoliter volume could be obtained⁸².

Besides cells, even large-sized multi-cellular organisms (e.g., *Caenorhabditis elegans*) may be compartmentalized inside droplets^{61, 89}. Starting from eggs, *C. elegans* hatches, grows to adult worms, and even gives birth to the next generation within droplets. Such droplet-based *C. elegans* assays will facilitate genetics and

neurochemistry study, as well as drug discovery in a high-throughput format at the organism level.

2.4.2 Protein study

As with what they'd like to know about genomics, scientists and clinicians now work hard toward proteomics, the study of proteins, which is critical in understanding disease mechanisms and determining effective treatment approaches. Droplet microfluidic systems are ideally suited for figuring out the structure of proteins, analyzing enzyme–substrate reactions, and identifying how proteins bind to or interact with one another. In screening protein crystallization conditions, in which, often, only a minimal amount of bio-molecules is available, the droplet system offers great advantages by consuming less reagents and increasing the number of assays (i.e., the high-throughput approach). With a droplet microfluidic system, crystallization conditions such as pH, temperature, crystallization reagents, and reagent concentrations can also be easily varied and stably controlled. Thus, the optimized conditions to obtain good-quality crystals may be quickly explored.

As recently shown, the flexible on-chip control of the nucleation and growth of protein crystals allows systematic investigation of crystallization kinetics and leads to the harvesting of crystals with good diffraction qualities¹⁰⁰. Shim et al. utilized a smartly designed platform, Phase Chip, to decouple the physical processes of protein crystal nucleation and growth¹⁰⁰. Droplets in a combinatoric sequence of protein solutions of different salt and protein concentrations are generated and

stored into individual chamber on chip. Through a dialysis membrane attached to each chamber, a reservoir is linked with the chamber. By varying the reservoir flow media and conditions, large and high-quality protein crystals may be obtained, and the role of supersaturation kinetics on nucleation and growth of protein crystals may be systematically studied. In another plug-based microfluidic platform, aggregation of protein (e.g., amyloidogenic peptides) was studied. Such measurement offered fundamental understanding of the biophysics of amyloidosis, which is significant in developing methods for detection and treatment of amyloid diseases¹⁰¹.

On the other hand, study of protein–protein interactions is critical in understanding protein functions, cultivating disease therapies, discovering new drug candidates, and developing effective diagnostic approaches. Srisa-Art et al. utilized a confocal fluorescence detection system to perform online measurements of streptavidin-biotin binding kinetics via fluorescence resonance energy transfer (FRET) between two dyes, Alexa Fluor 488 and 647¹⁰². In the same way, the group also analyzed the analysis of binding between Angiogenin and an anti-Angiogenin antibody¹⁰³. It was proved that the use of fluorescent dyes did not affect the enzyme activities as well as its binding activities with antibodies; thus, such labeling methods enable the high-throughput analysis of protein–protein interactions in droplet-based microfluidic systems.

Protein study with droplet microfluidic systems was also conducted via

self-assembled monolayers (SAM) at the fluorous–aqueous interface in a plug-based microfluidic system¹⁰⁴. Such controlled protein adsorption at the interface was utilized for membrane protein crystallization and proved to increase both the rate of nucleation and the quality of crystals formed, when compared with traditional approaches in which crystallization occurred inside plugs¹⁰⁵. Other than plug-based microfluidic systems, electrodynamic droplet manipulation methods such as EWOD and DEP have also been employed in the formation of artificial bilayer lipid membranes (BLMs), followed by electrical recording of membrane protein activity. The advantages of such systems include that the BLM can be formed simply by bringing two aqueous droplets surrounded by the lipid-containing organic phase together and the electrical measurements can be made throughout the BLM formation.

2.4.3 Emulsion polymerase chain reaction (ePCR)

The use of microfluidic droplets for emulsion polymerase chain reaction (ePCR) has been widely accepted due to many advantages including reduced reagent consumption, fast thermal cycling, high-throughput, and, most importantly, amplification in a bias-free manner. There has emerged a commercially available droplet microfluidic platform used for targeted sequencing for various biomedical applications¹⁰⁶. Recent studies on ePCR have focused on the improvement of throughput, exploitation of different heating techniques in temperature cycling, and the couple of single cell analysis into ePCR. Schaerli et al. presented a circular

platform with radial pattern channels, which conducted droplets through alternating temperature zones corresponding to denaturation, annealing, and extension for the thermal cycling process⁴¹. Since such PCR in moving droplets avoided temperature cycling of the entire device and allowed rapid head transfer, a fast, entire PCR cycle and, thus, a fast throughput may be achieved. In another platform with alternating temperature zone design, Kiss et al. also demonstrated improved system throughput¹⁰⁷. Another way to shorten the PCR process is to achieve fast thermal cycling by improving thermal heating of droplets. Due to water's permanent molecular dipole moment, which has large dielectric loss in the presence of a high-frequency electrical field, droplets tend to adsorb microwave power much more strongly than PDMS, glass, and oils. Issadore et al. utilized this to integrate the microwave dielectric heater into the microchannel to locally and rapidly heat water droplets in oil for PCR and achieved fast temperature cycling⁸⁰. Other than microwave dielectric heating, laser radiation may also be utilized to generate local and fast heating of droplets in oil. Faris et al. showed that such optical heating leads to extremely fast heating, with a completion of 40 amplification cycles in 370 seconds^{78, 79}. Thus, high-speed real-time PCR may be performed on a simple substrate without any set-up of microheaters.

Avoiding the complications linked to the thermocycling of PCRs, Mazutis et al. utilized an isothermal hyperbranched rolling circle amplification (HRCA) to achieve a high-throughput, on-chip analysis for clonal DNA amplification¹⁰⁸. In the same work, an in vitro translation (IVT) system was also employed for

quantifying the clonal amplified DNA in droplets by measuring the activity of the encoded enzymes after IVT. Such integration will broaden applications of droplet-based PCR in the rapid identification of gene products (proteomics), localization of mutations causing truncated gene products, protein folding studies, and incorporation of modified amino acids¹⁰⁹. Other than DNA molecules, single cells may also be encapsulated into individual droplets and analyzed through subsequent PCR processing. As shown by Kumaresan et al., bacteria and mammalian cells compartmented in droplets can be lysed with genomic DNA released into the droplet volume, followed by DNA sequencing and genetic analysis¹¹⁰. As usual in such a high-throughput system, reverse primer functionalized microbeads are incorporated in the droplets along with target DNA template or cell to ensure the amplification monoclonality and facilitate the analysis of amplified products.

2.4.4 Synthesis

The latest cases of synthesis through droplet microfluidic techniques include nanoparticle synthesis inside droplets; conversion of droplets to functional microspheres with or without material encapsulation; formation of unique structured microspheres, such as core shell particles and hollow microspheres; and generation of hybrid Janus microspheres. Nanoparticles can be synthesized by reaction inside droplets after droplet coalescence^{111, 112} or through the reaction at the droplet–oil phase boundary¹¹³. Benefiting from the fast mixing inside droplets,

controlled diffusion across the droplet–oil boundary and homogeneous reaction conditions over all droplets, the nanoparticles (e.g., magnetic iron oxide, Ba₂SO₄, and zeolite A) are more uniform than those obtained through synthesis in bulk solutions.

Microparticles may be formed by replacing the disperse phase with liquid monomers, polymers, or copolymers, and the generated droplets may then be converted to particles through UV polymerization^{114–118}, chemical crosslinking^{119, 120}, solvent evaporation^{121–123}, solvent diffusion^{124, 125}, or ionic gelation^{126, 127}. With the controlled reaction conditions, particles derived from this process have unique properties, such as macroporosity^{120, 121, 126}, controlled surface or skin¹¹⁵, controlled pore size^{114, 116}, and controlled internal structure¹¹⁸. By encapsulating different materials such as QDs, protein, and magnetite into the microparticles, even the microparticles themselves can serve as functional particles for drug carriers^{119–121, 126, 127}, smart drug delivery^{117, 122, 123}, catalytic reactions¹²⁵, enzyme immobilization^{114, 115}, tissue engineering^{114, 115}, chromatography¹¹⁶, and so on.

In the preparation of poly(lactic-co-glycolic acid; PLGA) biodegradable microparticles, Xu et al. conducted detailed experiments on both drug-loading and drug-release profiles, showing that microfluidic-prepared microparticles exhibit significantly reduced burst release and slower overall rates of drug release than conventional polydisperse microparticles¹²¹. In another study, Gong et al. utilized the decomposition of H₂O₂ during UV polymerization to generate

micrometer-sized internal voids within the microparticles formed¹¹⁶. It was also shown that such method ensured a homogeneous distribution of the pores inside the macroporous polymer microspheres. In addition to single step polymerization, Li et al. demonstrated that multi-step polymerization reactions could be designed and implemented to obtain uniquely structured microparticles¹¹⁸. The use of microfluidics enables the optimization of the composition of the reaction mixture by varying the flow rates of liquid reactants such that reactions of polyaddition and polycondensation may be arranged in a series through an internal reaction trigger. The resultant polymer particles may be optimized to have a unique interpenetrating polymer network (IPN) structure. Other than polymer microspheres, mesoporous silica microparticles can also be prepared through solvent evaporation¹²⁸ or solvent diffusion techniques¹²⁴. The synthesized silica particles could find diversified application in drug delivery and molecular and cellular encapsulation, as well as formation of lattices.

Core shell-structured microspheres can also be prepared, and the microfluidic synthesis even allows control over shell thickness and porosity of microspheres¹²⁹⁻¹³¹. Through reaction at the droplet–oil phase boundary, Gong et al. produced hollow titania microspheres with the droplet template¹²⁹. The controlled water diffusion and hydrolysis of titanium tetrabutoxide led to the controlled surface morphology of microspheres. Zhang et al. reported the preparation of hollow or porous poly(HEMA–MMA) microspheres with controlled structures¹³⁰. Employing proper initiators and different types of porogens can optimize

poly(HEMA–MMA) microspheres to have either a hollow or porous structure. The shell thickness of hollow microspheres or porosity of porous microspheres can also be controlled accordingly. In another work by Gong and his colleagues, magnetically functionalized chitosan core/shell microspheres were prepared through a dewetting technique¹³¹. It was shown that the incorporation of magnetite into the shell enabled the on-demand release of encapsulated materials by applying a magnetic field. Thus, smart drug delivery could be implemented with such magnetically functionalized core/shell chitosan microspheres.

Droplet microfluidics is also very suitable to generate anisotropic particles, or Janus particles¹³²⁻¹³⁵. With distinct compositions on two sides, Janus particles possess functional anisotropy, such as hydrophobic vs. hydrophilic, organic vs. inorganic, magnetic vs. non-magnetic, and so on. Various applications, such as emulsion stabilization, rheological studies, and building blocks for electronic paper, can be assigned to Janus particles. Most fabrication of Janus particles in droplet microfluidic platforms involves the co-flowing of different fluids in channel followed by polymerization or crosslinking^{132, 135}. Other than coflowing, induced phase separation of colloidal nanoparticles in droplets was also utilized by Shah et al¹³³. There is difficulty with co-flowing immiscible fluids with high interfacial tension difference in channel, and this limits the type of fluids that may be used for forming the two sides of Janus particles. To address such issues, Chen et al. developed the double emulsion technique to successfully incorporate two materials with vastly different properties into Janus particles, and excellent control over the

size, morphology, and monodispersity of particles has been demonstrated¹³⁴.

2.4.5 High-throughput screening

Droplet microfluidics is extremely suitable for high-throughput screening because it allows the precise control of nanoliter liquid volumes in a confined environment without the concern of evaporation and cross-contamination. The high-speed formation of monodisperse droplets creates homogeneous conditions within all droplets, which ensures the reproducibility requirement of screening. The latest development of droplet fusion, storage, incubation, and sorting techniques, as described earlier, facilitated the manipulation of droplets. The development of encapsulation⁹¹, sample separation¹³⁶, generation of reagent concentration¹³⁷, and droplet encoding⁹⁵ facilitated the control of droplet contents as well as its traceability. The coupling of MS^{51, 52, 55, 86}, CE⁵³, and fluorescence detection¹³⁸ allowed for direct probing of droplet contents with those well-developed, highly sensitive detection tools. Baret et al. developed a highly efficient microfluidic fluorescence-activated droplet sorter (FADS) capable of sorting droplets according to fluorescence and demonstrated the sorting of *E. coli*-containing droplets according to cell enzymatic activity¹³⁸. Compared with fluorescence-activated cell sorting (FACS) in a continuous stream, the isolation of droplets from each other ensured no cross-contamination, and thus, individual cells in droplet could be screened for their enzyme activity. Besides screening of bacterial cells according to their enzyme activities⁹³, mammalian cells may also be

sorted by employing FADS because they can survive in droplets^{58, 89}. Also benefiting from the isolation of droplets from each other, Joensson et al. described an enzymatic amplification method for the detection and analysis of cell-surface protein biomarkers on human monocytic cells so that a high signal resolution above background might be achieved¹³⁹.

A droplet system was also successfully employed for rapid bacterial detection and drug susceptibility screening. In a plug-based microfluidic platform, Boedicker et al. confined a single bacterial cell in a droplet and determined the susceptibility of bacteria to antibiotics¹⁴⁰. Owing to the high-throughput droplet microfluidic techniques combined with single-cell measurements, this drug screening could be conducted on a low-density bacterial sample without pre-incubation. For high-throughput screening with a complex drug library, drug coding was shown to be more efficient by Brouzes et al⁹⁵. In a droplet-based cytotoxicity screen, a drug library was fluorescently encoded (by droplets containing different concentrations of mitomycin C) and then screened for its cytotoxic effect on human monocytic U937 cells. Obviously, such a coding scheme permitted the identification of each member of the drug library and could be applied to other complex screening of large scaled libraries.

The coupling of MS to a droplet microfluidic platform allowed for on-line reading of the contents of droplets. Fidalgo et al. successfully conducted MS detection of droplets by polarizing the targeted droplet and then sending it back to

the aqueous phase for MS detection^{51, 52}. Pei et al⁸⁶, and Kelly et al.⁵⁵ also demonstrated the analysis of nanoliter plugs of samples separated by air or oil by ESI-MS. Both sensitivity and speed have been demonstrated to be practically suitable for detection of low-volume samples at high throughput. On the other hand, microfluidic droplets have been demonstrated as effective, too, in coupling two-dimensional separations in both time and space⁵³. After the first dimension high-resolution microscale separation, samples are segmented and encapsulated into nanoliter-volume droplets^{53, 136}. Then, in a pillar-assisted oil depletion and droplet-merging process, those droplets are loaded into the second dimension for comprehensive separations. Such coupling of droplets with two-dimensional separations greatly facilitates the high-throughput analysis of complex mixtures in proteomics, genomics, and metabolomics sciences.

Additionally, the throughput has been shown to be enhanced by generating droplets from different on-line streams representing different concentration gradients¹³⁷. High-density static droplet arrays are also formed on chip to enable the manipulation of droplets in batch processes^{57, 60, 100}. There have even been efforts to utilize droplet microfluidic devices as liquid dispensers to generate off-chip droplet arrays for large-scale screening experiments. With such high-throughput dispenser systems, an array of PCR reactions was demonstrated by Wang and Zhou et al¹⁴¹.

2.4.6 Other applications of droplets

In addition to the usages of droplets mentioned above, there are other recently developed applications, including liquid crystallography, nucleation study, electroporation of cells, and infochemistry encoding. Shui et al. utilized a nanochannel to generate femtolitre oil droplets and arrange them into three-dimensional close-packed face-centered cubics¹⁴². Such liquid droplet patterning provided an alternative to solid-state crystals for crystallography. With homogeneous conditions created across monodisperse droplets, arrayed droplets have been shown to be suitable for the nucleation and solidification study of aqueous solutions^{59, 143}. Laval et al. also utilized droplets for nucleation kinetics measurement and polymorphous investigation of aqueous solutions of potassium nitrate¹⁴⁴. It should also be noted that a novel technique termed *infochemistry* has been developed to encode information as optical pulses using droplets in a microfluidic device¹⁴⁵. The precise control over the flow of multiphase fluids offered by droplet microfluidic technique is the basis of such developments.

Chapter 3 Experimental Approaches

3.1. Methodology

Various experimental methodologies have been employed to develop droplet manipulation platforms and utilize droplets as miniaturized laboratories. The success of developing a miniaturized lab-on-chip system greatly relies on the correct use of materials, detailed understanding of droplet manipulation mechanisms, novel architectures, well-planned fabrication processes, and efficient monitoring and characterization. These methodologies are utilized and/or developed as they are related to the applications.

3.1.1 Droplet microfluidic system: fabrication and system integration

3.1.1.1 Soft lithography

Soft lithography techniques are used to make microfluidic structures through the replicating process. To make the replica mold, a negative photoresist, SU-8, is used to generate protruding patterns on silicon wafers. SU-8 is spin coated on silicon wafers followed by a baking process before UV exposure to transfer a pattern from mask to SU-8 on wafer. Then, baking and developing processes are applied to finally generate the pattern on the wafer. The thickness of the SU-8 layer is determined by the spin-coating parameters such as spin time, speed, and acceleration. This thickness will define the channel depth during the molding process.

PDMS (Corning, Sylgard 184) is the polymer material used during the molding

process. Typically, a PDMS precursor is mixed with a curing agent from the Sylgard 184 kit at a weight ratio of 10:1 followed by a thorough mixing and degassing. The degassed PDMS mixture is then poured onto the SU-8 mold and subjected to a curing at 80°C for 2 hours. The cured PDMS replica is peeled off from the mold, and holes for microfluidic inlets and outlets are cut using a customized syringe needle. To remove residues from the PDMS slab, the PDMS microstructure is washed with ethanol and, subsequently, DI water. This is followed by baking at 70°C for 1 hour.

(a) Designing & fabricating SU-8 master mold on silicon wafer

PDMS poured on the SU-8 master mold and was cured at 70°C for 1 hours

Cured PDMS layer was peeled off from master mold; Tubing inlets/outlets were cut

(b) Fabricating PDMS layer

Surface exposure to O₂ plasma (150mW RF, 1% oxygen, 20 s); Attach PDMS, glass layer together;

(c) Forming channel by PDMS-glass bonding

Scheme 3.1 Microfluidic device fabrication through PDMS replication and oxygen plasma bonding

3.1.1.2 Oxygen plasma bonding and post-processing for water in oil emulsion formation

For bonding the replicated PDMS layer with the glass slides to form the microfluidic structure, both the PDMS layer and glass slides are washed with ethanol and DI water followed by drying at 70°C for 1 hour. During oxygen plasma processing (150 mW RF, 1% oxygen, 20 s, Oxford Instruments, UK), the to-be-bonded sides of both the PDMS and glass slides are put face up in the plasma generator. After plasma treatment, the PDMS layer is quickly put together with the glass slide followed by even application of a gentle pressure on them. It takes about 30 minutes for the bonding to be firm. The oxygen plasma process leaves Si-OH groups on the surface of the PDMS slab. When the PDMS slab and a glass slide are placed in contact with each other, an irreversible bond is formed by the Si-O-Si bond.

PDMS is intrinsically hydrophobic, and its surface is rendered temporarily hydrophilic after oxygen plasma processing.¹⁴⁶ For generating water in oil emulsion, the channel has to be hydrophobic so that the channel surface is preferably wetted by the oil phase. In this case the freshly fabricated microfluidic platform will not be used immediately. In the long run, PDMS will have a water contact angle at around 110 degrees.

3.1.1.3 Obtaining microfluidic structures with high aspect ratio

In this design, a microfluidic structure with a high aspect ratio needs to be fabricated such that desired hydrodynamic resistance of channels may be obtained for droplet manipulation. Deep reactive ion etching (DRIE) is a widely used microfabrication process for highly anisotropic etching of silicon wafers to create deep trenches in wafers. However, the silicon surface after the DRIE process is rough and leads to poor bonding in forming the microfluidic chamber. While LIGA fabrication technique may be employed for creating high-aspect ratio structures, the process is quite tedious and time consuming. An alternative microfabrication technique is through Su-8 patterning to fabricate a master mold for soft lithography, but a process of multiple spincoating and exposure needs to be developed to obtain a structure of 400 μm in height.

The maximum structure height fabricated with SU-8 100 in a single-step photolithography process is 250 μm . In a two-step photolithography process to obtain a structure of 400 μm in height, a spincoating at 1000 rpm gives a SU-8 layer of about 250 μm in thickness. After post-exposure baking, another SU-8 layer of 150 μm in thickness is spin coated, followed by UV exposure for 95 seconds. There is no cohesive failure between the two layers during the following processing and usage of the master mold.

