APPLICATION OF MULTIFUNCTIONAL MICROFLUIDIC CHIPS TO SUSPENSION CELL-CELL COMMUNICATION RESEARCH

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Application of Multifunctional Microfluidic Chips to Suspension Cell-cell Communication Research

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ABSTRACT

A series of microfluidic chips were developed for the research of suspension cells. These microfluidic chips could be used to precisely manipulate cells and control cell environment at single and multiple cell levels. These microdevices provide novel platforms, open new avenues for cell biology research and contribute to breaking the bottleneck of biology research. Following is an outline of the contents and results of the thesis:

(1) On the basis of sandbag structures developed by our lab, the design of cell immobilization structures was improved. In one chip, a single cell array, containing approximate 600 single Jurkat cells, could be formed for eight-channel analysis in parallel. The responses of calcium-release activated Ca^{2+} (CRAC) channels to activators and inhibitors from the cell array were recorded and analyzed at the single cell level. The variation of CRAC channel response was observed. Although the variation of the CRAC channel response was common in single cells, the average values of 50 single cells could reflect the effects of inhibitors at high concentrations. These results demonstrated that our microfluidic chip could provide a platform to form single cell arrays for the multi-channel studies of the heterogeneity of calcium channel responses.

(2) A microfluidic microdevice was developed to exert mechanical stimulation on a single suspension cell for mechanosensation research. In this microfluidic chip, a single cell could be isolated from a population, and subsequently trapped. Mechanical stimulation could then be exerted on the trapped cell. Using this chip, the mechanosensation of HL60 cells

(leukemic cells) was studied. It was found that mechanical stimulation could trigger extracellular calcium to flow into HL60 cells through channels on cell membranes. The cytoskeleton, such as microfilament (consist of actins) and microtubules (consist of tubulins), was not a prerequisite for the mechanosensation of HL60 cells. Additionally, two function units for the entrapment of single cells and the exertion of mechanical stimulation were integrated into one chip for mechanosensation study in parallel. HL60 cells (leukemic cells) and Jurkat cells (lymphocytes) both responded to direct mechanical stimulation. These results demonstrate that the developed microfluidic device can be used to investigate the mechanosensation in a single suspension cell and so make it possible to conduct studies in parallel.

(3) Cell immobilization structures, a cell entrapment and a cell compressive component and microvalves were integrated in one microfluidic chip. In this microfluidic chip, suspension cell-cell communication could be in real time monitored. Using the chip，the influence of different factors, including fluid states (static or flowing) and mechanical stimulation modes (single or persistent), was evaluated. Additionally, calcium oscillations were observed in some of the recipient cells when mechanical stimulation was exerted on a small number (10 or 31) of donor cells in the compressive component.

In this thesis, a serial of components were developed to provide the capabilities of cell manipulation and the exactly control of chemical and physical environment. With the integration of these components in chips, biological analysis and cell research could be conducted, including CRAC channel analysis in parallel, the mechanosensation study at the single suspension cell level and suspension cell-cell communication. These results demonstrated that the application of microfluidic chips extended to the research of mechanosensation

and intercellular communication at the single cell level and the development of these microfluidic chips also open the new avenue for suspension cell research, which could be conducted using traditional methods.

TABLE OF CONTENS

ABSTRACT i

LIST OF PUBLICATIONS iv

ACKNOWLEDGEMENTS v

TABLE OF CONTENS vi

LIST OF ABBREVIATIONS ix

LIST OF FIGURES x

LIST OF TABLES xiii

CHAPTER 1 INTRODUCTION AND RESEARCH OBJECTIVE 1

1.1 WHAT IS MICROFLUIDICS 2

1.2 HISTORY OF MICROFLUIDICS 2

1.3 CHARACTERISTICS OF MICROFLUIDICIS 5

1.4 APLLICATION OF MICROFLUIDICS TO CELL RESEARCH 11

1.4.1 Extracellular environment control 11

1.4.2 Application of Droplets to cell research 26

1.4.3 Cell manipulation in cell research 28

1.4.4 Electrical detectors for monitoring cell activity in microfluidics 32

1.5 CELL-CELL COMMUNICATION 36

1.5.1 Introduction of cell-cell communication 36

1.6 STRUCTURE AND OBJECTIVES OF THE THESIS 42

CHAPTER 2 MICROFLUIDIC FORMATION OF SINGLE CELL ARRAY FOR PARALLEL ANALYSIS OF CRAC CHANNEL 44

2.1 INTRODUCTION 45

2.2 MATERIALS AND METHODS 47

3.2.1 Microfluidic chip fabrication 47

3.2.2 Cell culture and reagents 47

3.2.3 Cell docking ····· 48
3.2.4 Single cell response to CRAC channel activitor and inhibitor ····· 48
3.2.5 Fluorescent image acquisition ····· 49
2.3 RESULT AND DISCUSSION ····· 51
2.3.1 Microfluidic cell array formation ····· 51
2.3.