3.1.1.4 Treatment of SU-8 mold for PDMS release

To promote PDMS release during the molding of high-aspect ratio structures, a mold release coating needs to be applied on the mold surface such that the

PDMS may be smoothly peeled off without damaging the structure. Typically, vapor deposition of trimethylchlorosilane (TMCS; Sigma–Aldrich) is used to silanize the SU-8 mold to prevent the PDMS from adhering to the photoresist. The mold is placed in a sealed container with a few drops of TMCS inside for 3 minutes for the TMCS to evaporate and coat the mold. After the surface treatment, the master mold is ready for PDMS replication.

3.1.1.5 Electrode fabrication

Electrodes are necessary when electrical fields need to be generated for droplet manipulations. Electrodes on glass may be made through either a lift-off process or etching process. A lift-off process is used here for patterning Au film on glass as electrodes. Typically, photoresist is patterned on a glass wafer with the area where the metal is to be deposited exposed. Then, an adhesion layer (e.g., 50 nm platinum) followed by an Au layer (e.g., 150 nm) is deposited through a sputtering process. The whole wafer is then put in acetone with gentle agitation for a few hours until the sacrificial photoresist layer is cleared. Finally, the wafer is washed three times with ethanol and one time with water, followed by drying.

For ITO-coated glass, the desired ITO pattern is obtained by the etching of the ITO layer with a patterned photoresist as the protecting layer. To enhance the adhesion between the ITO surface and photoresist, the ITO surface should be thoroughly cleaned and subjected to a pre-bake for 30 minutes at 175°C before photoresist spincoating. A HCl : H₂O : HNO₃ (4:2:1 by volume) solution is put in an anti-acid in a fume hood for ITO etching, and the etching is conducted at room

temperature. The etching rate is estimated to be about 20 nm/min. After the etching process, the ITO glass slide is then put in acetone with agitation for removing the photoresist layer. Finally, the slide is washed three times with ethanol and one time with water followed by drying.

3.1.1.6 Applying voltage and wire connection

For circuit connections among microfabricated electrodes and power supply equipments, copper wire is placed in contact with gold or ITO electrodes, and silver paste is manually dropped on top of the copper wire and electrodes. After curing in the oven for two hours at 70°C, the silver paste becomes dry, and a solid connection between copper wire and electrodes is obtained. A wire clamp is then used to connect the copper wire to the power supply.

3.1.1.7 Image extraction and data mining

Droplet-moving speed may be estimated from video taken by a microscopic imaging system composed of Nikon SMZ1500 microscope and Nikon Coolpix 5000 digital camera. Dimensions of microdroplets can also be measured from the microscopic images. After video capturing, a series of images showing droplet trapping, fusion, and release are extracted from video files by using the commercialized software VideoCrop. Since the video-capturing speed is known, the time taken for each step of droplet manipulations may be estimated from the movie file.

3.1.2 Microfluidic droplet control

3.1.2.1 Surfactants

Emulsions are not stable systems and droplets always tend to coalescence. The addition of a small quantity of surfactants can help to prevent coalescence of droplets and greatly increase the stability of emulsions. Surfactants are categorized as ionic and nonionic according to their polar group. The molecules of an ionic surfactant bear a charge and, thus, may lead to droplet–droplet and droplet–channel wall electrostatic interactions. Thus, nonionic surfactants are more prevalent in stabilizing emulsions. Surfactants are also assigned with a trimethylchlorosilane (HLB) number to indicate the balance between their hydrophilic (water-loving) and lipophilic (water-hating) molecule groups. Low HLB surfactants are more water-hating and, thus, are normally used for water in oil emulsions. In this report, Span 80 (sorbitan monooleate) is the most often used surfactant dissolved in the oil phase for the formation of aqueous droplets, and it has a HLB number of 4.3.

3.1.2.2 Droplet formation at T-junction

Although many methods are available for generating droplets on chip, the most common one is the use of T-junction geometry formed by the intersection of two perpendicularly arranged inlet channels, as shown in Figure 3.1. Two immiscible fluid phases, the disperse phase and continuous phase, are introduced into channels and merged at the T-junction, where the shear stress generated by the continuous phase tends to deform the liquid thread of the disperse phase, whereas the interfacial tension at the fluid phase interface tends to restore the deformation.

The resultant droplet breakup may be explained by several key parameters, including the T-junction geometry, Reynolds number (Re), capillary number (Ca), and the flow rates of both the continuous phase and disperse phase.

Figure 3.1 Sequential images showing the formation of water (in red)-in-oil emulsion droplet at a T-junction

Fluid flow in channel may be characterized by the Reynolds number, which gives the relative importance of inertia and viscous stresses and may be expressed as follows¹⁴⁷:

$$Re = \frac{\rho U l}{\mu} \quad \text{Equation (3.1)}$$

where ρ is the density of the fluid, U is the speed of fluid flow in channel, l is the characteristic dimension of the microchannel, and μ denotes the dynamic viscosity of the fluid. Flow in microchannel normally leads to $Re \ll 1$, indicating the negligible inertial effects.

The capillary number indicates the competition between the viscous stresses and capillary pressure and may be described as follows¹⁴⁷:

$$Ca = \frac{\mu U l}{\gamma} \quad \text{Equation (3.2)}$$

where μ and U are the viscosity and speed of the carrier fluid, respectively, and γ denotes the interfacial tension at the interface of fluid phases.

When the capillary number is large (e.g., $Ca = 1$), the shear force exerted on the liquid thread of the disperse phase is large enough to deform it and cause it to break up to form a droplet. The size of the produced droplets may be estimated by¹²

$$r \approx \frac{\gamma}{\mu \dot{\epsilon}} \quad \text{Equation (3.3)}$$

where $\dot{\epsilon}$ is the shear rate which is determined by the flow rate of the continuous phase. Obviously the droplet size can be controlled by varying the flow rate of the continuous phase.

At relevant low rate of flow, the much smaller value of the capillary number indicates that during droplet formation, the shear force exerted on the liquid thread of the disperse phase is not large enough to significantly deform it, and in this way, liquid plugs are formed in channel. The liquid thread of the disperse phase elongates into the channel and blocks most of the channel, leading to an increase

of pressure across the thread tip. The growing liquid thread is pushed downstream and, eventually, the neck connecting the liquid thread to its main stream becomes thinner until the neck breaks as a result of the formation of a droplet. A scaling law is given for the size of the plugs as follows¹⁴⁸:

$$L/W = 1 + \alpha Q_{in} / Q_{out} \quad \text{Equation (3.4)}$$

where L is the length of the plug, W is the width of channel, and Q_{in} and Q_{out} are the flow rates of the disperse and continuous phases, respectively.

It may be seen that the plug size decreases linearly with the flow rate of the continuous phase.

3.1.2.3 Droplet manipulation by DEP forces

Figure 3.2 Positive DEP force exerted on a polarized particle in a nonuniform electrical field

Dielectric materials can be polarized by an applied electrical field. In a

non-uniform electrical field, the interaction between the electrical field and induced dipole moment lead to a force named dielectrophoretic force exerted on polarized particles. In the case that the particles are more polarizable than the surrounding medium, the DEP force tends to direct the particles toward the region where the maximum electrical field is located, as shown in Figure 3.2. For water droplets in oil phase, water is more polarizable than oil because the dielectric permittivity of water is much higher than that of oil (dielectric permittivity: $\epsilon_{H_2O} \approx 81$, $\epsilon_{Oil} \approx 2.5$). Thus, in a non-uniform electrical field, a DEP force arises and tends to direct droplets toward higher electrical fields (positive DEP). The design of electrodes to generate DEP force on droplets is, thus, to create a non-uniform electrical field with desired field distribution and magnitude. Usually, a sharp-shaped electrode is employed because the highest electrical field strength could be located at the tip of the electrode.

The DEP force exerting on the droplet may be estimated as follows¹⁴⁹:

$$\vec{F}_{DEP} = 4\pi\epsilon r^3 \text{Re}[F_{CM}] \nabla \vec{E}^2 \quad \text{Equation (3.5)}$$

where E , ϵ , and r are the electrical field intensity, the dielectric constant of the continuous phase, and the radius of the droplet, respectively, and $\text{Re}[F_{CM}]$ denotes the Clausius-Mossotti factor, which is close to 1 for water drops in oil. Obviously, the polarity of the electrical field applied has no effect on the direction of the DEP force.

3.1.2.4 Simulation of electrical field distribution

Since the gradient of electrical field determines both the direction and the magnitude of the DEP force on droplets, it is desirable to obtain a highly divergent electrical field by the optimization of design of the electrodes. The electrical field strength needed for the effective manipulation of droplets is usually quite high, in the range of $10^4 \sim 10^6$ V/m. It is possible, and very helpful, to obtain the distribution of the electrical field by simulation using finite element software such as COMSOL MULTIPHYSICS or MAXWELL. The simulation could indicate where the highest electrical field strength is located and the scale of the electrical field gradient at any specific point.

In addition, the Maxwell stress tensor may be integrated over the surface of a droplet positioned in the electrical field to obtain the DEP force exerted on the droplet. This helps to predict the behavior of droplets under various physical conditions and, in turn, assists in better design of the devices.

3.1.2.5 Droplet charging and manipulation by Coulomb force

It has been reported that a droplet in contact with an electrode can obtain a certain amount of electrical charge, and in the presence of an applied-DC electrical field, the charged droplet can move between two electrodes due to the Columbic interactions.¹⁵⁰ An experiment was conducted to verify the droplet-charging process and the movement of a charged droplet in an electrical field. A customized cell consisting of two stainless-steel flat electrodes with a 6 mm distance was made, as shown in Figure 3.3. The two steel electrodes were vertically fixed in the cell by

filling one half of the cell with PDMS followed by a process of curing at 80°C for 1 hour. Light mineral oil was then added to the cell to fill it up. The two stainless-steel electrodes were 30 mm, 15 mm, and 2 mm in length, width, and thickness, respectively.

Figure 3.3 Schematic drawing showing the customized cell for the verification of the droplet-charging process and the movement of a charged droplet in an electrical field

To verify the droplet-charging process and the movement of a charged droplet in an electrical field, a water droplet was micropipetted into the oil in between the two electrodes in the cell, and then a 1,000 V DC voltage was applied across the two electrodes. It was found that the droplet moved a distance to get in contact with one of the electrodes and moved away from the electrode immediately toward the opposite electrode. Once in contact with the opposite electrode, the droplet

again reversed its direction of motion toward the opposite electrode. When the droplet was in contact with the electrodes, slight deformation occurred. As long as the DC voltage was applied, the oscillating movement was continuous. The experimental results indicated that droplets could obtain electrical charges when in contact with DC-driven electrodes and subjected to Columbic force in the applied electrical field.

3.1.2.6 Charge relaxation in oil phase

The charge obtained by droplets by contacting the electrodes could leak away in the oil phase, although the charge leakage in oil is a slow process. The charge relaxation time in the continuous phase may be estimated by the following equation¹⁵¹ :

$$\tau = \varepsilon / \sigma \quad \text{Equation (3.6)}$$

where ε and σ are the relative permittivity and conductivity of the oil phase, respectively.

An estimation of the charge relaxation time in silicon oil is 47 s.

3.1.2.7 Fluid resistance

To obtain desired flow properties in microchannel, fluid resistance needs to be taken into consideration. When dealing with droplet breakup and droplet sorting, fluid resistance plays critical role. Since the cross-section of channel fabricated through the soft lithography process is normally rectangular, to characterize the flow in channel, the hydraulic radius (R_H) or characteristic dimension needs to be

determined. This is given as¹⁵²:

$$R_H = \frac{2A}{P} = \frac{ab}{a+b} \quad \text{Equation (3.7)}$$

where A is the cross sectional area, P is the wetted perimeter for a channel, and a and b are the height and width of the rectangular channel, respectively.

The flow resistance of a microchannel with the length of L may then be estimated as¹⁵³

$$R = 12\mu L \left[\left(1 + \frac{5}{6}\alpha \right) ab R_H^2 \right]^{-1} \quad \text{Equation (3.8)}$$

where μ is the dynamic viscosity of fluid; R is the flow resistance of the channel path; L is the length of the channel path; a and b are the depth and width of the microchannel, respectively, and $\alpha = \frac{a}{b}$ or $\frac{b}{a}$ such that $0 \leq \alpha \leq 1$; and R_H is the hydraulic radius of the microchannel defined as the ratio of the channel's cross-sectional area to its perimeter as in Equation 3.7.

The flow rate in a microchannel is proportional to the pressure change (ΔP) and inversely proportional to the fluid flow resistance, given as

$$Q = \frac{\Delta P}{R} \quad \text{Equation (3.9)}$$

where Q is the flow rate of the fluid in channel. Interestingly, this water circuit is analogical to an electrical circuit where the water pressure is equivalent to the voltage, the flow rate is equivalent to the current, and the resistance to flow is like the resistance to current.

Since the pressure drop across a channel of certain length may be estimated as $\Delta P = QR$, it is possible to build a channel network to control the droplet

movement within it. When external force is applied to the droplets, an active manipulation of droplets may be achieved. In a later study, a parallel channel with different flow resistances was designed for actively moving droplets into branched channels. The channel structure and detailed dimensions are shown in Figure 3.4.

Figure 3.4 Channel structure and its detailed dimensions for controlling droplet movement in a branched network

It was intentionally arranged that in the branched flow shown in Figure 3.4, the flow resistance of path 1 and path 2 are much different (path 1—not going through the microwell; path 2—going through the microwell). Ignoring the flow resistance of the large round area (microwell opening) for path 2 and ignoring the flow resistance due to the 90 degree turn for path 1, the flow resistances for path1 and path 2 may be estimated using Equation 3.8, where for path 1, the dimensions of L , a , and b are 715 μm , 400 μm , and 300 μm , respectively, and for path 2,

dimensions of L , a , and b are $285\text{ }\mu\text{m}$, $400\text{ }\mu\text{m}$, and $100\text{ }\mu\text{m}$, respectively. With

$\mu = 10\text{mPa}\cdot\text{s}$, the ratio of flow resistance of path 1 to that of path 2 is

$$\frac{R_1}{R_2} \approx \frac{1}{10}$$

and the flow resistance for R_1 is

$$R_1 = 1.5 \times 10^{10} \text{Pa}\cdot\text{s}\cdot\text{m}^{-3}$$

As a result of the flow resistance difference between paths 1 and 2, droplets prefer to flow through the main channel path instead of the microwell path, as shown in Figure 3.5.

Figure 3.5 Sequential images showing that droplets prefer to flow through the main channel path instead of the microwell path.

3.1.2.8 Stokes drag force

A spherical microdroplet moving in a viscous oil phase experiences a drag force which can be estimated by the Stokes's Law¹⁵⁴,

$$F = 6 \pi \mu r U \quad \text{Equation (3.10)}$$

where μ is the dynamic viscosity of the oil phase, r is the radius of the droplet, and U is the droplet velocity related to the oil flow velocity.

3.1.2.9 Surface tension

For a static microdroplet submerged in oil phase, the curvature of the water-oil interface generates a pressure difference across the two phases called Laplace pressure. Such pressure can be defined by the Young-Laplace equation^{155, 156}:

$$\Delta P = \gamma_{wo} \left(\frac{1}{R_1} + \frac{1}{R_2} \right) \quad \text{Equation (3.11)}$$

where ΔP is the pressure difference across the water and oil phases, γ_{wo} is the interfacial tension between the water and oil phases, and R_1 and R_2 represent the radii of curvature of the interface.

The Young-Laplace equation is very useful for obtaining the relationship between the interfacial tension and the pressure within a static droplet. Considering a droplet brought into contact with a micropipette, by slowly increasing the suction pressure, P_p , the droplet could be deformed and enter into the micropipette and reach an equilibrium stage, as shown in Figure 3.6.

Figure 3.6 Equilibrium stage of a droplet aspirated into a micropipette

For the part of the droplet left in the bulk solution,

$$P_{in} = \frac{2\gamma_{wo}}{R_d} \quad \text{Equation (3.12)}$$

For the part of the droplet aspirated into the micropipette,

$$P_{in} - P_p = \frac{2\gamma_{wo}}{R_p} \quad \text{Equation (3.13)}$$

Then a relationship between the applied micropipette pressure and the deformation of droplet can be acquired:

$$P_p = 2\gamma_{wo} \left(\frac{1}{R_d} - \frac{1}{R_p} \right) \quad \text{Equation (3.14)}$$

In the case of using a microchannel instead of a micropipette to capture the droplet, the deformation of the droplet in the channel will be constrained by the channel width or height depending on the channel dimensions. Then, the curvature of the droplet in that direction will be half of the channel width or height.

3.1.3 Characterization and applications of beads converted from droplets

3.1.3.1 Scanning electron microscope and energy dispersive spectrum

A scanning electron microscope (SEM; SIRION SEM, FEI, Netherlands) was used to characterize the surface morphology of the chitosan microbead and Au nanoparticles on microbead. Energy dispersive spectrum (EDS) analysis was done with SEM to confirm the formation of Au nanoparticles on chitosan microbeads.

3.1.3.2 Raman spectroscopy

A Raman microscope CRM200 (Witek, Germany) was used to probe Rhodamine 6G (R6G) immobilized on Au nanoparticle-coated chitosan microbeads in a PDMS/glass-based microfluidic device. The microfluidic device with R6G immobilized chitosan microbeads was placed under the Raman microscope system. The confocal Raman microscope system was excited by a 488 nm Ar ion laser, and the laser light was adjusted to focus on the chitosan bead in the microwell. Raman signals were captured with data acquisition time in an interval of 10 seconds with two accumulations. The Raman peaks of R6G Spectra recorded on the Au nanoparticle substrate on the chitosan microbead were compared to the reported characteristic spectra of R6G. As reported earlier, the appearance of intense Raman signals at about 1188, 1362, 1505, 1572. and 1572 cm^{-1} may be assigned to the totally symmetric modes of in-plane C-C stretching vibrations associated with R6G¹⁵⁷.

3.2. Materials and equipments

3.2.1. Reagents and chemicals

Chitosan (CS; medium molecular weight, degree of deacetylation 87%), hydrochloroauric acid (HAuCl_4), and surfactant Span 80 (sorbitan monooleate) were purchased from Sigma-Aldrich. Silicon oil and light mineral oil (M5904) were purchased from Fluka. Photoresist SU-8 100 and its developer were purchased from MicroChem Corp, USA. Poly(dimethylsiloxane) elastomer kits (Sylgard 184) were purchased from Dow Corning, USA. Also, 96% ethanol, hydrochloric acid (37%), nitric acid (50%), acetic acid (> 99.5% purity), and hydrogen peroxide were purchased from Merck KGaA, Germany. All other used chemicals were of analytical grades and obtained from common commercial suppliers.

3.2.2. Equipment

Syringe pumps (Harvard 11 Plus, Harvard Apparatus, Holliston, MA) were used for water/oil phase injection into the microchannel.

A confocal Raman microscope system (WITec CRM 200, Germany) equipped with a 488 nm laser was used for Raman spectrum measurements.

A field emission-scanning electron microscope (FESEM; SIRION SEM, FEI, Netherlands) was used to characterize the surface morphology of chitosan microbeads and Au nanoparticles on the microbead. EDS analysis was done with FESEM to confirm the formation of Au nanoparticles on microbeads.

Contact angle measurement of the PDMS substrate was carried out on FTÅ 200, First Ten Angstroms, USA.

Fluorescence detection of mercury ions in droplets was performed by a ProXPRESS 2-D Proteomic Imaging System (PerkinElmer Inc., USA).

A microscopic imaging system composed of a Nikon SMZ1500 microscope and Nikon Coolpix 5000 digital camera was used to capture images and record videos of the droplet microfluidics. Dimensions of the produced microdroplets were measured from the microscopic images.

A Milli-Q Plus system (Millipore Co.) was used to prepare pure water for chemical reactions.

A UV machine (UV Flood from Epoxy & Equipment Technology Pte Ltd, Singapore) with wavelengths over 310 nm ~ 380 nm was used to irradiate chitosan microbeads for the synthesis of Au nanoparticles.

A double-sided contact and proximity mask aligner system (Karl Suss, MA-6, Germany) was used for the photolithography process. The UV source is broadband, emitting between 350nm and 450nm (i- and h-line).

Magnetron sputtering equipment (Coaxial MSS3A, England) was employed to prepare gold thin film as electrodes on glass slides.

Plasma bonding of PDMS and glass is performed with a March PX-500™ Cleaning System (March Plasma Systems, Concord, CA) operated at a radio frequency (RF) of 13.56 MHz.

Chapter 4 Efficient On-demand Compound Droplet Formation: From Microfluidics to Microdroplets as Miniaturized Laboratories^a

4.1. Introduction

In this chapter, a novel, economical, on-demand microfluidic method of producing compound microdroplets is reported to facilitate the use of droplets as microreactors. One of the advantages associated with a microfluidic droplet system is that it realizes adding reagents to droplets by controlled droplet fusion, whereas this is extremely difficult to implement in bulk emulsion. To implement droplet-based reactions, the conventional way is to make use of specific microfluidic structures such as pillar⁴⁶, modified channel surface properties¹⁵⁸, and branched channels to bring droplets together, followed by film drainage, rupturing, and then coalescence. Electrical charging of droplets is also utilized to assign opposite charges to paired droplets during droplet formation and then to bring them together for fusion¹⁵⁹. However, pillar-assisted droplet fusion requires precise control of the flow of oil phase such that the hydrodynamic pressure releases the final produced droplet. In the passive droplet fusion initiated by the changes of

^a Reproduced in part with permission from [W. Wang, C. Yang, C. M. Li., *Small*, 5 (2009), 1149-1152,.] Copyright[2009] Wiley-VCH Verlag GmbH & Co. KGaA

channel surface properties or geometry, the concentration of surfactants around droplets is a concern⁴⁸ and even may not be allowed for the presence of surfactants⁴⁹. The approach of droplet fusion by charge is more effective and does not concern the use of surfactants. But the complicated channel structures (6 inlets) inhibit its wide use.

The new approach described here achieves one-step droplet formation and droplet fusion on a microfluidic platform. Each droplet generated through this method contains two or more different solution contents for reaction or probing. The picoliter volume droplets produced have all the benefits associated with droplet microreactors, and the platform easily can be further integrated into lab-on-chip systems.

4.2. Platform development and experimental methods

Compound droplet generation is achieved through the implementation of EHD forces during droplet formation. In brief, a pair of embedded Pt-wire and ITO electrodes produces a non-uniform electrical field in a microchannel with a high electrical field around the tip of the Pt wire. Such a non-uniform electrical field polarizes the water–oil interface and results in instability of the liquid film formed by water–oil–water phases. The two water streams are, thus, attracted to the Pt electrode and fused at the tip of the Pt electrode, followed by droplet formation. The formed compound droplets can move downstream with mixing or reaction inside.

Obviously, by use of this method, fusion of water streams may be triggered on demand while generating droplets with single-solution or multi-solution contents simply controlled by turning the electrical field on and off. This electrokinetically assisted one-step stream fusion and droplet formation allows for effectively controlled, high-throughput droplet-based microreactions.

Figure 4.1 a) The layout of the microfluidic platform. (a: oil phase; b: Pt wire; c: water phase one; d: water phase two; e: Elongation of water thread; f: Compound

droplet formed;). b) Schematic diagram of the channel and electrodes position [Channel width = 200 or 500 μm , Pt wire diameter = 25 μm (not in scale), channel depth = 40 μm]

A double T-junction microfluidic platform was designed to incorporate two aqueous streams with one or several composition(s) into a flow of oil phase. Shear and pressure forces cause the elongated aqueous threads to break up to form a droplet, and the droplet size and generation frequency can be controlled by a combination of channel dimension and flow ratio of water/oil phases¹⁶⁰. Figure 4.1 shows the layout of the microfluidic platform and the embedded electrodes. A soft lithographic technique was used to fabricate the microchannel structure with PDMS as building blocks¹⁶¹. The microchannel had a depth of 40 μm and a width of 200 μm at the double T-junction area. ITO patterned on glass served as one of the electrodes and part of the ITO electrode was in contact with oil phase. A Pt wire (25 μm in diameter, from Goodfellow Cambridge Limited) embedded in the microfluidic channel served as the opposite electrode and also formed a partial isolation between the two water streams. The ITO electrode on glass was obtained by standard lithography process followed by etching in a HCl : H₂O : HNO₃ (4:2:1 by volume) solution. To embed a Pt wire electrode in the microchannel, the Pt wire was first stretched and fixed on an ITO-patterned glass wafer. A standard photolithography process was used to pattern 45 μm x 20 μm (width x height) photoresist along the Pt wire that was centered along the width direction of the

channel. The microfluidic droplet platform was formed by aligning the PDMS channels with the patterned ITO glass slide, and bonding was achieved by oxygen plasma pretreatment. The channels for water phase and oil phase had dimensions of 200 μm wide and 40 μm high at the channel junction region. Acetone was injected into the microchannel to release the Pt wire at the double T-junction area by undercutting photoresist. Finally, the microchannel was washed by flushing with DI water. A non-uniform electrical field could be generated by applying a DC voltage (~ 180 V) across the ITO and Pt wire electrodes. The flow rates for the droplet formation were 1.6 $\mu\text{l}/\text{min}$ for the continuous oil phase and 0.25 $\mu\text{l}/\text{min}$ for the dispersed aqueous phase.

Figure 4.2 The formation of alternative droplets in the absence of electrical field

4.3. Formation of droplets in the absence of electrical field

In the absence of the electrical field, droplets were formed alternatively at each side of the Pt wire in a repeated manner as shown in Figure 4.2. Under the effects of

the pressure, shear, and surface tension forces, the water thread was elongated into the oil flow path. The growing water thread was pushed by the oil flow downstream, and the neck connecting the water thread to its inlet became thinner until the neck broke. Finally, a water droplet was formed and moved with the oil flow, and the water thread retracted back to its inlet. This process occurred alternatively or sometime randomly at each inlet of the water phase. The pressure build-up due to the elongation of one side of the water threads hindered the development of elongation of another side of the water threads.

Figure 4.3 The coalescence of two water stream fronts and formation of

two-solution-in-one compound droplets in the presence of electrical field; The voltage applied across Pt wire electrode and ITO electrode is 180 V). Scale bar – 200 μm

4.4. Formation of compound droplets and its principles

When introducing an electrical field by applying a DC voltage (e.g., 180 V) across the ITO and Pt-wire electrodes, the scenario of the droplet formation occurred in another mode. Two water threads grew from both sides. Two tips of both water threads were attached to the Pt wire where the two elongated water threads merged together. Pressure of the upstream oil phase was built up due to the almost full blockage of the channel by the two elongated water threads. Water threads elongated further downstream until the necks connecting the water threads to their inlets broke. Hence, a compound droplet from two water threads was formed and flowed downstream, while mixing and/or reaction could occur inside such two-in-one compound droplets. This process could be repeated in a much synchronous manner as shown in Figure 4. For easy observation, non-ionic dyes (for food industry) were incorporated in both water streams. The experiments were also done with pure DI water and the same phenomenon was observed.

Electrocoalescence of droplets was achieved by inducing an electrical field across the channel to polarize the interfaces of adjacent droplets leading to droplet fusion.¹⁶²⁻¹⁶⁴ By applying an electric pulse above 1.5 kV, Fidalgo et al. demonstrated

the use of electrocoalescence for selectively merging a droplet from a stream of droplets with a continuous aqueous flow for separation and detection⁵¹. Here, it was proposed that the attachment of two water threads to the Pt wire electrode was mainly due to electrohydrodynamic forces, too. It is known that the dielectric permittivity and conductivity of water are much higher than those of oil (e.g., dielectric permittivity: $\varepsilon_{H_2O} \approx 81$, $\varepsilon_{oil} \approx 2.5$). Under the non-uniform electrical field, water threads are more polarizable, and the positive dielectrophoretic force causes the water thread toward the higher electrical field where the Pt wire tip is located. The EHD body force, F_E , generated by an electrical field with strength E in a fluid of dielectric permittivity ε , density ρ , and uniform temperature T is expressed as^{165, 166}

$$F_E = \rho_e E - \frac{1}{2} E^2 \nabla \varepsilon + \frac{1}{2} \nabla \left[E^2 \frac{\partial \varepsilon}{\partial \rho} \rho \right] \quad \text{Equation (4.1)}$$

where ρ_e is the local free charge density in the fluid, and $\rho_e E$ is the Coulomb force exerted by the electrical field upon the free charges. The second and third terms correspond to the dielectrophoretic force and electrostrictive force, respectively.

The electric charge density ρ_e is given by¹⁶⁷

$$\rho_e = E \cdot \nabla \varepsilon - \frac{\varepsilon}{\sigma_E} E \cdot \nabla \sigma_E \quad \text{Equation (4.2)}$$

where σ_E is the conductivity of the water phase.

As there are significant changes in both conductivity and electric permittivity across the water-oil interface, electric charges can be built up at the interface and, hence, the Coulomb force can be produced. In addition, the force term $-\frac{1}{2}E^2\nabla\epsilon$ in Equation 4.1 is due to the spatial variation of permittivity, which, again, is significant at the water-oil interface owing to the permittivity jump (from 81 for the water to 2.5 for the oil phase). Such a force is in the direction of decreasing permittivity that points from the water phase to the oil phase regardless of the polarity of the electrical field.

As indicated, both the Coulomb force and the DEP force are proportional to the square of the electrical field intensity, suggesting a significant role of the electrical field. Maxwell (Ansoft Corporation) software was used to analyze the electrical field strength around the tip of the Pt-wire electrode in the microfluidic channels. In Figure 4.4, the simulation results show that the field strength at the tip of the Pt wire is much larger than that of any other areas around the double T-junction. Water threads under the positive DEP have a tendency to be attracted to the high-electrical field region. When the water stream front moves close to the tip of the Pt wire, the EHD force can overcome the external pressure force, leading to the attachment of water streams to the Pt wire tip. Additional experiments showed that reversing the polarity of the voltage applied across the electrodes had no effect on the attraction of water streams toward the tip of the Pt wire electrode.

This clearly indicated that the attraction force is mainly due to the electrical polarization effects.

Figure 4.4 Numerical simulation of electrical field around the tip of the Pt-wire electrode. The white bar represents the Pt-wire electrode located in the center of the channel for the oil phase. Y-axis: center line of flow channel of oil phase; X axis: center line of flow channel of water phase.

The transition from droplet formation in the absence of electrical field to the water thread coalescence followed by the formation of two-in-one compound droplets under the electrical field effect occurred almost instantaneously, and there was no obvious delay observed. For highly purified water, the electrolytic conductivity at 20°C was $\gamma > 4 \times 10^{-6} (\Omega m)^{-1}$. The time constant of charge redistribution or relaxation could then be determined by the ratio of the electrical permittivity to the conductivity, which gives a value of $\sim 1.8 \times 10^{-4}$ seconds for water

here. Taking a droplet formation rate as high as 100 Hz, the charge relaxation time (1.8×10^{-4} s) was much faster than the time of the droplet formation (0.01 s) in the present fabricated device.

Coalescence of the two water stream fronts was induced by dynamically destabilizing the interfacial oil film between the two water stream threads, which were brought together to a very close distance by a competition flow (striving to reach the point of maximum field intensity) in the constricted microchannel. The applied electrical field polarized the two water stream fronts, promoted contact between water threads, and exerted EHD forces across the interfacial film.¹⁶⁸ In experiments, the threshold voltage (180 V) required to attract water stream threads to the Pt-wire electrode generated an electrical field of about 105 Vm^{-1} to induce coalescence. Another experiment for coalescence of two water droplets (120 μm in diameter each) in a stationary oil phase with the same setup of electrodes showed that 10 V DC voltage could give consistent coalescence. It was believed that the oil flow-related pressure and shear forces would compete with the EHD force, and hence, a higher voltage was needed to achieve the droplet coalescence.

4.5. Conclusions

In summary, this research demonstrated a novel technique for targeted coalescence of two water stream fronts in a microchannel followed by formation of compound droplets. In the designed double-T microfluidic structure, both water

stream threads were attracted toward the high electrical field region where the Pt wire tip was located. Then, the coalescence of two stream fronts was induced by dynamically destabilizing of the water–oil–water film facilitated by a non-uniform electrical field. Mixing within the two-in-one compound droplet could occur when the droplets flowed downstream. The generation frequency of the compound droplets as microreactors could meet the demand of the high-throughput requirement of a lab-on-chip system. Change from non-fusion to selective fusion could be achieved almost instantaneously. This new technique could be easily integrated into other microfluidic platforms for sorting, mixing, and detection functions. Moreover, by introducing multiple reagents at both inlets of water phase, targeted multi-step microreaction or/and detection could be implemented, and an indexed droplets array could be generated. It was believed that the ability of in-site fusion of different streams and production of compound droplets on demand fills a void to facilitate the efficient use of droplet-based microreactors.

Chapter 5 On-Demand Microfluidic Droplet Trapping and Fusion for On-Chip Static Droplet Assays^b

5.1. Introduction

In this chapter, on-demand droplet trapping and droplet fusion through novel approaches are successfully demonstrated to form a static droplet assay on chip for timelapse study of droplet-based microreactions.

One of the main advantages of droplet microfluidics is the ability to add reagents to selected droplets such that chemical or biochemical reactions may be initiated. However, all the time, droplet fusions are limited to paired droplets only^{48, 111, 169}. This is due to the extreme difficulties to effectively manipulate droplets and bring them together for fusion. To fully utilize droplets as miniaturized reaction vessels, it is very desirable to implement parallel processing of droplets and to acquire vast multiplexing of data sets through on-demand fusions within a droplet library. The on-demand droplet manipulations developed in this part of the research enabled such desired implementations.

A PDMS microfluidic platform was developed for generation, trapping, and fusion of droplets with application in droplet-based on-chip assays. DC electrical fields were utilized for selectively trapping droplets into microwells associated with a microchannel. Furthermore, as the trapped microdroplet no longer moved with the

^b Reproduced in part with permission from [W. Wang, C. Yang, C. M. Li., Lab On a Chip, 9 (2009), 1504-1506.] Copyright[2009] Royal Society of Chemistry

bulk flow (i.e., oil phase), on-demand fusion of a selected droplet with the trapped droplet in the microwell could be achieved with the aid of an applied electrical field. The benefits of such a microfluidic device are manifold: Droplets may be selectively trapped into microwells, and the trapped droplets may be safely stored in the microwells, giving rise to flexibility in manipulation of such droplets. The fusion of a selected droplet with the trapped droplet allows not only for introduction of additional reagents/samples into a targeted droplet, but also for attaining controllable chemical reactions within a droplet. Importantly, since the fused droplet is trapped in the microwell, in situ monitoring may be easily achieved, and real-time study of the chemical reaction process inside droplets becomes possible without the need for a high-speed camera. This greatly simplifies droplet-based detection. With such an effectively controllable on-demand droplet-trapping and droplet fusion method, microfluidic-based lab-on-chip systems for droplet reaction assays may be implemented. It is expected that the method and technique presented in this study can greatly facilitate the wide application of droplet-based microfluidics as a powerful tool to study and optimize biological and chemical reactions (e.g., enzyme kinetic study, protein folding, crystallization, etc.).

Figure 5.1 a) Schematic drawing of the microfluidic platform showing the detailed structure of the channels, microwell, and electrodes; shaded areas represent electrodes embedded in the platform; b) With no electrical field applied, droplet moves along the main channel; c) With electrical field applied, droplet is pulled into the microwell and then trapped there; d) Voltage needed to implement droplet trapping for different flow conditions; e) Detail dimensions of electrodes and droplet trapping structure; Scale bar in b) and c) - - $300 \mu m$

5.2. On-demand droplet trapping and fusion

As schematically shown in Figure 5.1a, droplet generation, trapping, and fusion

were achieved with a PDMS-based microfluidic platform, in which silicon oil purchased from Fluke was used as the oil phase, while using DI water with dissolved non-ionic dye from the food industry for easy visualization as the aqueous phase. The microfluidic structure was fabricated using a soft lithographic technique with PDMS as building blocks¹⁶¹. ITO electrodes on glass were obtained by a standard lithography process followed by ITO etching in an HCl : H₂O : HNO₃ (4:2:1 by volume) solution. The microfluidic platform was formed by aligned bonding of PDMS slip with the ITO-patterned glass assisted by oxygen plasma pretreatment. As shown in Figure 5.1e, the ITO electrode embedded in the microwell was located with its 60 degree tip at the center of the microwell. The opposite electrode had a rectangular shape (width = 360 μm) with its lower edge 210 μm away from the center of the main channel. The thickness of the ITO thin film was around 150 nm. The T-junction for droplet formation had a dimension of 150 μm (channel width) for the disperse phase and 100 μm (channel width) for the continuous phase at the phase-merging region. All microwells had a dimension of 450 μm in diameter and joint with the main channel with 300 μm channel width. The distance between the center line of the main channel and the center of the microwell was 360 μm . All channels had the same depth: 400 μm . For the droplet formation, the flow rates were 120 ~ 300 $\mu\text{l/hour}$ for the continuous phase and 10 $\mu\text{l/hour}$ for the aqueous phase. For the droplet trapping, a DC voltage pulse (1000 ~ 2000 DC volts) was applied across each pair of ITO electrodes.

For droplet generation at the T-junction, Rayleigh–Plateau instability may cause

the elongated aqueous phase threads to break up, while the size of the droplets and frequency of droplet generation may be controlled by the effects of channel dimensions and flow ratio of two liquid–fluid phases^{170, 171}. The generated droplets flow downstream and pass by microwells one by one, as shown in Figure 5.1b. The geometries of the microwell and the main channel were specifically designed such that the droplet flowing through the main channel encounters less hydrodynamic resistance than the droplet flowing through the microwell. An analysis for the hydrodynamic resistance of these two flowing paths indicates how the droplet flow is determined, as shown in section 3.1.2.7), giving the ratio of flow resistance of path 1 to that of path 2 as

$$\frac{R_1}{R_2} \approx \frac{1}{10}$$

The flow resistance for path 1 is

$$R_1 = 1.5 \times 10^{10} \text{ Pa} \cdot \text{s} \cdot \text{m}^{-3} \quad (\mu = 10 \text{ mPa} \cdot \text{s})$$

As a result, droplets prefer to flow through the main channel path instead of the microwell path as shown in Figure 5.1b.

To selectively trap a droplet into the microwell, a DC voltage step was applied across two ITO electrodes positioned near or within the microwells as shown in Figure 5.1a. In this way, a non-uniform electrical field was generated, with the highest electrical field at the tip of the bottom electrode in the microwell. Droplets passing by the microwell are polarized and, thus, are subject to DEP force, which affects their motion. A general expression for the DEP force exerted on a spherical particle in a dielectric medium is given by¹⁴⁹

$$\vec{F}_{DEP} = 4\pi\epsilon r^3 \text{Re}[F_{CM}] \nabla \vec{E}^2$$

where E is the electrical field intensity, ϵ is the dielectric constant of the continuous phase, r is the radius of the particle and $\text{Re}[F_{CM}]$ denotes the Clausius-Mossotti factor, which is close to 1 for water drops in oil in this experiment.

In this experiment, since the dielectric permittivity of water is much higher than that of oil ($\epsilon_{H_2O} \approx 81$, $\epsilon_{oil} \approx 2.5$), a positive DEP force can pull the aqueous droplet toward the high-electrical field region so that the droplet can be trapped into the microwell, as shown in Figure 5.1c. The motion of droplet is dependent on the total external force F_{ext} exerted on the droplet, and such force is determined by integrating the hydrodynamic stress tensor and the Maxwell stress tensor over the surface of droplet¹⁷². However, precisely determining the hydrodynamic stress tensor and the Maxwell stress tensor is difficult because of the complex hydrodynamic flow and electrical fields. By varying the flow rates of aqueous and oil phases, the voltage level needed to enable the droplet trapping at different flow conditions ($Q_{oil}/Q_{aqueous}$) was obtained, as shown in Figure 5.1d. Since a higher applied voltage can generate a stronger DEP force, the voltages imposed in Figure 1d) can certainly ensure successful droplet trapping. Instead the voltage level applied being increased, high-frequency AC voltage, or even DC voltage, pulses by simple on-off operation of the power supply can have the same effect on improving droplet trapping. It is worthy of note that the effect of increased voltage results from both increased flow rate and decreased droplet size.

Figure 5.2 Sequential images showing the trapped droplet fused with a passing droplet (1–3). The fused droplet is trapped in the microwell again (4–5). After removing the electrical field, no more fusion occurs (6).

In a similar way, through the application of a DC electrical field, a selected droplet may be fused with the trapped droplet, and Figure 5.2 shows sequential images of the two-droplet fusion process. Once a droplet is chosen for fusion, a DC electrical field is switched on. As analyzed earlier, the presence of the DEP force can push the selected droplet toward the tip of the bottom electrode in the microwell, where the selected droplet and the trapped droplet meet together, leading to the occurrence of droplet fusion. Therefore, additional samples/reagents may be introduced from the selected droplet into the trapped droplets for mixing and chemical reaction. After the fused droplet is trapped in the microwell, the electrical field is turned off so that the next droplet will not be trapped in the microwell and, hence, will pass by the microwell, as shown in Figure 5.2. This ensures that only select droplet-carrying, predetermined samples/reagents may be fused with the trapped droplets for mixing and chemical reaction. Additionally, when needed, the fusion may be continuously triggered and the microwell may be completely filled.

Figure 5.3 a) Schematic drawing showing one droplet trapped in the microwell. b) Calculated minimum velocity of the main flow needed for the trapped droplet to escape from the narrow neck for droplets of various radii; as long as the flow velocity does not exceed this value for a given droplet, the trapped droplet will be safely stored in the microwell.

5.3. Microfluidic structure design for droplet trapping, fusion and storage

It is worthy of note that the geometric dimensions of the microwell, the main, and the branch channels are crucial for successfully trapping and fusing droplets in the microwell. The choice of these geometric dimensions should be made in such a way that the trapped droplet will not escape from the narrow neck of the microwell. Here, the Young-Laplace equation may be used to determine whether the trapped droplet can deform and, thus, escape from the narrow neck. Considering a trapped droplet, as shown in Figure 5.3a, for preventing the trapped droplet from escaping from the narrow neck, it is necessary to have

$$P_{in} - P_2 < \gamma \left(\frac{1}{r_{drop}} + \frac{2}{w} \right) \quad \text{Equation (5.1)}$$

where P_{in} and P_2 are the local pressure indicated in Figure 5.3a, r_{drop} and $w/2$ represent the radii of curvature of the part of the droplet extended in the narrow neck of the microwell. Because of the small volume of the part of droplet extended into the neck, the r_{drop} may be approximately chosen as the radius of the droplet with no extended part in the narrow neck.

Since the hydrodynamic pressure drops along the two flowing paths shown in Figure 5.3a are the same, an identity may be obtained:

$$(P_{in} - P_2) + (P_2 - P_3) = (P_{in} - P_1) + (P_1 - P_3)$$

$$\text{Then } (P_{in} - P_2) = (P_{in} - P_1) + (P_1 - P_3) - (P_2 - P_3) \quad \text{Equation (5.2)}$$

$$\text{For path 1, } P_1 - P_3 = Q_1 R_{path1} < Q_{main} R_{path1} \quad \text{Equation (5.3)}$$

where Q_1 and Q_2 are the flow rates of path 1 and path 2, respectively and $Q_{main} = Q_1 + Q_2$ represents the flow rate of oil phase before the microwell junction.

Applying Young's Laplace equation for the part of droplet left in the microwell,

$$P_m - P_1 = \gamma \frac{2}{r_{drop}} \quad \text{Equation (5.4)}$$

Here because of small volume of the part of the droplet extended into the neck, the r_{drop} can be approximately chosen as the radius of the droplet with no extended part in the narrow neck.

For the part of droplet extended in the narrow neck,

$$P_2 - P_3 = Q_{path2} R_{path2} > 0 \quad \text{Equation (5.5)}$$

Here $Q_{path2} R_{path2} > 0$ as the neck channel is not fully blocked by the droplet.

Combining Equation 5.3, 5.4 and 5.5 gives

$$(P_m - P_1) + (P_1 - P_3) - (P_2 - P_3) < Q_{main} R_{path1} + \gamma \frac{2}{r_{drop}} \quad \text{Equation (5.6)}$$

Combining Equation 5.2 and 5.6 gives

$$P_m - P_2 = (P_m - P_1) + (P_1 - P_3) - (P_2 - P_3) < Q_{main} R_{path1} + \gamma \frac{2}{r_{drop}} \quad \text{Equation (5.7)}$$

Equations 5.1 and 5.7 show that as long as the following is satisfied

$$P_m - P_2 = (P_m - P_1) + (P_1 - P_3) - (P_2 - P_3) < Q_{main} R_{path1} + \gamma \frac{2}{r_{drop}} \leq \gamma \left(\frac{1}{r_{drop}} + \frac{2}{w} \right)$$

$$\text{Equation (5.8)}$$

the condition for non-escaping of the droplet is fulfilled. Equation 5.8 leads to

$$Q_{main} \leq \frac{\gamma}{R_{path1}} \left(\frac{1}{r_{drop}} + \frac{2}{w} - \frac{2}{r_{drop}} \right) = \frac{\gamma}{R_{path1}} \left(\frac{2}{w} - \frac{1}{r_{drop}} \right) \quad \text{Equation (5.9)}$$

R_{path1} was calculated as $1.5 \times 10^{10} Pa \cdot s \cdot m^{-3}$, as shown in section 3.1.2.7 (fluid resistance). With the interfacial tension γ as $35 mN \cdot m^{-1}$, it can be found that

$$\frac{\gamma}{R_{path1}} = 2.3 \times 10^{-12} m^4 \cdot s^{-1}$$

Therefore, a plot of the velocity of flow $\left(\frac{Q_{main}}{Channel_Depth \times Channel_Width} \right)$ vs.

r_{drop} can be obtained as shown in Figure 5.3b

$$(velocity_of_flow = 1900 \left(0.02 - \frac{1}{r_{drop}} \right) cm/s, \text{ where } r_{drop} \text{ is in } \mu m).$$

As seen in Figure 5.3b, for the droplets ranging from 60 μm to 150 μm in radius, as long as the fluid velocity in the main channel is below 6 cm/s, the trapped droplet cannot escape from the narrow neck. In the experiment, the generated droplets were in the range between 60 μm and 150 μm , and the bulk fluid velocity in the main channel never reached 1 cm/s. Thus, the trapped droplets could be always kept in the microwell. For a fused droplet with a radius larger than 200 μm , it was also shown that an even higher flow rate is needed to push the droplet through the neck.

Equation 5.9 gives a guideline for design of the droplet trapping structure. It is necessary to simultaneously manipulate both the channel depth and the neck width to obtain an optimized design for droplet storage.

5.4. Static droplet array

Since each microwell for trapping and fusing droplets may function alone, an array of completely independent microwells for droplet trapping and fusion, as well as reaction monitoring and analyses, may be built. Electrodes are attached to individual microwells for independent control of droplet trapping and fusion. By manipulation of droplet-loading or droplet-generation schemes, droplets can carry different reagents, and a multiplex on-chip droplet reaction assay can be developed. Figure 5.4 shows such an array of microwells for droplet trapping, fusion, reaction, and storage, which may be used for on-demand droplet manipulation and analysis.

Figure 5.4 An array of microwells for droplet fusion and thus for individual monitoring and analyzing of mixing or chemical reactions

5.5. Conclusions

In summary, for the first time, on-demand microfluidic droplet trapping and fusion were demonstrated. By applying a controllable DC electrical field, a droplet could be selectively moved into a microwell. In a similar way, a targeted droplet passing by a microwell could be fused with an already-trapped droplet in the microwell. The design of this kind of microwell allowed the droplets to be selectively moved into the microwell, and the trapped droplet could be safely

stored in the microwell. Additional droplet fusion control allowed us to introduce sample/reagents into the trapped droplet when needed. Since the fused droplet was stored in the microwell, this allowed for in situ and real-time study of chemical reactions inside a droplet. Such an on-chip microreaction system provides a powerful tool to study and optimize fast (e.g., millisecond) kinetic biological and chemical reactions.

Chapter 6 On-demand Droplet Release for Droplet-Based Microfluidic System^c

6.1. Introduction

In this chapter, on-demand droplet release from microwells was successfully implemented and combined with droplet-trapping/fusion functions to make an ideal and integrated droplet-based microfluidic system.

Droplet-based reactions have been demonstrated by alternatively generating droplets of two reagents followed by droplet merging, and the resultant droplets are normally identical in contents^{48, 111, 169}. As an emerging technology, droplet microfluidics is expected to perform more complex biological/chemical reactions with effective methods to bring droplets with different reagents together. In the author's previously reported research, a PDMS microfluidic platform with submicrowells connected to the main channel for droplet trapping and fusion was developed⁵⁴. Such on-demand droplet trapping and fusion techniques enabled droplet fusion among a droplet library. However, there is a need to move the post-reaction droplets in the microwells back to the main channel, or droplet release. Through droplet release, the microwells may be used for the next round of droplet reactions, and vast multiplex data sets may then be obtained from a series of microwells. In addition, the post-reaction droplet released to the main channel

^c Reproduced in part with permission from [W. Wang, C. Yang, Y.S. Liu, C. M. Li., Lab On a Chip, 10 (2010), 559-562.] Copyright[2009] Royal Society of Chemistry

may be further manipulated and analyzed such that the reactions within droplets may be investigated and continuously controlled. Recently, both ME⁵¹ and CE⁵³ were successfully coupled to droplet microfluidic systems to bring enhanced analytical resolution in proteomics and genomics. The integration of these highly sensitive detections to the author's current system also required controlled release of post-reaction droplets downstream. To avoid loss of any information, the droplet release process should be able to preserve all contents of the droplet.

In this chapter, an on-demand droplet release scheme was developed and combined with the droplet trapping and fusion technique into a single microfluidic platform. The release was fast and had no significant disturbance on the flow in main channel. The released droplets were unbroken without loss of any contents in the droplets. Most importantly, the release was on-demand and offered the future ability to trace and process individual droplets released when employing MS or CE detections.

Figure 6.1 a) Schematic drawing showing the structure of microfluidic platform and layout of microwell/electrodes. b) Sequential images showing the droplet-trapping process (scale bar is 300 μm). c) Sequential images showing the droplet fusion process (scale bar is 300 μm). d) Driving voltages needed for droplet trapping under different flow conditions (Q_{water} fixed at 10 $\mu\text{l}/\text{hour}$).

6.2. Integration of on-demand droplet trapping and fusion

Figure 6.1a schematically shows the microfluidic platform to achieve all the droplet manipulations including droplet trapping, fusion, and release. The microchannel structure is the same as what the author reported and is also fabricated using the soft lithography technique⁵⁴. The electrodes shown in yellow are made from gold thin film deposited on a glass slide by sputtering. A 30 nm chrome layer was sputtered before the deposit of gold thin film as an adhesion layer between the gold film and glass. The patterned gold electrodes were obtained by the liftoff micro-fabrication process. The microfluidic platform was then formed by aligned bonding of the PDMS slip with the gold-patterned glass assisted by oxygen plasma pretreatment. The Au electrode embedded in the microwell is located with its top edge 100 μm away from the center of the microwell. The opposite top electrode has a rectangular shape (width = 2000 μm) with its lower edge of 840 μm away from the center line of the main channel. The bottom right electrode, as in Figure 6.1a, is 800 μm in width and 685 μm and 900 μm away from the center of microwell. For generation of aqueous droplets, oil flow (as the continuous phase) and aqueous solution flow (as the disperse phase) are introduced into the two inlets of the platform, as shown in Figure 6.1a. It is known that the Rayleigh–Plateau instability generated on the elongated aqueous phase thread can cause breakup of the thread to continuously form aqueous droplets. With the current three-electrode design, both droplet trapping and fusion are triggered by applying DC step voltages across the two electrodes at the bottom to generate a

non-uniform electrical field. Figures 6.1b and 6.1c show the sequential images captured during droplet trapping and fusion, respectively.

The driving voltages for droplet trapping under various flow conditions are shown in Figure 6.1d. The general trend is that a higher driving voltage is required to trap a water droplet as the flow rate ratio of the continuous oil phase to the dispersed water phase increases. This is due to the fact that droplets experience larger hydrodynamic inertial forces when increasing the oil-to-water flow rate ratio. Thus, a larger electrical force (and a higher applied voltage) is needed to overcome such hydrodynamic force to achieve droplet trapping.

6.3. Droplet release by increasing flow of oil phase

Since we are more interested in droplet release, two droplet release methods are proposed and demonstrated here. The first method is to release a trapped droplet through a narrow neck (connecting the microwell to the downstream channel) by increasing the flow of the oil phase. The second one is on-demand in nature and is achieved through the incorporation of EHD force by applying DC step voltages across the top electrode and the short-connected two bottom electrodes.

As theoretically studied previously, droplets trapped in a microwell may be successfully stored there as long as the flow of oil phase does not exceed a threshold limit. By increasing the flow of the oil phase, the release of droplets through the narrow neck was indeed found, as shown as in Figure 6.2. For droplet release achieved by increasing oil flow rate, the positive aspect is that all droplets

in different diameters may be released very quickly. However, a number of drawbacks exist: First, all droplets in the microwells are cleared simultaneously without the option for selective release of specific droplets. Second, large droplets that escape from the neck may be split into small droplets, as shown in Figure 6.2b, which is definitely not desirable if further analysis is needed. Third, the released droplets move very fast under the high flow of the oil phase, thereby making them impossible to be traced.

Figure 6.2 Sequential images (extracted from video, time interval is estimated as 125 ms) showing release of droplet trapped in microwell by increased flow of continuous phase.

6.4. On-demand droplet release and its mechanism

The second method, however, can overcome the drawbacks encountered during

the droplet release by increasing the flow of oil phase, and it is an on-demand method to release droplets. It can keep all the contents of the droplet during release, and thus, the droplet can be readily traced for further analysis. This method is based on the use of the EHD force that is generated by applying a DC step voltage across the top electrode and the short-connected two bottom electrodes. As shown in Figures 6.3a and 6.3b, once a DC voltage is applied, droplets move toward the upper exit, then go into the main channel, and finally join the flow in the main stream. Even if the droplet dimension is larger than the upper exit size, the droplet still can deform itself and get through, as shown in Figure 6.3(b). The entire droplet release undergoes such a mild process that no droplet splitting occurs. Besides that, the release process is quite fast, thus making the released droplet nicely join the series of droplets in the main stream.

It was proposed that two kinds of EHD forces are involved in on-demand droplet release. One is the DEP force due to the presence of the applied non-uniform electrical field. Another is the Coulomb force due to the electrical charges obtained by the droplet when in contact with the electrode embedded in microwell. During droplet release, the bottom two electrodes are short-connected and the voltage is applied across them and the top electrode to obtain the desired non-uniformity of the electric field for a stronger DEP force.

Figure 6.3 a) Sequential images showing release of small-sized droplet trapped in microwell; b) Sequential images showing release of large-sized droplet trapped in microwell; c) Driving voltages needed for the release of droplets in different diameters;

Figure 6.4 a) Simulated distribution of electrical field around droplet trapped in microwell; b) Oscillation movement of droplet between two plate electrodes; c) Droplet constrained by the orifice (r_0 in radius for protruding portion);

The DEP force exerting on the droplet can be estimated from¹⁴⁹

$$\vec{F}_{DEP} = 4\pi\epsilon r^3 \text{Re}[F_{CM}] \nabla \vec{E}^2 \quad \text{Equation (6.1)}$$

where E , ϵ , and r are the electrical field intensity, the dielectric constant of the continuous phase, and the radius of the droplet, respectively, and $\text{Re}[F_{CM}]$ denotes the Clausius–Mossotti factor, which is close to 1 for water drops in oil in this experiment. By carrying out a three-dimensional simulation with finite-element software (COMSOL MULTIPHYSICS), the distribution of the electrical field around a droplet may be obtained, assuming a 300 μm diameter with a position in the center of the microwell under an applied DC voltage of 1640 V (see Figure 6.4). It clearly indicates that the electrical field on the upper side of the droplet is higher than that on the lower side of the droplet. This shows that there is a positive DEP force that can drag the droplet toward the upper exit to release it from the microwell. To calculate the DEP force exerted on the droplet, the Maxwell stress tensor is integrated over the surface of the spherical droplet to obtain $F_{DEP} = [1.05, 3.48, 2.54] \times 10^{-5} \text{ N}$. The estimated DEP force in the y-direction is toward the upper exit of the microwell, which results in the movement of the droplet upwards in the presence of the electrical field.

Besides the DEP force exerted on the droplets, it was believed the Coulomb force also played a role during droplet release. It has been reported that a droplet in contact with an electrode can obtain a certain amount of electrical charge, and the charged droplet can experience an oscillating motion between two electrodes due to the Columbic interactions¹⁵⁰. This phenomenon was verified by the movement of a water droplet in oil phase between two stainless-steel flat electrodes. Briefly, a

droplet of 200 μm in diameter was placed between the two steel plate-type electrodes with 6 mm distance. The two steel plates as electrodes were 30 mm, 15 mm, and 2 mm in length, width, and thickness, respectively. By applying a 1,000 V DC voltage across the two steel plate-type electrodes, oscillating motion of the droplet between two electrodes was observed, as shown in Figure 6.4b, which was observed from the top of the platform. The experimental results amply demonstrate that droplets can obtain electrical charges when in contact with DC-driven electrodes.

For a to-be-released droplet in the microwell, the amount of charges acquired by the droplet once in contact with the electrode may be estimated from¹⁷³

$$q = \left[\frac{\pi^2}{6} \right] 4 \pi r^2 \varepsilon_1 \varepsilon_0 E_0 \quad \text{Equation (6.2)}$$

where r is the radius of the droplet, E_0 is the electrical field and ε_1 is the dielectric constant of the continuous phase. The induced electrostatic force is then given by

$$F = qE_0 = \left[\frac{\pi^2}{6} \right] 4 \pi r^2 \varepsilon_1 \varepsilon_0 E_0^2 \quad \text{Equation (6.3)}$$

It is worthy of a note that the charges, once acquired by the droplets from an electrode, mostly can be kept at the droplet-oil interface. This is because of the extremely slow process of the charge leakage in the oil phase. It is known that the charge relaxation time in the continuous phase can be estimated by

$$\tau = \varepsilon / \sigma \quad \text{Equation (6.4)}$$

where ε and σ are the relative permittivity and conductivity of the continuous phase, respectively. Taking silicon oil as the continuous phase in this experiment, it can be found that the value of τ is 47 s. In comparison to the short droplet release time (about 1 s), such a charge relaxation time is at least one order of magnitude longer, suggesting that Equation 6.3 with a constant charge density mode may be used here to calculate the Columbic force on the droplet. Then, the estimated electrostatic forces are 41 μN and 73 μN for 300 μm and 400 μm droplets, respectively. It also can be seen that both the DEP force and the electrostatic force are of the same order of magnitude, indicating that both are important in the droplet release process.

When the droplet deforms to get through the orifice, its curvature change leads to the change of pressure difference across the oil–water interface, and this may be explained by the interfacial force. The condition of deforming the droplet and releasing it from the microwell by electrical field is that the sum of DEP force and the Columbic force can overcome the interfacial tension force (F_γ) and the hydrodynamic drag (F_r). Mathematically, this condition may be expressed as

$$4\pi\varepsilon r^3 \text{Re} [F_{CM}] \nabla \bar{E}^2 + \left[\frac{\pi^2}{6} \right] 4\pi r^2 \varepsilon_1 \varepsilon_0 E_0^2 = F_\gamma + F_r \quad \text{Equation (6.5)}$$

The droplet deformation through the orifice results in interaction on the oil-water-orifice three-phase contact line, leading to an interfacial tension force, which tends to hold the droplet at the orifice. The scale of the interfacial tension force acting on the deformed droplet can be estimated by $F_r = 2\gamma(r_0 + l)$, with γ as the interfacial tension between the silicone oil and water, r_0 as the width of the

orifice , and t as the channel height. Substituting the values of $\gamma = 30 \text{ mN} / \text{m}$ (measured using an FTA 200 tensiometer), $r_0 = 260 \mu\text{m}$ and $t_0 = 400 \mu\text{m}$, one can compute the interfacial tension force as $39.6 \mu\text{N}$, which falls in the same order of magnitude as the DEP force and Columbic force obtained earlier. For the hydrodynamic force F_r , the Stokes drag expression is used to estimate $F_r = 6\pi\mu r U_p$, with μ as the dynamic viscosity of the oil phase and U_p as the droplet velocity related to the oil flow velocity. One can estimate such hydrodynamic force as $0.38 \mu\text{N}$ by using the values of $\mu = 10 \text{ mPa.s}$, $U_p = 1 \text{ cm} / \text{s}$, and $r = 200 \mu\text{m}$ into the Stokes drag equation. It is noted that the hydrodynamic force is much lower than the interfacial tension force under these experimental flow conditions. The droplet release processes for both small and large droplets, in comparison to the dimension of orifice, are shown in the Figure 6.3. For a droplet smaller than the orifice size, a much lower driving voltage is needed; this can be explained as less blocking of the droplet by the orifice. Since the droplets may be released quite quickly into a stream of droplets flowing in the main channel without greatly affecting the main stream flow, this can be utilized to obtain multi-droplets in a desired sequence for indexing or addressing purpose. A droplet series may be easily segmented by droplets of different sizes, colors, or other properties. When combining with droplet sorting and screening, such function will be significantly useful, and it is an additional advantage for this reported method for on-demand droplet release.

Figure 6.5 Mercury ion detection through droplet reactions in arrayed microwells on chip. (Tests 1 and 2 resulted in fluorescence after the reaction between probe droplet [RB-immobilized Au nanoparticle solution] and target droplet [$10\ \mu\text{M}$ mercury ion solution].) Scale bar is $500\ \mu\text{m}$.

As a demonstration, the present droplet-based microfluidic platform was utilized for the detection of mercury ions in water with aid of gold nanoparticles. A solution of $0.6\ \text{nM}$ RB-AuNP-PDCA was prepared according to what was reported as the probe solution (Gold colloid, G1652 from Sigma), and $100\ \mu\text{M}$ mercury ions in pure water was used as the to-be-detected solution.¹⁷⁴ In an array of microwells on the microfluidic platform, aqueous droplets carrying RB-immobilized Au nanoparticles coalesced with to-be-detected water droplets, followed by incubation for 10 minutes for the detection of mercury ions. According to the literature¹⁷⁴, when mercury ions were present, RB molecules were released from the Au nanoparticles, and the restored fluorescence of RB molecules could be detected by a ProXPRESS 2D Proteomic Imaging System (PerkinElmer Inc.), as shown in Figure 6.5. Owing to the capability for on-demand manipulation of droplets at each individual microwell among the array of microwells, both control and test reactions may be conducted side by side and repeated for different tests as many times as desired. The on-demand droplet

trapping, fusion, and release allow us to carry out such scaled-down and parallel detections/reactions on a single microfluidic chip. However, the droplets in this experiment can also be released by increasing the oil phase flow rate since there is no concern for the breakup of droplets. When integrating a mass spectrometer or capillary electrophoresis technique in the present microfluidic system for the analysis of the post-reaction of released droplets, on-demand droplet release is necessary since all contents within a droplet can be preserved for precise data acquirement.

The mercury ion detection experiments were also used to verify if there was any contamination issue for the electrodes, since the droplets might have contacted the electrode surface. After repeated ion detection experiments were carried out ten times, as described above, the fluorescent images of all electrodes were obtained and checked under a fluorescent microscope. It was noted that there was no obvious fluorescence from all electrodes, and there was not much difference between the electrodes in the control microwell and those in the test microwell. It may be understood that the presence of surfactants caused the stable thin oil film between the droplets and the electrode surface and, thus, prevented the direct contact of electrodes with the trapped droplets, eliminating the concern of electrode contamination.

6.5. Conclusions

In summary, a novel on-demand droplet release technique has been developed

and integrated with on-demand droplet trapping and fusion techniques in a single microfluidic platform for reaction and detection. Droplet release was realized by employing both DEP force and Columbic force assisted by electrodes embedded on chip. Droplets released in this approach could join the droplet stream in the main channel while keeping all contents in the released droplets for further analysis. Additionally, this approach could also be used for droplet indexing and addressing with droplets in different sizes, colors, or fluorescence as segments in a stream of droplets. By integrating all droplet manipulations within a single microfluidic platform, individually controlled and parallel microreactions with ease of monitoring could be conducted, as demonstrated in the detection of mercury ions. This technique could greatly facilitate the wide usage of droplets as naturally well-defined miniaturized laboratories.

Chapter 7 Droplet Microfluidic Preparation of Au Nanoparticle-Coated Chitosan Microbeads for Flow-Through Surface-Enhanced Raman Scattering Detection^d

7.1. Introduction

Droplet-based microfluidics has attracted significant interest for exploiting naturally well-defined cell-like droplets as miniaturized laboratories^{4, 8, 175-177} and their broad applications in chemistry, medicine, and molecular biology, such as cell compartmentalization^{58, 95, 139, 178}, DNA amplification (PCR)¹⁷⁹, enzyme kinetics study^{93, 137}, protein crystallization¹⁸⁰ and chemical synthesis^{181, 182}. However, one of the critical limitations is that droplets are usually in the form of aqueous contents surrounded by an oil phase and, thus, cannot function as rigid substrates, which greatly hinders operations to adopt conventional microfluidic processes on solid substrates, such as surface modification, binding, washing, and so on¹⁸³ for analytical applications. One possible solution is to convert the droplets to microparticles for a microbead-based microfluidic detection platform, and various available surface modifications can be used to introduce multiple functionalities on microparticles¹⁸³ through such a conversion.

The bead-based detection can greatly improve the surface-to-volume ratio

^d Reproduced in part with permission from [W. Wang, C. Yang, X.Q. Cui, Q.L. Bao, C. M. Li., *Microfluidics and Nanofluidics*, 9 (2010), MNF80110, Copyright[2010] Springer

for high target-capturing efficiency and the detection sensitivity is increased accordingly^{183, 184}. With miniaturized beads in channels, the detection-spotting region is also much narrower and real-time monitoring may be easily performed. In addition, a bead-based system may be implemented in an array format with beads physically separated from each other for multiple detections¹⁸⁵⁻¹⁸⁹. However, the conventional way to make bead-based detections requires preparation of beads offline followed by loading them into the desired microfluidic channels and/or microwells¹⁸³. Currently, the loading mainly relies on manual loading assisted by specific micromanipulators¹⁸⁶⁻¹⁸⁹, which is tedious, time consuming, and labor intensive. In addition, there is a concern about contamination caused by the subsequent sealing/bonding process if the pre-immobilized probe molecules on the beads are sensitive to the sealing/bonding reagents subsequently used. It remains a great challenge to *in situ* load single beads into various predefined positions in sealed channels.

The conventional way to prepare polymer beads such as alginate, poly(vinyl alcohol) (PVA), dextran, and chitosan beads, also involves ionic or chemical crosslinking reactions¹⁹⁰⁻¹⁹² but results in complete or partial loss of the functional groups of the polymers, then severely affecting its further functionalizations, such as the probe attachment. It is desirable to fabricate solidified polymer beads with well-preserved functional groups.

In this research, a droplet microfluidic technique was used to prepare solid,

functional microbeads at predefined locations in microchannels. With a manipulation technique recently developed by us⁵⁴, droplets containing polymers such as chitosan were generated, trapped, and fused to on-chip form polymer droplets with desired sizes. The droplets were then solidified on-chip to generate polymer microbeads by continuous loss of their water contents. Except the novel *in situ* bead formation and local positioning, the additional superiority of the approach here to the existing bead-based detection techniques is that the functional groups of the beads, the amino groups of chitosan, could be reserved for further development since they cannot be lost in the physical solidification process. In this work, the well-preserved amino groups are to complex with AuCl_4^- for further synthesis of Au nanoparticles on the beads by photoreduction. The Au/polymer beads are further used for highly sensitive on-chip SERS detection of R6G, a dye extensively used in biotechnology and bioanalytical applications such as fluorescence microscopy, flow cytometry, and ELISA.

SERS is a powerful analytical tool in the detection and characterization of molecules due to its high sensitivity produced by an enhanced local electromagnetic field from the surface plasma resonance of a nanoscale roughed metallic surface. Since SERS activity is highly dependent on the metallic surface roughness, fabrication of a suitably rough substrate is essential for Raman enhancement. Therefore, the *in situ* synthesis of Au nanoparticles on polymer microbeads provides an alternative route to obtain a highly rough surface in

microchannel for highly sensitive SERS detection of molecules.

7.2. Preparation and characterization

Chitosan (CS; medium molecular weight; degree of deacetylation, 87%) and hydrochloroauric acid (HAuCl_4) were purchased from Sigma-Aldrich. Silicon oil was purchased from Fluka and used as the oil, or continuous, phase.

Two syringe pumps (Harvard 11 Plus, Harvard Apparatus, Holliston, MA) were used for water/oil phase injection. A microscopic imaging system composed of a Nikon SMZ1500 microscope and Nikon Coolpix 5000 digital camera was used to capture images and record videos of the droplet microfluidics. Dimensions of the produced microdroplets were measured from the microscopic images. All aqueous solutions were prepared using pure water made with a Milli-Q Plus system (Millipore Co.).

A scanning electron microscope (SEM; SIRION SEM, FEI, Netherlands) was used to characterize the surface morphology of the chitosan microbead and Au nanoparticles on microbead. Energy dispersive spectrum (EDS) analysis was done with SEM to confirm the formation of Au nanoparticles on chitosan microbeads. A UV machine (UV Flood from Epoxy & Equipment Technology Pte Ltd, Singapore) with wavelengths over 310 nm ~ 380 nm was used to irradiate chitosan microbeads. SERS signals were obtained using a confocal Raman microscope system (WITec CRM 200, Germany).

Figure 7.1 a) Schematic drawing of the microfluidic platform showing the detailed structure of the channels, microwells, and electrodes; yellow areas represent electrodes embedded in the platform;. (b) Sequential images showing droplet trapping and fusion processes to form a large-sized chitosan droplet in the microwell: (1) in the absence of an electrical field, droplets move along the main channel; (2) with an electrical field applied, the droplet passing the microwell is pulled into the microwell and then trapped there; (3 ~ 5) with the electrical field applied, droplet coalescence occurs to form a large-sized droplet; (6) the formed large droplet is still trapped in the microwell. After removing the applied electrical field, no more droplet coalescence occurred. Scale bar was 300 μm , $Q_{\text{oil}} = 220 \mu\text{l/hour}$, and $Q_{\text{water}} = 20 \mu\text{l/hour}$.

7.2.1 On-chip synthesis of chitosan microbeads

Chitosan microbeads were synthesized through droplet generation, trapping, fusion, and water diffusion to the oil phase (WDTO) in a PDMS microfluidic device, as schematically shown in Figure 7.1a. The microfluidic structure was fabricated using the soft lithographic technique with PDMS as the construction material¹⁶¹. A 150 nm ITO thin film was coated on glass and further fabricated as

electrodes through photolithography and wet etching techniques. In brief, the ITO-coated glass was patterned with a thin layer (12 μm) of AZ9260 photoresist through a standard photolithography process. The photoresist-patterned glass substrate was soaked in an ITO etchant solution of $\text{HCl} : \text{H}_2\text{O} : \text{HNO}_3$ (4 : 2 : 1 by volume) for 6 minutes followed by rinsing with de-ionized water. The unprotected ITO coating was then etched away, and the photoresist layer was removed with acetone to reveal the patterned ITO electrodes. The PDMS and the ITO glass were aligned under microscope and bonded together via oxygen plasma pretreatment. The bottom electrode was positioned with its sharp tip at the center of the microwell as shown in Figure 7.1a, and the top electrode was 1200 μm away from the bottom electrode. The sharp electrode could have high electrical field strength at its sharp point to drag the fused droplet into the microwell during the droplet fusion.

A T-junction with channel inlet widths of 150 and 100 μm for the disperse and the continuous phases, respectively was employed to produce the droplets. The microwells connecting with the main channels (300 μm channel width) were made to have a diameter of 450 μm . The distance between the center line of the main channel and the microwell center was 360 μm . All channels were made to have a same depth of 400 μm . For droplet generation, chitosan (2% w/v) solution and silicon oil were used as the disperse phase and continuous phase, respectively.

Chitosan (2% w/v) solution was prepared by dissolving 3g of chitosan in

100ml of 1.5 vol.-% acetic acid solution followed by 2 hours sonication for complete dissolution. The solution was filtered with a medium-porous filter to remove non-dissolved chitosan, and then was used as the disperse phase to mix with silicon oil, the continuous phase, in an emulsion process to generate water droplets in oil. At the T-junction in the microfluidic device, Rayleigh–Plateau instability caused the elongated aqueous phase threads to break up for continuously generating chitosan droplets. The size of the droplets was determined by the channel dimensions and flow ratio of two liquid–fluid phases and, thus, was controllable^{170, 171}.

Through a droplet trapping and fusion process as shown in Figure 7.1b, a chitosan droplet in a predetermined volume was trapped in a predefined microwell. The geometries of the microwell and the main channel were specifically designed in such a way that the trapped droplet could be secured in the microwell without escaping. The oil-surrounded droplets could not contaminate the electrodes. With the continuous injection of the continuous phase, the trapped chitosan droplet gradually became solidified since its water content was lost to the continuous phase via diffusion. After the chitosan microbeads formed *in situ*, they were flushed by flow-through of ethanol in the device.

7.2.2 On-chip synthesis of Au nanoparticle-coated chitosan microbeads

To flow-through functionalize the *in situ* formed chitosan microbeads,

HAuCl₄ solution (0.5 mM) was introduced at a flow rate of 500 μ l/hour into different microwells, where AuCl₄⁻ was reduced to Au nanoparticles on the trapped chitosan microbead surface under UV irradiation. (PDMS is transparent in the 230 nm -700 nm wavelength range). The bottom of the microfluidic device (glass side) was in contact with a steel bar, most of which was immersed in an ice-water bath to prevent thermal burst of the chitosan microbeads from UV exposure. Three kinds of Au nanoparticle-coated chitosan microbeads were prepared by UV irradiation for 1,000, 1,500, and 2,000 seconds, respectively. During fabrication of the Au nanoparticle-coated microbeads, some of the produced Au nanoparticles formed in the solution could exist in the microwell or diffuse into the main channel. After UV exposure, the microchannels were washed again by flowing ethanol in.

7.2.3 Collection of on-chip generated microbeads

The *in situ* generated Au nanoparticle-coated microbeads could be collected from the microwells by reversing the fluid flow in the channels, into which DI water was introduced at a flow rate of 2 ml/hour from its outlet. Then, beads could be collected from the inlets of the channel by micropipetting for further characterizations. Before SEM characterization, all beads were washed three times with acetone and one time with water, followed by drying.

By unloading all formed beads from channels, the microfluidic device could be used for the next round of synthesis and detection, as discussed later.

7.2.4 Characterization of on-chip prepared Au nanoparticle-coated chitosan microbeads

SEM, equipped with an EDS, was used to characterize the surface morphology of on chip-prepared Au nanoparticle-coated chitosan microbeads. Samples were prepared by mounting chitosan microbeads on metal grids using a double-sided adhesive tape and were then coated with platinum under a vacuum before characterization.

7.2.5 On-chip single bead based SERS detection of R6G

A solution of 100 nM R6G in ethanol was flowed into the microchannel where Au nanoparticle-coated chitosan microbeads were embedded. After 30 minutes of incubation, a microfluidic device was placed under the Raman microscope system. The confocal Raman microscope system was excited by a 488 nm Ar ion laser, and the laser light was adjusted to focus on the chitosan bead in the microwell. Raman signals were then captured with data acquisition time in an interval of 10 seconds with two accumulations.

7.2.6 Swelling test of Au nanoparticle-coated microbeads

Au nanoparticle-coated microbeads were left in DI water for 2 hours. The diameter changes of the beads were monitored under a microscopic imaging system.

7.3. Results and discussion

The formation and transport of droplets is shown in image 1 of Figure. 7.1b, indicating that without additional interference, the droplets can only flow through the main channels and cannot be trapped in the microwells. This is because the unique design of the microwell and the main channel is such that the main channel could have lower hydrodynamic resistance than the microwell. However, by applying a DC step voltage of 1,800 V across the two ITO electrodes associated with each microwell, a droplet passing by the microwell can be pulled into the microwell as shown in image 2 of Figure 7.1b. The trapping is actually enabled by a DEP force that is generated by the polarization of droplets in the non-uniform electrical field around the microwell. The DEP force exerted on the droplet can be estimated as $\vec{F}_{DEP} = 4\pi\epsilon r^3 \text{Re}[F_{CM}] \nabla \vec{E}^2$ ¹⁹³, where E , ϵ , and r are the electrical field intensity, the dielectric constant of the continuous phase, and the radius of the droplet, respectively; $\text{Re}[F_{CM}]$ denotes the Clausius–Mossotti factor, which is close to 1 for water drops in oil. As indicated by the equation, the DEP force is proportional to the square of the electrical field intensity. Trapping or fusion is simply controlled on-demand by turning the electrical field on or off.

Figure 7.2 Three-dimensional numerical simulation of the distribution of electrical field around the microwell using the finite element software (COMSOL MULTIPHYSICS; DC = 1,800 V applied across paired electrodes).

The electrical field also determines the direction of DEP force by its distribution. As shown in Figure 7.2, we obtained the distribution of the electrical field around the microwell by three-dimensional simulation using the AC/DC module of the finite element software (COMSOL MULTIPHYSICS). A DC voltage of 1,800 V was assigned across the paired electrodes for the simulation. The simulation clearly indicates the highest electrical field strength is located at the sharp point of the electrode positioned in the microwell on a scale of 10^5 V/m. As we know, the dielectric permittivity of water is much higher than that of the

surrounding medium (oil; dielectric permittivity: $\epsilon_{H_2O} \approx 81$, $\epsilon_{Oil} \approx 2.5$). In the non-uniform electrical field, since water is more polarizable than oil, a positive DEP force always tends to direct the droplets toward the highest electrical field, where the microwell is located. The design of the sharp electrode in the microwell can assist the generation of a non-uniform electrical field when voltage is applied across electrodes.

Figure 7.1b shows the fusion of droplets, indicating that the DEP force also can drive the fused droplet into the microwell. As shown in images 3 and 4 of Figure 7.1b, the droplet fusion is induced by continuously applying a DC electrical field and may be triggered as many times as desired as long as the produced droplet is constrained in the microwell. Thus, the microwell can be completely filled, and the number of droplets fused is determined by the volume of the microwell that can be tailored by the design. Under microscope, the droplets may be clearly observed to start the fusion at the opening of the microwell and then move in to complete the fusion process through the hydrodynamic force and DEP force. The dimensions of the fusion-generated droplet can be controlled by the applied fusion times and the flow rates of both disperse and continuous phases. Figure 7.1 demonstrates how 300 μm diameter droplets are produced with optimized flow conditions and one-time fusion. Such a dimension is just suitable for the droplet shrinkage process, as shown later. The fused microdroplets are retained in the microwells due to its size being larger than the tiny downstream

channel, while the interfacial tension between oil and water makes it round, thus preventing its escape from deformation.

(a)

(b)

Figure 7.3 a) Diameter of chitosan droplet decreased with time due to WDTO. b) Au nanoparticle-coated chitosan microbead synthesized in the microwell (scale bar = 300 μm).

Figure 7.4 EDS pattern of the Au nanoparticle-coated chitosan microbeads

Figure 7.3a shows the experimentally determined relation of the droplet diameter change versus the shrinkage time in the droplet solidification process. In the first 9 hours of the solidification process, the dimension of the chitosan droplet almost linearly decreased from 300 μm to 120 μm in diameter and then approaches a plateau value without further significant decrement. A water loss process has been recently observed during manipulation of static droplets in oil due to WDTO⁶⁰. Clearly, the droplet solidification process that occurred in our flow biphasic system should be ascribed to the same phenomena. The water loss into a static oil phase is normally very slow. In our microfluidic system, the continuous flow of the oil phase actually facilitates the water loss process since the oil phase is renewed continuously. In this work, the WDTO process is innovatively used to shrink the polymer droplets on chip for *in situ* fabrication of arrayed polymer

beads. The finally formed solidified chitosan beads ($\sim 120\ \mu\text{m}$ in diameter) in the microwells could not escape from the neck connecting the microwell to the downstream channel and were used for further functionalization.

Figure 7.3b illustrates the in situ synthesized Au nanoparticle-coated chitosan microbead through a photoreduction method. The EDS element analysis spectra of SEM in Figure 7.4 indicate that the microbead surface is mainly composed of Au, clearly confirming the existence of Au nanoparticles on the polymer microbead. The AuCl_4^- needs to complex with the amino group of chitosan and, then, is further photo-reduced to form an Au-nanoparticle coating on the chitosan microbead surface. The Au nanoparticles are formed by UV photo-induced reduction of AuCl_4^- ¹⁹⁴. AuCl_4^- complexes with chitosan and is reduced to Au atoms as the nuclei under UV irradiation, followed by subsequent deposition of photo-reduced gold atoms to finally form Au nanoparticles on the surface of chitosan microbeads. The confirmed Au nanoparticle deposition on the chitosan discussed above demonstrates that the physical drying process (WDTO) used in this work for formation of polymer beads avoids chemical cross-linking, which would consume the amino groups of chitosan and, thus, prevent its complexation with AuCl_4^- for Au nanoparticle coating on the chitosan microbeads.

Figure 7.5 Density and size of Au nanoparticles formed on chitosan microbead. a) On microbead 1 prepared by 1000s UV irradiation for photoreduction. b) On microbead 3 prepared by UV irradiation for 2000s during synthesis. c) On microbead 2 prepared by UV irradiation for 1500s during synthesis. d) Au nanoparticle-coated chitosan microbeads observed by SEM (scale bar [white] = 20 μm).

Figure 7.6 SERS of R6G enhanced by Au nanoparticle-coated chitosan microbead 2 and SERS obtained from R6G solution only as a reference (No Au NP)

The morphology, especially the density and size of the Au nanoparticles on the surface of chitosan microbeads produced with different UV irradiation times, was examined by SEM. The result is shown in Figure 7.5a, b, and c. Figure 5d illustrates the typical image of the sampled Au nanoparticle-coated chitosan microbeads. The Au nanoparticles on microbead 1 prepared with 1,000 s UV irradiation (Figure 7.5a) display a size range of 5 nm–10 nm with no metal cluster or dense particles formed. The Au nanoparticles coated on the microbead 2 surface by 1,500 seconds UV irradiation show densely aggregated metal clusters, and most nanoparticles fall in the range of 20 nm–90 nm in diameter (Figure 7.5c). With a UV irradiation time of 2,000 s, only large-sized nanoparticles (> 150 nm) are densely formed, and all particles are aggregated together to form large clusters on

the microbead surface (microbead 3). Obviously, a longer time of UV irradiation results in larger size, higher aggregation, and denser packing of Au nanoparticles on the polymer bead surface.

The three different Au nanoparticle substrates formed on the polymer microbeads in Figure 7.5 exhibit different effects on the SERS activity. Figure 7.6 shows that a successful enhancement of the Raman scattering effect is achieved by an Au nanoparticle substrate on microbead 2 during detection of 100 nM R6G. The characteristic Raman Spectra of the R6G immobilized bead 2 have intense characteristic Raman signals at about 1,188, 1,362, 1,505, 1,530, and 1,572 cm^{-1} , which all can be assigned to the totally symmetric modes of in-plane C-C stretching vibrations associated with R6G, as reported¹⁵⁷. Very likely, the enhancement of SERS on the Au nanoparticles surface on microbead 2 is not only contributed to by the high density of nanoparticle clusters to allow adsorption of more molecules/analytes, but also comes from the suitable aggregated nanoparticle clusters with many crevices, at which the collective surface plasmon may be trapped to create particularly large local electrical fields for significantly enhancing the SERS signal. Solutions with different R6G concentrations (100 nM, 50 nM, and 10 nM) were used to study the SERS detection limit on microbead 2. As shown in Figure 7.6, there are still detectable Raman features with 10 nM R6G, while further reduction of R6G solution leads to no more enhanced Raman signal. The sensitivity obtained here is even comparable with SERS enhancement with the

silver nanostructured substrate¹⁹⁵.

On microbead 1, only individual nanoparticles are coated on its surface without forming nanoparticle clusters. Lacking “hot spots,” as on microbead 2, the substrate on microbead 1 indeed shows little enhancement of the Raman scattering effect for detection of R6G. The Au nanoparticle surface formed on microbead 3 by prolonged photoreduction time represents another type of substrate, which has metal cluster but with larger Au nanoparticles (>150 nm) in comparison to those of the substrate (20–90 nm) on microbead 2. It was revealed that such a surface with highly aggregated, large-sized nanoparticle clusters also inhibits the effectiveness of SERS, and little SERS enhancement effect is observed in detection of R6G. These results show that there is an optimal range of Au nanoparticle cluster sizes (20–90 nm) on the microbeads for the strongest SERS enhancement, which is in good agreement with the reported work that the size of metal nanoparticles should be in the range of 20–60 nm¹⁹⁶ for effective SERS enhancement.

It is worthy of note that chitosan beads tend to swell in an aqueous solution; however, the Au nanoparticle-coated microbeads show no obvious swelling during the swelling tests. This can be explained by the fact that the Au nanoparticles bound with the chitosan microbeads could greatly increase the mechanical strength of the microbeads and, thus, could prevent their swelling. There is an additional advantage of this microbead-based detection platform. After SERS detection, the

microbeads trapped in microwells can be unloaded by simply flowing pure DI water in from the channel outlet. With the unique architecture of the junction neck between the microchannel and the microwell, the normal fluid flow makes the neck junction serve as a blockage of microbeads, while the reverse flow crafts the neck becoming an inlet of fluid to the microwell. Fluid going to the microwell forces the microbeads moving out of the microwells. Due to the droplet shrinking from drying in microwells, the produced microbeads are always smaller than the orifice ($>200\text{ }\mu\text{m}$) to be released out reversely. Such easy salvage makes the platform more attractive for users. Thus, the microfluidic chip-based SERS detection platform here indeed demonstrates its superior advantage of low reagent consumption, fast mass transport, efficient immobilization, time-reduced washing process, reuse ability, and high detection sensitivity.

7.4 Conclusions

On-chip fabrication of arrayed Au-nanoparticle coated chitosan microbeads by a droplet microfluidic device for highly sensitive SERS detection of molecules has been successfully demonstrated. Without manual loading of beads into channel, the developed microfluidic droplet technique allows *in situ* conversion of polymer droplets to solid polymer microbeads. The physical solidification process preserves all polymer functional groups to generate novel functionalized microbeads. While all microbeads are formed and *in situ* positioned in individual microwells, arrayed

sensing is performed. Additionally, owing to the unique design of the microfluidic structure, the microbeads produced on chip can be unloaded from the channel and the platform may be reused for the next round of synthesis and detection. The on-chip conversion of droplets to localized functional microbeads provides a universal approach to *in situ* fabricate novel functional beads for powerful arrayed bead-based microfluidic molecular detections.

Chapter 8 General Conclusion and Future Work

8.1. General conclusion

This thesis gives a thorough review of the latest development of the field of droplet microfluidics. The main contribution of this research is that effective control techniques, such as on-demand compound droplet formation, on-demand droplet trapping, fusion, and release are developed to facilitate the use of droplets as miniaturized laboratories have been developed.

On-demand compound droplet formation was developed as an effective scheme to trigger droplet-based reactions. Different from the conventional method of droplet-based reactions, where paired droplets are synchronously formed, transported, and physically brought together for fusion, the developed method utilized EHD forces during droplet formation to polarize the water streams to achieve one-step stream fusion and droplet formation. With this method, fusion of water streams could be triggered on demand while generating droplets with single- or multi-solution content is simply controlled by turning the electrical field on or off. The on-demand direct formation of compound droplets eliminates stringent dependence on the periodic generation of droplet pairs and tedious schemes to bring droplets together for fusion.

Going one step further, on-demand droplet trapping and fusion techniques were developed to enable droplet fusions within a droplet library. All the time, droplet fusions are limited to paired droplets only. This is due to the extreme

difficulties of effectively manipulating droplets and bringing them together for fusion. To fully utilize droplets as miniaturized reaction vessels, it is very desirable to implement parallel processing of droplets and acquire vast multiplexing of data sets through on-demand fusions among a droplet library. The developed on-demand droplet fusion and trapping in this research enables such desired manipulations. Like multi-wells of 96 plates, arrayed microwells are designed to be attached to the main channel for temporary storage of droplets. Hydrodynamic flow resistance is utilized to differentiate the droplet flow paths; the main channel has less flow resistance, while the microwell path has higher flow resistance. In this way, the target droplet may be selectively moved into the microwell by a manipulation force, while the rest flow through the main channel only. DEP force is utilized in the manipulation because a large force can be generated and the response of droplets to DEP force is quite fast. Trapping of droplets creates ready-for-reaction arrayed reactors. On-demand droplet fusion is, then, to conduct multiplexed reactions among a droplet library. The additional advantage is that the fusion-generated droplets are still statically positioned in the microwell; thus, time-lapsed monitoring of reactions inside droplets can be easily conducted without the need for a high-speed camera. Additionally, droplet fusion can be triggered as many times as desired as long as the produced droplet is constrained in the microwell.

The on-demand droplet release technique was developed and combined with the droplet trapping and fusion techniques in order to integrate it into a single

microfluidic platform. Although the reactions were conducted in the microwells, the droplets were the reaction vessels, instead of the microwells. Since there was no direct contact between the oil-surrounded droplet and the microwell wall, it is desirable to release the droplet into the main channel such that the contamination-free microwell may be reused. In addition, the post-reaction droplet released to the main channel may be further manipulated and analyzed. Thus, in this research, an on-demand droplet release scheme was developed and showed very encouraging results. The release was fast and had no significant disturbance on the flow in main channel. The released droplets were unbroken, without loss of any contents in the droplet. Most importantly, the release was on demand, and thus, it could be conducted at the right time. By integrating droplet trapping, fusion, and release functions within a single microfluidic platform, individually controlled and parallel microreactions can be continuously conducted with ease of monitoring, as demonstrated in the detection of mercury ions. The developed droplet manipulation schemes are expected to greatly facilitate the wide usage of droplets as naturally well-defined, miniaturized laboratories.

Droplets are usually in the forms of aqueous contents surrounded by an oil phase; thus, they cannot function as rigid substrates and adopt conventional microfluidic processes on solid substrates, such as surface modification, binding, washing, and so on, for analytical applications. When the droplets are converted to microparticles, microbead-based microfluidic detection platforms may be formed, and thus, various available surface modifications can be used to introduce multiple

functionalities on those microparticles. In this part of the research, an in situ conversion of chitosan droplets to arrayed functionalized microbeads was demonstrated, and those functionalized microbeads were then used for highly sensitive SERS detection of molecules. This droplet microfluidic method facilitated the generation of novel functional beads for substrate-based molecule detections.

In brief, this research developed the direct formation of compound droplets to facilitate the droplet-based reactions. For reactions among a droplet library, on-demand droplet trapping and fusion were innovated. Together with on-demand droplet release, an integration of all droplet manipulations within a single microfluidic platform was demonstrated to facilitate the wide use of microdroplets as naturally well-defined microreactors.

8.2. Future work

In this PhD project, DEP force was utilized for droplet trapping and fusion because of its large force scale and short response time. However, the electrodes needed for such kind of manipulation occupied a lot of space and limited further miniaturization of the device. The recent advances in light-induced droplet manipulation might be utilized for optic-based droplet trapping, fusion, and release. Manipulation through remotely introduced laser light might eliminate the need for an electrode array. The laser focusing area can be measured down to micrometer for more precise control of droplets in small dimensions. It is worthy of the effort

to couple optic-based droplet manipulation and the current platform for developing an even more promising droplet trapping, fusion, and release platform.

On the other hand, it is desirable to integrate on-demand droplet formation into the current platform. Available methods, such as electrowetting or DEP-based droplet formation could be utilized for the current system by coupling reservoirs of different samples or reagents to the inlets of main channel. Such on-demand formation should offer control over when to generate droplets and how much volume the droplets should be. More complicated and precise controlled reactions could then be conducted conveniently.

Additionally, conventional droplet detection based on fluorescence normally requires the preloading of fluorescence labels into droplets. Future study could be directed toward the utilization of non-fluorescence-based droplet detection to monitor what occurs inside the droplets. The to-be-developed detection should be sensitive with a short response time, not affecting or affected by the emulsion system. The current PDMS and glass-based platform has good optical properties, such that detections such as UV-Vis and Raman Spectroscopy can be integrated into the platform. Current research has explored the conversion of polymer droplets to polymer microbeads for flow-through-based detection. Such SERS detection might be realized by directly coupling microbeads into droplets and loading into the microwells. Since SERS detection is highly sensitive, it is worthy to integrate it into the current platform.

On-demand droplet trapping, fusion, and release provide all of the

manipulations needed for multiplexed droplet-based reactions. The further exploration of more advanced detection and on-demand droplet generation will empower the current system and make it both a scientific and commercial success.

Abbreviate

3D: three-dimensional

BLM: bilayer lipid membranes

Ca : capillary number

CE: capillary electrophoresis

CHO: Chinese hamster ovary

CS: Chitosan

DEP: dielectrophoretic

DI: deionized

DRIE: Deep reactive-ion etching

EDS: Energy-dispersive spectrum

EGFP: enhanced green fluorescent protein

EHD: electrohydrodynamic

ESI: electronic supplementary information

EWOD: electrowetting-on-dielectric

FESEM: Field emission scanning electron microscope

FRET: fluorescence resonance energy transfer

ePCR: Emulsion polymerase chain reaction

ESI-MS: electrospray ionization mass spectrometry

FACS: fluorescence-activated cell sorting

FADS: fluorescence-activated droplet sorter

GA: glutaraldehyde

GFP: green fluorescence protein

HLB: trimethylchlorosilane
HRCA: hyperbranched rolling circle amplification
IC: integrated circuit
IDT: interdigital transducer
IPN: interpenetrating polymer network
IVT: in vitro translation
MS: mass spectrometry
PCR: polymerase chain reaction
PDMS: poly (dimethylsiloxane)
PLGA: poly (lactic-co-glycolic acid)
PVA: Poly (vinyl alcohol)
QD: quantum dot
R6G: Rhodamine 6G
RB: rhodamine B
 Re : Reynolds number
RF: radio frequency
SAM: self-assembled monolayers
SAW: Surface acoustic wave
SEM: Scanning electron microscope
SERs: surface-enhanced Raman scattering
Span 80: sorbitan monooleate
SPRi: surface plasmon resonance imaging
TMCS: trimethylchlorosilane

Reference

1. Huebner, A.; Sharma, S.; Srisa-Art, M.; Hollfelder, F.; Edel, J. B.; Demello, A. J., Microdroplets: A sea of applications? *Lab on a Chip* **2008**, *8*, (8), 1244-1254.
2. Griffiths, A. D.; Tawfik, D. S., Miniaturising the laboratory in emulsion droplets. *Trends in Biotechnology* **2006**, *24*, (9), 395-402.
3. Taly, V.; Kelly, B. T.; Griffiths, A. D., Droplets as microreactors for high-throughput biology. *Chembiochem* **2007**, *8*, (3), 263-272.
4. Song, H.; Chen, D. L.; Ismagilov, R. F., Reactions in droplets in microfluidic channels. *Angewandte Chemie-International Edition* **2006**, *45*, (44), 7336-7356.
5. Bibette, J.; Calderon, F. L.; Poulin, P., Emulsions: basic principles. *Reports on Progress in Physics* **1999**, *62*, (6), 969-1033.
6. Roland, I.; Piel, G.; Delattre, L.; Evrard, B., Systematic characterization of oil-in-water emulsions for formulation design. *International Journal of Pharmaceutics* **2003**, *263*, (1-2), 85-94.
7. Welin-Berger, K.; Bergenstahl, B., Inhibition of Ostwald ripening in local anesthetic emulsions by using hydrophobic excipients in the disperse phase. *International Journal of Pharmaceutics* **2000**, *200*, (2), 249-260.
8. Teh, S. Y.; Lin, R.; Hung, L. H.; Lee, A. P., Droplet microfluidics. *Lab on a Chip* **2008**, *8*, (2), 198-220.
9. Joanicot, M.; Ajdari, A., Applied physics - Droplet control for microfluidics. *Science* **2005**, *309*, (5736), 887-888.
10. Tawfik, D. S.; Griffiths, A. D., Man-made cell-like compartments for molecular evolution. *Nature Biotechnology* **1998**, *16*, (7), 652-656.
11. Xu, J. H.; Li, S. W.; Tan, J.; Wang, Y. J.; Luo, G. S., Preparation of highly monodisperse droplet in a T-junction microfluidic device. *Aiche Journal* **2006**, *52*, (9), 3005-3010.
12. Thorsen, T.; Roberts, R. W.; Arnold, F. H.; Quake, S. R., Dynamic pattern formation in a vesicle-generating microfluidic device. *Physical Review Letters* **2001**, *86*, (18), 4163-4166.
13. Nisisako, T.; Torii, T.; Higuchi, T., Droplet formation in a microchannel network. *Lab on a Chip* **2002**, *2*, (1), 24-26.
14. Priest, C.; Herminghaus, S.; Seemann, R., Generation of monodisperse gel emulsions in a microfluidic device. *Applied Physics Letters* **2006**, *88*, (2).
15. Anna, S. L.; Bontoux, N.; Stone, H. A., Formation of dispersions using "flow focusing" in microchannels. *Applied Physics Letters* **2003**, *82*, (3), 364-366.
16. Zhou, C. F.; Yue, P. T.; Feng, J. J., Formation of simple and compound drops in microfluidic devices. *Physics of Fluids* **2006**, *18*, (9).
17. Tan, Y. C.; Cristini, V.; Lee, A. P., Monodispersed microfluidic droplet generation by shear focusing microfluidic device. *Sensors and Actuators B-Chemical* **2006**, *114*, (1), 350-356.
18. Woodward, A.; Cosgrove, T.; Espidel, J.; Jenkins, P.; Shaw, N., Monodisperse emulsions from a microfluidic device, characterised by diffusion NMR. *Soft Matter* **2007**, *3*, (5), 627-633.
19. Yobas, L.; Martens, S.; Ong, W. L.; Ranganathan, N., High-performance flow-focusing geometry for spontaneous generation of monodispersed droplets. *Lab on a Chip* **2006**, *6*, (8), 1073-1079.

20. Abate, A. R.; Romanowsky, M. B.; Agresti, J. J.; Weitz, D. A., Valve-based flow focusing for drop formation. *Applied Physics Letters* **2009**, 94, (2).
21. Lee, C. Y.; Lin, Y. H.; Lee, G. B., A droplet-based microfluidic system capable of droplet formation and manipulation. *Microfluidics and Nanofluidics* **2009**, 6, (5), 599-610.
22. Wu, H. W.; Huang, Y. C.; Wu, C. L.; Lee, G. B., Exploitation of a microfluidic device capable of generating size-tunable droplets for gene delivery. *Microfluidics and Nanofluidics* **2009**, 7, (1), 45-56.
23. Lai, C. W.; Lin, Y. H.; Lee, G. B., A microfluidic chip for formation and collection of emulsion droplets utilizing active pneumatic micro-choppers and micro-switches. *Biomedical Microdevices* **2008**, 10, (5), 749-756.
24. Nguyen, N. T.; Ting, T. H.; Yap, Y. F.; Wong, T. N.; Chai, J. C. K.; Ong, W. L.; Zhou, J.; Tan, S. H.; Yobas, L., Thermally mediated droplet formation in microchannels. *Applied Physics Letters* **2007**, 91, (8).
25. Gu, H.; Malloggi, F.; Vanapalli, S. A.; Mugele, F., Electrowetting-enhanced microfluidic device for drop generation. *Applied Physics Letters* **2008**, 93, (18).
26. Bransky, A.; Korin, N.; Khoury, M.; Levenberg, S., A microfluidic droplet generator based on a piezoelectric actuator. *Lab on a Chip* **2009**, 9, (4), 516-520.
27. Xu, J.; Attinger, D., Drop on demand in a microfluidic chip. *Journal of Micromechanics and Microengineering* **2008**, 18, (6).
28. Lin, B. C.; Su, Y. C., On-demand liquid-in-liquid droplet metering and fusion utilizing pneumatically actuated membrane valves. *Journal of Micromechanics and Microengineering* **2008**, 18, (11).
29. Galas, J. C.; Bartolo, D.; Studer, V., Active connectors for microfluidic drops on demand. *New Journal of Physics* **2009**, 11.
30. Zeng, S. J.; Li, B. W.; Su, X. O.; Qin, J. H.; Lin, B. C., Microvalve-actuated precise control of individual droplets in microfluidic devices. *Lab on a Chip* **2009**, 9, (10), 1340-1343.
31. Wang, K. L.; Jones, T. B.; Raisanen, A., DEP actuated nanoliter droplet dispensing using feedback control. *Lab on a Chip* **2009**, 9, (7), 901-909.
32. Gong, J.; Kim, C. J., All-electronic droplet generation on-chip with real-time feedback control for EWOD digital microfluidics. *Lab on a Chip* **2008**, 8, (6), 898-906.
33. Prakash, R.; Kaler, K., DEP actuation of emulsion jets and dispensing of sub-nanoliter emulsion droplets. *Lab on a Chip* **2009**, 9, (19), 2836-2844.
34. Frenz, L.; Blouwolff, J.; Griffiths, A. D.; Baret, J. C., Microfluidic Production of Droplet Pairs. *Langmuir* **2008**, 24, (20), 12073-12076.
35. Chokkalingam, V.; Herminghaus, S.; Seemann, R., Self-synchronizing pairwise production of monodisperse droplets by microfluidic step emulsification. *Applied Physics Letters* **2008**, 93, (25).
36. Khan, S. A.; Duraiswamy, S., Microfluidic emulsions with dynamic compound drops. *Lab on a Chip* **2009**, 9, (13), 1840-1842.
37. Hashimoto, M.; Shevkoplyas, S. S.; Zasonska, B.; Szymborski, T.; Garstecki, P.; Whitesides, G. M., Formation of Bubbles and Droplets in Parallel, Coupled Flow-Focusing Geometries. *Small* **2008**, 4, (10), 1795-1805.
38. Yap, Y. F.; Tan, S. H.; Nguyen, N. T.; Murshed, S. M. S.; Wong, T. N.; Yobas, L., Thermally mediated control of liquid microdroplets at a bifurcation. *Journal of Physics D-Applied*

- Physics* **2009**, 42, (6).
39. Saint Vincent, M. R. D.; Wunenburger, R.; Delville, J. P., Laser switching and sorting for high speed digital microfluidics. *Applied Physics Letters* **2008**, 92, (15).
 40. Franke, T.; Abate, A. R.; Weitz, D. A.; Wixforth, A., Surface acoustic wave (SAW) directed droplet flow in microfluidics for PDMS devices. *Lab on a Chip* **2009**, 9, (18), 2625-2627.
 41. Zhang, K.; Liang, Q. L.; Ma, S.; Mu, X. A.; Hu, P.; Wang, Y. M.; Luo, G. A., On-chip manipulation of continuous picoliter-volume superparamagnetic droplets using a magnetic force. *Lab on a Chip* **2009**, 9, (20), 2992-2999.
 42. Long, Z. C.; Shetty, A. M.; Solomon, M. J.; Larson, R. G., Fundamentals of magnet-actuated droplet manipulation on an open hydrophobic surface. *Lab on a Chip* **2009**, 9, (11), 1567-1575.
 43. Pipper, J.; Inoue, M.; Ng, L. F. P.; Neuzil, P.; Zhang, Y.; Novak, L., Catching bird flu in a droplet. *Nature Medicine* **2007**, 13, (10), 1259-1263.
 44. Vig, A. L.; Kristensen, A., Separation enhancement in pinched flow fractionation. *Applied Physics Letters* **2008**, 93, (20).
 45. Niu, X. Z.; Gielen, F.; deMello, A. J.; Edel, J. B., Electro-Coalescence of Digitally Controlled Droplets. *Analytical Chemistry* **2009**, 81, (17), 7321-7325.
 46. Niu, X.; Gulati, S.; Edel, J. B.; deMello, A. J., Pillar-induced droplet merging in microfluidic circuits. *Lab on a Chip* **2008**, 8, (11), 1837-1841.
 47. Zagnoni, M.; Cooper, J. M., On-chip electrocoalescence of microdroplets as a function of voltage, frequency and droplet size. *Lab on a Chip* **2009**, 9, (18), 2652-2658.
 48. Mazutis, L.; Baret, J. C.; Griffiths, A. D., A fast and efficient microfluidic system for highly selective one-to-one droplet fusion. *Lab on a Chip* **2009**, 9, (18), 2665-2672.
 49. Christopher, G. F.; Bergstein, J.; End, N. B.; Poon, M.; Nguyen, C.; Anna, S. L., Coalescence and splitting of confined droplets at microfluidic junctions. *Lab on a Chip* **2009**, 9, (8), 1102-1109.
 50. Jung, Y. M.; Kang, I. S., A novel actuation method of transporting droplets by using electrical charging of droplet in a dielectric fluid. *Biomicrofluidics* **2009**, 3, (2).
 51. Fidalgo, L. M.; Whyte, G.; Bratton, D.; Kaminski, C. F.; Abell, C.; Huck, W. T. S., From microdroplets to microfluidics: Selective emulsion separation in microfluidic devices. *Angewandte Chemie-International Edition* **2008**, 47, (11), 2042-2045.
 52. Fidalgo, L. M.; Whyte, G.; Ruotolo, B. T.; Benesch, J. L. P.; Stengel, F.; Abell, C.; Robinson, C. V.; Huck, W. T. S., Coupling Microdroplet Microreactors with Mass Spectrometry: Reading the Contents of Single Droplets Online. *Angewandte Chemie-International Edition* **2009**, 48, (20), 3665-3668.
 53. Niu, X. Z.; Zhang, B.; Marszalek, R. T.; Ces, O.; Edel, J. B.; Klug, D. R.; Demello, A. J., Droplet-based compartmentalization of chemically separated components in two-dimensional separations. *Chemical Communications* **2009**, (41), 6159-6161.
 54. Wang, W.; Yang, C.; Li, C. M., On-demand microfluidic droplet trapping and fusion for on-chip static droplet assays. *Lab on a Chip* **2009**, 9, (11), 1504-1506.
 55. Kelly, R. T.; Page, J. S.; Marginean, I.; Tang, K. Q.; Smith, R. D., Dilution-Free Analysis from Picoliter Droplets by Nano-Electrospray Ionization Mass Spectrometry. *Angewandte Chemie-International Edition* **2009**, 48, (37), 6832-6835.
 56. Roman, G. T.; Wang, M.; Shultz, K. N.; Jennings, C.; Kennedy, R. T., Sampling and

- Electrophoretic Analysis of Segmented Flow Streams Using Virtual Walls in a Microfluidic Device. *Analytical Chemistry* **2008**, 80, (21), 8231-8238.
57. Schmitz, C. H. J.; Rowat, A. C.; Koster, S.; Weitz, D. A., Dropspots: a picoliter array in a microfluidic device. *Lab on a Chip* **2009**, 9, (1), 44-49.
 58. Koster, S.; Angile, F. E.; Duan, H.; Agresti, J. J.; Wintner, A.; Schmitz, C.; Rowat, A. C.; Merten, C. A.; Pisignano, D.; Griffiths, A. D.; Weitz, D. A., Drop-based microfluidic devices for encapsulation of single cells. *Lab on a Chip* **2008**, 8, (7), 1110-1115.
 59. Edd, J. F.; Humphry, K. J.; Irimia, D.; Weitz, D. A.; Toner, M., Nucleation and solidification in static arrays of monodisperse drops. *Lab on a Chip* **2009**, 9, (13), 1859-1865.
 60. Huebner, A.; Bratton, D.; Whyte, G.; Yang, M.; deMello, A. J.; Abell, C.; Hollfelder, F., Static microdroplet arrays: a microfluidic device for droplet trapping, incubation and release for enzymatic and cell-based assays. *Lab on a Chip* **2009**, 9, (5), 692-698.
 61. Shi, W. W.; Qin, J. H.; Ye, N. N.; Lin, B. C., Droplet-based microfluidic system for individual *Caenorhabditis elegans* assay. *Lab on a Chip* **2008**, 8, (9), 1432-1435.
 62. Boukellal, H.; Selimovic, S.; Jia, Y. W.; Cristobal, G.; Fraden, S., Simple, robust storage of drops and fluids in a microfluidic device. *Lab on a Chip* **2009**, 9, (2), 331-338.
 63. Verneuil, E.; Cordero, M. L.; Gallaire, F.; Baroud, C. N., Laser-Induced Force on a Microfluidic Drop: Origin and Magnitude. *Langmuir* **2009**, 25, (9), 5127-5134.
 64. Cordero, M. L.; Burnham, D. R.; Baroud, C. N.; McGloin, D., Thermocapillary manipulation of droplets using holographic beam shaping: Microfluidic pin ball. *Applied Physics Letters* **2008**, 93, (3).
 65. Chiou, P. Y.; Park, S. Y.; Wu, M. C., Continuous optoelectrowetting for picoliter droplet manipulation. *Applied Physics Letters* **2008**, 93, (22).
 66. Chuang, H. S.; Kumar, A.; Wereley, S. T., Open optoelectrowetting droplet actuation. *Applied Physics Letters* **2008**, 93, (6).
 67. Park, S. Y.; Kalim, S.; Callahan, C.; Teitell, M. A.; Chiou, E. P. Y., A light-induced dielectrophoretic droplet manipulation platform. *Lab on a Chip* **2009**, 9, (22), 3228-3235.
 68. Park, S.; Pan, C.; Wu, T. H.; Kloss, C.; Kalim, S.; Callahan, C. E.; Teitell, M.; Chiou, E. P. Y., Floating electrode optoelectronic tweezers: Light-driven dielectrophoretic droplet manipulation in electrically insulating oil medium. *Applied Physics Letters* **2008**, 92, (15).
 69. Lee, J.; Teh, S. Y.; Lee, A.; Kim, H. H.; Lee, C.; Shung, K. K., Single beam acoustic trapping. *Applied Physics Letters* **2009**, 95, (7).
 70. Abdelgawad, M.; Freire, S. L. S.; Yang, H.; Wheeler, A. R., All-terrain droplet actuation. *Lab on a Chip* **2008**, 8, (5), 672-677.
 71. Liu, J. J.; Lin, J. M.; Knopp, D., Using a circular groove surrounded inlet to generate monodisperse droplets inside a microfluidic chip in a gravity-driven manner. *Journal of Micromechanics and Microengineering* **2008**, 18, (9).
 72. Frenz, L.; Blank, K.; Brouzes, E.; Griffiths, A. D., Reliable microfluidic on-chip incubation of droplets in delay-lines. *Lab on a Chip* **2009**, 9, (10), 1344-1348.
 73. Sista, R.; Hua, Z. S.; Thwar, P.; Sudarsan, A.; Srinivasan, V.; Eckhardt, A.; Pollack, M.; Pamula, V., Development of a digital microfluidic platform for point of care testing. *Lab on a Chip* **2008**, 8, (12), 2091-2104.
 74. Beyzavi, A.; Nguyen, N. T., Modeling and optimization of planar microcoils. *Journal of Micromechanics and Microengineering* **2008**, 18, (9).

75. Beyzavi, A.; Nguyen, N. T., One-dimensional actuation of a ferrofluid droplet by planar microcoils. *Journal of Physics D-Applied Physics* **2009**, 42, (1).
76. Surenjav, E.; Priest, C.; Herminghaus, S.; Seemann, R., Manipulation of gel emulsions by variable microchannel geometry. *Lab on a Chip* **2009**, 9, (2), 325-330.
77. Um, E.; Park, J. K., A microfluidic abacus channel for controlling the addition of droplets. *Lab on a Chip* **2009**, 9, (2), 207-212.
78. Kim, H.; Dixit, S.; Green, C. J.; Faris, G. W., Nanodroplet real-time PCR system with laser assisted heating. *Optics Express* **2009**, 17, (1), 218-227.
79. Kim, H.; Vishniakou, S.; Faris, G. W., Petri dish PCR: laser-heated reactions in nanoliter droplet arrays. *Lab on a Chip* **2009**, 9, (9), 1230-1235.
80. Issadore, D.; Humphry, K. J.; Brown, K. A.; Sandberg, L.; Weitz, D. A.; Westervelt, R. M., Microwave dielectric heating of drops in microfluidic devices. *Lab on a Chip* **2009**, 9, (12), 1701-1706.
81. Hsieh, A. T. H.; Pan, P. J. H.; Lee, A. P., Rapid label-free DNA analysis in picoliter microfluidic droplets using FRET probes. *Microfluidics and Nanofluidics* **2009**, 6, (3), 391-401.
82. Funfak, A.; Cao, J. L.; Wolfbeis, O.; Martin, K.; Kohler, J., Monitoring cell cultivation in microfluidic segments by optical pH sensing with a micro flow-through fluorometer using dye-doped polymer particles. *Microchimica Acta* **2009**, 164, (3-4), 279-286.
83. Niu, X. Z.; Zhang, M. Y.; Peng, S. L.; Wen, W. J.; Sheng, P., Real-time detection, control, and sorting of microfluidic droplets. *Biomicrofluidics* **2007**, 1, (4).
84. Liu, S. J.; Gu, Y. F.; Le Roux, R. B.; Matthews, S. M.; Bratton, D.; Yunus, K.; Fisher, A. C.; Huck, W. T. S., The electrochemical detection of droplets in microfluidic devices. *Lab on a Chip* **2008**, 8, (11), 1937-1942.
85. Han, Z. Y.; Li, W. T.; Huang, Y. Y.; Zheng, B., Measuring Rapid Enzymatic Kinetics by Electrochemical Method in Droplet-Based Microfluidic Devices with Pneumatic Valves. *Analytical Chemistry* **2009**, 81, (14), 5840-5845.
86. Pei, J.; Li, Q.; Lee, M. S.; Valaskovic, G. A.; Kennedy, R. T., Analysis of Samples Stored as Individual Plugs in a Capillary by Electrospray Ionization Mass Spectrometry. *Analytical Chemistry* **2009**, 81, (15), 6558-6561.
87. Malic, L.; Veres, T.; Tabrizian, M., Two-dimensional droplet-based surface plasmon resonance imaging using electrowetting-on-dielectric microfluidics. *Lab on a Chip* **2009**, 9, (3), 473-475.
88. Evanko, D., Living droplets. *Nature Methods* **2008**, 5, (7), 580-581.
89. Clausell-Tormos, J.; Lieber, D.; Baret, J. C.; El-Harrak, A.; Miller, O. J.; Frenz, L.; Blouwolff, J.; Humphry, K. J.; Koster, S.; Duan, H.; Holtze, C.; Weitz, D. A.; Griffiths, A. D.; Merten, C. A., Droplet-based microfluidic platforms for the encapsulation and screening of mammalian cells and multicellular organisms. *Chemistry & Biology* **2008**, 15, (5), 427-437.
90. Chabert, M.; Viovy, J. L., Microfluidic high-throughput encapsulation and hydrodynamic self-sorting of single cells. *Proceedings of the National Academy of Sciences of the United States of America* **2008**, 105, (9), 3191-3196.
91. Edd, J. F.; Di Carlo, D.; Humphry, K. J.; Koster, S.; Irimia, D.; Weitz, D. A.; Toner, M., Controlled encapsulation of single-cells into monodisperse picolitre drops. *Lab on a Chip*

- 2008, 8, (8), 1262-1264.
92. Abate, A. R.; Chen, C. H.; Agresti, J. J.; Weitz, D. A., Beating Poisson encapsulation statistics using close-packed ordering. *Lab on a Chip* **2009**, 9, (18), 2628-2631.
 93. Huebner, A.; Olguin, L. F.; Bratton, D.; Whyte, G.; Huck, W. T. S.; de Mello, A. J.; Edel, J. B.; Abell, C.; Hollfelder, F., Development of quantitative cell-based enzyme assays in microdroplets. *Analytical Chemistry* **2008**, 80, (10), 3890-3896.
 94. Hufnagel, H.; Huebner, A.; Gulch, C.; Guse, K.; Abell, C.; Hollfelder, F., An integrated cell culture lab on a chip: modular microdevices for cultivation of mammalian cells and delivery into microfluidic microdroplets. *Lab on a Chip* **2009**, 9, (11), 1576-1582.
 95. Brouzes, E.; Medkova, M.; Savenelli, N.; Marran, D.; Twardowski, M.; Hutchison, J. B.; Rothberg, J. M.; Link, D. R.; Perrimon, N.; Samuels, M. L., Droplet microfluidic technology for single-cell high-throughput screening. *Proceedings of the National Academy of Sciences of the United States of America* **2009**, 106, (34), 14195-14200.
 96. Zhan, Y. H.; Wang, J.; Bao, N.; Lu, C., Electroporation of Cells in Microfluidic Droplets. *Analytical Chemistry* **2009**, 81, (5), 2027-2031.
 97. Kim, C.; Lee, K. S.; Kim, Y. E.; Lee, K. J.; Lee, S. H.; Kim, T. S.; Kang, J. Y., Rapid exchange of oil-phase in microencapsulation chip to enhance cell viability. *Lab on a Chip* **2009**, 9, (9), 1294-1297.
 98. Um, E.; Lee, D. S.; Pyo, H. B.; Park, J. K., Continuous generation of hydrogel beads and encapsulation of biological materials using a microfluidic droplet-merging channel. *Microfluidics and Nanofluidics* **2008**, 5, (4), 541-549.
 99. Morimoto, Y.; Tan, W. H.; Tsuda, Y.; Takeuchi, S., Monodisperse semi-permeable microcapsules for continuous observation of cells. *Lab on a Chip* **2009**, 9, (15), 2217-2223.
 100. Shim, J. U.; Cristobal, G.; Link, D. R.; Thorsen, T.; Fraden, S., Using microfluidics to decouple nucleation and growth of protein crystals. *Crystal Growth & Design* **2007**, 7, (11), 2192-2194.
 101. Meier, M.; Kennedy-Darling, J.; Choi, S. H.; Norstrom, E. M.; Sisodia, S. S.; Ismagilov, R. F., Plug-Based Microfluidics with Defined Surface Chemistry to Miniaturize and Control Aggregation of Amyloidogenic Peptides. *Angewandte Chemie-International Edition* **2009**, 48, (8), 1487-1489.
 102. Srisa-Art, M.; deMello, A. J.; Edel, J. B., High-throughput DNA droplet assays using picoliter reactor volumes. *Analytical Chemistry* **2007**, 79, (17), 6682-6689.
 103. Srisa-Art, M.; Kang, D. K.; Hong, J.; Park, H.; Leatherbarrow, R. J.; Edel, J. B.; Chang, S. I.; deMello, A. J., Analysis of Protein-Protein Interactions by Using Droplet-Based Microfluidics. *Chembiochem* **2009**, 10, (10), 1605-1611.
 104. Kreutz, J. E.; Li, L.; Roach, L. S.; Hatakeyama, T.; Ismagilov, R. F., Laterally Mobile, Functionalized Self-Assembled Monolayers at the Fluorous-Aqueous Interface in a Plug-Based Microfluidic System: Characterization and Testing with Membrane Protein Crystallization. *Journal of the American Chemical Society* **2009**, 131, (17), 6042-+.
 105. Gerdt, C. J.; Elliott, M.; Lovell, S.; Mixon, M. B.; Napuli, A. J.; Staker, B. L.; Nollert, P.; Stewart, L., The plug-based nanovolume Microcapillary Protein Crystallization System (MPCS). *Acta Crystallographica Section D-Biological Crystallography* **2008**, 64, 1116-1122.
 106. Blow, N., Genomics: catch me if you can. *Nature Methods* **2009**, 6, (7), 539-542.

107. Kiss, M. M.; Ortoleva-Donnelly, L.; Beer, N. R.; Warner, J.; Bailey, C. G.; Colston, B. W.; Rothberg, J. M.; Link, D. R.; Leamon, J. H., High-Throughput Quantitative Polymerase Chain Reaction in Picoliter Droplets. *Analytical Chemistry* **2008**, 80, (23), 8975-8981.
108. Mazutis, L.; Araghi, A. F.; Miller, O. J.; Baret, J. C.; Frenz, L.; Janoshazi, A.; Taly, V.; Miller, B. J.; Hutchison, J. B.; Link, D.; Griffiths, A. D.; Ryckelynck, M., Droplet-Based Microfluidic Systems for High-Throughput Single DNA Molecule Isothermal Amplification and Analysis. *Analytical Chemistry* **2009**, 81, (12), 4813-4821.
109. Katzen, F.; Chang, G.; Kudlicki, W., The past, present and future of cell-free protein synthesis. *Trends in Biotechnology* **2005**, 23, (3), 150-156.
110. Kumaresan, P.; Yang, C. J.; Cronier, S. A.; Blazei, R. G.; Mathies, R. A., High-throughput single copy DNA amplification and cell analysis in engineered nanoliter droplets. *Analytical Chemistry* **2008**, 80, (10), 3522-3529.
111. Frenz, L.; El Harrak, A.; Pauly, M.; Begin-Colin, S.; Griffiths, A. D.; Baret, J. C., Droplet-based microreactors for the synthesis of magnetic iron oxide nanoparticles. *Angewandte Chemie-International Edition* **2008**, 47, (36), 6817-6820.
112. Pan, Y. C.; Yao, J. F.; Zhang, L. X.; Xu, N. P., Preparation of Ultrafine Zeolite A Crystals with Narrow Particle Size Distribution Using a Two-Phase Liquid Segmented Microfluidic Reactor. *Industrial & Engineering Chemistry Research* **2009**, 48, (18), 8471-8477.
113. Li, S. W.; Xu, H. H.; Wang, Y. J.; Luo, G. S., Controllable preparation of nanoparticles by drops and plugs flow in a microchannel device. *Langmuir* **2008**, 24, (8), 4194-4199.
114. Dubinsky, S.; Zhang, H.; Nie, Z. H.; Gourevich, I.; Voicu, D.; Deetz, M.; Kumacheva, E., Microfluidic synthesis of macroporous copolymer particles. *Macromolecules* **2008**, 41, (10), 3555-3561.
115. Dubinsky, S.; Il Park, J.; Gourevich, I.; Chan, C.; Deetz, M.; Kumacheva, E., Toward Controlling, the Surface Morphology of Macroporous Copolymer Particles. *Macromolecules* **2009**, 42, (6), 1990-1994.
116. Gong, X. Q.; Wen, W. J.; Sheng, P., Microfluidic Fabrication of Porous Polymer Microspheres: Dual Reactions in Single Droplets. *Langmuir* **2009**, 25, (12), 7072-7077.
117. Choi, C. H.; Jung, J. H.; Kim, D. W.; Chung, Y. M.; Lee, C. S., Novel one-pot route to monodisperse thermosensitive hollow microcapsules in a microfluidic system. *Lab on a Chip* **2008**, 8, (9), 1544-1551.
118. Li, W.; Pham, H. H.; Nie, Z.; MacDonald, B.; Guenther, A.; Kumacheva, E., Multi-step microfluidic polymerization reactions conducted in droplets: The internal trigger approach. *Journal of the American Chemical Society* **2008**, 130, (30), 9935-9941.
119. Rondeau, E.; Cooper-White, J. J., Biopolymer microparticle and nanoparticle formation within a microfluidic device. *Langmuir* **2008**, 24, (13), 6937-6945.
120. Huang, K. S.; Lu, K.; Yeh, C. S.; Chung, S. R.; Lin, C. H.; Yang, C. H.; Dong, Y. S., Microfluidic controlling monodisperse microdroplet for 5-fluorouracil loaded genipin-gelatin microcapsules. *Journal of Controlled Release* **2009**, 137, (1), 15-19.
121. Xu, Q. B.; Hashimoto, M.; Dang, T. T.; Hoare, T.; Kohane, D. S.; Whitesides, G. M.; Langer, R.; Anderson, D. G., Preparation of Monodisperse Biodegradable Polymer Microparticles Using a Microfluidic Flow-Focusing Device for Controlled Drug Delivery. *Small* **2009**, 5, (13), 1575-1581.
122. Yang, C. H.; Huang, K. S.; Lin, Y. S.; Lu, K.; Tzeng, C. C.; Wang, E. C.; Lin, C. H.; Hsu, W.

- Y.; Chang, J. Y., Microfluidic assisted synthesis of multi-functional polycaprolactone microcapsules: incorporation of CdTe quantum dots, Fe₃O₄ superparamagnetic nanoparticles and tamoxifen anticancer drugs. *Lab on a Chip* **2009**, 9, (7), 961-965.
123. Abraham, S.; Park, Y. H.; Lee, J. K.; Ha, C. S.; Kim, I., Microfluidic synthesis of reversibly swelling porous polymeric microcapsules with controlled morphology. *Advanced Materials* **2008**, 20, (11), 2177-+.
 124. Lee, I.; Yoo, Y.; Cheng, Z.; Jeong, H. K., Generation of Monodisperse Mesoporous Silica Microspheres with Controllable Size and Surface Morphology in a Microfluidic Device. *Advanced Functional Materials* **2008**, 18, (24), 4014-4021.
 125. Xiang, Z. Y.; Lu, Y. C.; Zou, Y.; Gong, X. C.; Luo, G. S., Preparation of microcapsules containing ionic liquids with a new solvent extraction system. *Reactive & Functional Polymers* **2008**, 68, (8), 1260-1265.
 126. Xu, J. H.; Li, S. W.; Tan, J.; Luo, G. S., Controllable preparation of monodispersed calcium alginate microbeads in a novel microfluidic system. *Chemical Engineering & Technology* **2008**, 31, (8), 1223-1226.
 127. Yang, C. H.; Lin, Y. S.; Huang, K. S.; Huang, Y. C.; Wang, E. C.; Zhong, J. Y.; Kuo, C. Y., Microfluidic emulsification and sorting assisted preparation of monodisperse chitosan microparticles. *Lab on a Chip* **2009**, 9, (1), 145-150.
 128. Carroll, N. J.; Rathod, S. B.; Derbins, E.; Mendez, S.; Weitz, D. A.; Petsev, D. N., Droplet-based microfluidics for emulsion and solvent evaporation synthesis of monodisperse mesoporous silica microspheres. *Langmuir* **2008**, 24, (3), 658-661.
 129. Gong, X. Q.; Wang, L. M.; Wen, W. J., Design and fabrication of monodisperse hollow titania microspheres from a microfluidic droplet-template. *Chemical Communications* **2009**, (31), 4690-4692.
 130. Zhang, H.; Ju, X. J.; Xie, R.; Cheng, C. J.; Ren, P. W.; Chu, L. Y., A microfluidic approach to fabricate monodisperse hollow or porous poly(HEMA-MMA) microspheres using single emulsions as templates. *Journal of Colloid and Interface Science* **2009**, 336, (1), 235-243.
 131. Gong, X. Q.; Peng, S. L.; Wen, W. J.; Sheng, P.; Li, W. H., Design and Fabrication of Magnetically Functionalized Core/Shell Microspheres for Smart Drug Delivery. *Advanced Functional Materials* **2009**, 19, (2), 292-297.
 132. Prasad, N.; Perumal, J.; Choi, C. H.; Lee, C. S.; Kim, D. P., Generation of Monodisperse Inorganic-Organic Janus Microspheres in a Microfluidic Device. *Advanced Functional Materials* **2009**, 19, (10), 1656-1662.
 133. Shah, R. K.; Kim, J. W.; Weitz, D. A., Janus Supraparticles by Induced Phase Separation of Nanoparticles in Droplets. *Advanced Materials* **2009**, 21, (19), 1949-1953.
 134. Chen, C. H.; Shah, R. K.; Abate, A. R.; Weitz, D. A., Janus Particles Templated from Double Emulsion Droplets Generated Using Microfluidics. *Langmuir* **2009**, 25, (8), 4320-4323.
 135. Zhao, L. B.; Pan, L.; Zhang, K.; Guo, S. S.; Liu, W.; Wang, Y.; Chen, Y.; Zhao, X. Z.; Chan, H. L. W., Generation of Janus alginate hydrogel particles with magnetic anisotropy for cell encapsulation. *Lab on a Chip* **2009**, 9, (20), 2981-2986.
 136. Edgar, J. S.; Milne, G.; Zhao, Y. Q.; Pabbati, C. P.; Lim, D. S. W.; Chiu, D. T., Compartmentalization of Chemically Separated Components into Droplets. *Angewandte Chemie-International Edition* **2009**, 48, (15), 2719-2722.

137. Damean, N.; Olguin, L. F.; Hollfelder, F.; Abell, C.; Huck, W. T. S., Simultaneous measurement of reactions in microdroplets filled by concentration gradients. *Lab on a Chip* **2009**, 9, (12), 1707-1713.
138. Baret, J. C.; Miller, O. J.; Taly, V.; Ryckelynck, M.; El-Harrak, A.; Frenz, L.; Rick, C.; Samuels, M. L.; Hutchison, J. B.; Agresti, J. J.; Link, D. R.; Weitz, D. A.; Griffiths, A. D., Fluorescence-activated droplet sorting (FADS): efficient microfluidic cell sorting based on enzymatic activity. *Lab on a Chip* **2009**, 9, (13), 1850-1858.
139. Joensson, H. N.; Samuels, M. L.; Brouzes, E. R.; Medkova, M.; Uhlen, M.; Link, D. R.; Andersson-Svahn, H., Detection and Analysis of Low-Abundance Cell-Surface Biomarkers Using Enzymatic Amplification in Microfluidic Droplets. *Angewandte Chemie-International Edition* **2009**, 48, (14), 2518-2521.
140. Boedicker, J. Q.; Li, L.; Kline, T. R.; Ismagilov, R. F., Detecting bacteria and determining their susceptibility to antibiotics by stochastic confinement in nanoliter droplets using plug-based microfluidics. *Lab on a Chip* **2008**, 8, (8), 1265-1272.
141. Wang, J. B.; Zhou, Y.; Qiu, H. W.; Huang, H.; Sun, C. H.; Xi, J. Z.; Huang, Y. Y., A chip-to-chip nanoliter microfluidic dispenser. *Lab on a Chip* **2009**, 9, (13), 1831-1835.
142. Shui, L. L.; Kooij, E. S.; Wijnperle, D.; van den Berg, A.; Eijkel, J. C. T., Liquid crystallography: 3D microdroplet arrangements using microfluidics. *Soft Matter* **2009**, 5, (14), 2708-2712.
143. Stan, C. A.; Schneider, G. F.; Shevkoplyas, S. S.; Hashimoto, M.; Ibanescu, M.; Wiley, B. J.; Whitesides, G. M., A microfluidic apparatus for the study of ice nucleation in supercooled water drops. *Lab on a Chip* **2009**, 9, (16), 2293-2305.
144. Laval, P.; Crombez, A.; Salmon, J. B., Microfluidic Droplet Method for Nucleation Kinetics Measurements. *Langmuir* **2009**, 25, (3), 1836-1841.
145. Hashimoto, M.; Feng, J.; York, R. L.; Ellerbee, A. K.; Morrison, G.; Thomas, S. W.; Mahadevan, L.; Whitesides, G. M., Infochemistry: Encoding Information as Optical Pulses Using Droplets in a Microfluidic Device. *Journal of the American Chemical Society* **2009**, 131, (34), 12420-12429.
146. Bodas, D.; Khan-Malek, C., Hydrophilization and hydrophobic recovery of PDMS by oxygen plasma and chemical treatment - An SEM investigation. *Sensors and Actuators B-Chemical* **2007**, 123, (1), 368-373.
147. Atencia, J.; Beebe, D. J., Controlled microfluidic interfaces. *Nature* **2005**, 437, (7059), 648-655.
148. Garstecki, P.; Fuerstman, M. J.; Stone, H. A.; Whitesides, G. M., Formation of droplets and bubbles in a microfluidic T-junction - scaling and mechanism of break-up. *Lab on a Chip* **2006**, 6, (3), 437-446.
149. Pohl, H. A., Dielectrophoresis. *In Cambridge University Press* **1978**.
150. Jung, Y. M.; Oh, H. C.; Kang, I. S., Electrical charging of a conducting water droplet in a dielectric fluid on the electrode surface. *Journal of Colloid and Interface Science* **2008**, 322, (2), 617-623.
151. Sjoblom, J., Emulsions and emulsion stability *Taylor & Francis, Chapter 15, 556* **2006**.
152. O.Wilkes, J., Fluid Mechanics for Chemical Engineers. *Prentice Hall International Series* **2006**, 151.
153. Zimmermann, M.; Schmid, H.; Hunziker, P.; Delamarche, E., Capillary pumps for

- autonomous capillary systems. *Lab on a Chip* **2007**, 7, (1), 119-125.
154. O.Wilkes, J., Fluid Mechanics for Chemical Engineers. *Prentice Hall International Series* **2006**, 198.
155. Adamson, A. W., Physical Chemistry of Surfaces, 4th ed. *Wiley: New York*; **1982**, Chapter 1.
156. Sugiura, S.; Nakajima, M.; Iwamoto, S.; Seki, M., Interfacial tension driven monodispersed droplet formation from microfabricated channel array. *Langmuir* **2001**, 17, (18), 5562-5566.
157. Hildebrandt, P.; Stockburger, M., Surface-Enhanced Resonance Raman-Spectroscopy of Rhodamine-6g Adsorbed on Colloidal Silver. *Journal of Physical Chemistry* **1984**, 88, (24), 5935-5944.
158. Fidalgo, L. M.; Abell, C.; Huck, W. T. S., Surface-induced droplet fusion in microfluidic devices. *Lab on a Chip* **2007**, 7, (8), 984-986.
159. Link, D. R.; Grasland-Mongrain, E.; Duri, A.; Sarrazin, F.; Cheng, Z. D.; Cristobal, G.; Marquez, M.; Weitz, D. A., Electric control of droplets in microfluidic devices. *Angewandte Chemie-International Edition* **2006**, 45, (16), 2556-2560.
160. Zheng, B.; Tice, J. D.; Ismagilov, R. F., Formation of droplets of alternating composition in microfluidic channels and applications to indexing of concentrations in droplet-based assays. *Analytical Chemistry* **2004**, 76, (17), 4977-4982.
161. McDonald, J. C.; Duffy, D. C.; Anderson, J. R.; Chiu, D. T.; Wu, H. K.; Schueller, O. J. A.; Whitesides, G. M., Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis* **2000**, 21, (1), 27-40.
162. Priest, C.; Herminghaus, S.; Seemann, R., Controlled electrocoalescence in microfluidics: Targeting a single lamella. *Applied Physics Letters* **2006**, 89, (13).
163. Chabert, M.; Dorfman, K. D.; Viovy, J. L., Droplet fusion by alternating current (AC) field electrocoalescence in microchannels. *Electrophoresis* **2005**, 26, (19), 3706-3715.
164. Singh, P.; Aubry, N., Transport and deformation of droplets in a microdevice using dielectrophoresis. *Electrophoresis* **2007**, 28, (4), 644-657.
165. A.Cemal Eringen, G. A. M., *Electrodynamics of Continua, Fluids and Complex Media*. Springer-Verlag, New York: 1990; Vol. 2, p 553.
166. Saville, D. A., Electrohydrodynamics: The Taylor-Melcher leaky dielectric model. *Annual Review of Fluid Mechanics* **1997**, 29, 27-64.
167. Allen, P. H. G.; Karayiannis, T. G., Electrohydrodynamic Enhancement of Heat-Transfer and Fluid-Flow. *Heat Recovery Systems & Chp* **1995**, 15, (5), 389-423.
168. Eow, J. S.; Ghadiri, M.; Sharif, A. O.; Williams, T. J., Electrostatic enhancement of coalescence of water droplets in oil: a review of the current understanding. *Chemical Engineering Journal* **2001**, 84, (3), 173-192.
169. Hung, L. H.; Choi, K. M.; Tseng, W. Y.; Tan, Y. C.; Shea, K. J.; Lee, A. P., Alternating droplet generation and controlled dynamic droplet fusion in microfluidic device for CdS nanoparticle synthesis. *Lab on a Chip* **2006**, 6, (2), 174-178.
170. Plateau, J., *ACAD SCI BRUXELLES M* **1849**, 23, (5).
171. RAYLEIGH, L., *P R SOC LONDON* **1879**, 29, (71).
172. Israelachvili, J. N., *Intermolecular and Surface forces*. 2nd ed.; Academic, San Diego: 1991.
173. Lebedev, N. N.; Skalskaya, I. P., Force Acting on a Conducting Sphere in Field of a Parallel Plate Condenser. *Soviet Physics-Technical Physics* **1962**, 7, (3), 268-&.
174. Huang, C. C.; Chang, H. T., Selective gold-nanoparticle-based "turn-on" fluorescent sensors

- for detection of mercury(II) in aqueous solution. *Analytical Chemistry* **2006**, 78, (24), 8332-8338.
175. Wang, W.; Yang, C.; Li, C. M., Efficient On-Demand Compound Droplet Formation: From Microfluidics to Microdroplets as Miniaturized Laboratories *Small* **2009**, DOI: 10.1002/sml.200801598.
 176. Mukhopadhyay, R., Diving into droplets. *Analytical Chemistry* **2006**, 78, (5), 1401-1404.
 177. Belder, D., Microfluidics with droplets. *Angewandte Chemie-International Edition* **2005**, 44, (23), 3521-3522.
 178. Grodrian, A.; Metze, J.; Henkel, T.; Martin, K.; Roth, M.; Kohler, J. M., Segmented flow generation by chip reactors for highly parallelized cell cultivation. *Biosensors & Bioelectronics* **2004**, 19, (11), 1421-1428.
 179. Beer, N. R.; Hindson, B. J.; Wheeler, E. K.; Hall, S. B.; Rose, K. A.; Kennedy, I. M.; Colston, B. W., On-chip, real-time, single-copy polymerase chain reaction in picoliter droplets. *Analytical Chemistry* **2007**, 79, (22), 8471-8475.
 180. Zheng, B.; Roach, L. S.; Ismagilov, R. F., Screening of protein crystallization conditions on a microfluidic chip using nanoliter-size droplets. *Journal of the American Chemical Society* **2003**, 125, (37), 11170-11171.
 181. Chan, E. M.; Alivisatos, A. P.; Mathies, R. A., High-temperature microfluidic synthesis of CdSe nanocrystals in nanoliter droplets. *Journal of the American Chemical Society* **2005**, 127, (40), 13854-13861.
 182. Song, Y. J.; Hormes, J.; Kumar, C., Microfluidic synthesis of nanomaterials. *Small* **2008**, 4, (6), 698-711.
 183. Lim, C. T.; Zhang, Y., Bead-based microfluidic immunoassays: The next generation. *Biosensors & Bioelectronics* **2007**, 22, (7), 1197-1204.
 184. Liu, W. T.; Zhu, L.; Qin, Q. W.; Zhang, Q.; Feng, H. H.; Ang, S., Microfluidic device as a new platform for immunofluorescent detection of viruses. *Lab on a Chip* **2005**, 5, (11), 1327-1330.
 185. Sato, K.; Yamanaka, M.; Takahashi, H.; Tokeshi, M.; Kimura, H.; Kitamori, T., Microchip-based immunoassay system with branching multichannels for simultaneous determination of interferon-gamma. *Electrophoresis* **2002**, 23, (5), 734-739.
 186. Wen, J. H.; Yang, X. H.; Wang, K. M.; Tan, W. H.; Zhou, L. J.; Zuo, X. B.; Zhang, H.; Chen, Y. Q., One-dimensional microfluidic beads array for multiple mRNAs expression detection. *Biosensors & Bioelectronics* **2007**, 22, (11), 2759-2762.
 187. Yang, X. H.; Zhao, X.; Zuo, X. B.; Wang, K. M.; Wen, J. H.; Zhang, H., Nucleic acids detection using cationic fluorescent polymer based on one-dimensional microfluidic beads array. *Talanta* **2009**, 77, (3), 1027-1031.
 188. Zhang, H.; Yang, X.; Wang, K.; Tan, W.; Zhou, L.; Zuo, X.; Wen, J.; Chen, Y., Detection of single-base mutations using 1-D microfluidic beads array. *Electrophoresis* **2007**, 28, (24), 4668-4678.
 189. Zhang, H.; Yang, X. H.; Wang, K. M.; Tan, W. H.; Li, H. M.; Zuo, X. B.; Wen, H. H., On-chip oligonucleotide ligation assay using one-dimensional microfluidic beads array for the detection of low-abundant DNA point mutations. *Biosensors & Bioelectronics* **2008**, 23, (7), 945-951.
 190. Jameela, S. R.; Jayakrishnan, A., GLUTARALDEHYDE CROSS-LINKED CHITOSAN

- MICROSPHERES AS A LONG-ACTING BIODEGRADABLE DRUG-DELIVERY VEHICLE - STUDIES ON THE IN-VITRO RELEASE OF MITOXANTRONE AND IN-VIVO DEGRADATION OF MICROSPHERES IN RAT MUSCLE. *Biomaterials* **1995**, 16, (10), 769-775.
191. Russo, R.; Malinconico, M.; Santagata, G., Effect of cross-linking with calcium ions on the physical properties of alginate films. *Biomacromolecules* **2007**, 8, (10), 3193-3197.
 192. Shu, X.; Zhu, K. J., A novel approach to prepare tripolyphosphate/chitosan complex beads for controlled release drug delivery. *International Journal of Pharmaceutics* **2000**, 201, (1), 51-58.
 193. H.A, P., Dielectrophoresis. In Cambridge University Press: 1978.
 194. Yonezawa, Y.; Sato, T.; Kawabata, I., Photoinduced Formation of Cold Metal-Film from Metal Salt of Chitosan. *Chemistry Letters* **1994**, (2), 355-358.
 195. Qiu, T.; Wu, X. L.; Shen, J. C.; Xia, Y.; Shen, P. N.; Chu, P. K., Silver fractal networks for surface-enhanced Raman scattering substrates. *Applied Surface Science* **2008**, 254, (17), 5399-5402.
 196. Kneipp, K.; Kneipp, H.; Kneipp, J., Surface-enhanced Raman scattering in local optical fields of silver and gold nanoaggregates - From single-molecule Raman spectroscopy to ultrasensitive probing in live cells. *Accounts of Chemical Research* **2006**, 39, (7), 443-450.

Publications

1. **W. Wang**, C. Yang, Y.S. Liu, C. M. Li : On-demand Droplet Release for Droplet-Based Microfluidic System, *Lab-on-a-Chip*, DOI: 10.1039/B924929J, accepted
2. **W. Wang**, C. Yang, C. M. Li : On-demand Microfluidic Droplet Trapping and Fusion for On-chip Static Droplet Assays, *Lab-on-a-Chip* , 2009, 9, 1504-1506, DOI: 10.1039/b903468d
3. **W. Wang**, C. Yang, C. M. Li : Efficient On-Demand Compound Droplet Formation: From Microfluidics to Microdroplets as Miniaturized Laboratories, *Small* , 2009, 5, 1149-1152, DOI: 10.1002/smll.200801598
4. **W. Wang**, C. Yang, X.Q. Cui, Q.L. Bao, C. M. Li : Droplet Microfluidic Preparation of Au Nanoparticles-coated Chitosan Microbeads for Flow-through Surface-enhanced Raman Scattering Detection, *Microfluidics and Nanofluidics*, MNF80110, accepted
5. L. Yu, C. M. Li, J. Gao, Y.S.Liu, **W. Wang** : Flow-through Functionalized PDMS Microfluidic Channels with Dextran Derivative for ELISAs, *Lab-on-a-Chip* , 2009, 9, 1243-1247, DOI:10.1039/B816018J
6. Q.L. Bao, C. M. Li, L. Liao, H.B. Yang, **W. Wang**, C. Ke, Q.L. Song, H.F. Bao, T. Yu, K.P. Loh, J. Guo: Electrical Transport and Photovoltaic Effects of Core-shell CuO/C-60 Nanowire Heterostructure. *Nanotechnology*, DOI: 10.1088/0957-4484/20/6/065203
7. R. Li, Y. Gan, Q.L. Song, Z.H. Zhu, J.S. Shi, H.B. Yang, **W. Wang**, C.M. Li: Bidirectional Mediation of TiO₂ Nanowires Field Effect Transistor by Dipole Moment from Purple Membrane, *Nanoscale*, accepted
8. C. M. Li, **W. Wang** : Microfluidic Droplet-based Lab-On-a Chip System - On-Chip Nanoliter Droplet Trapping, Fusion, Release and Detection. *International Electron Devices and Materials Symposia (IEDMS)*, 2009
9. C. M. Li, **W. Wang** : A Complete Droplet Based Miniaturized Lab-on-a-Chip with On-demand Trapping, Fusion, and Releasing. *Patent application*, 2010.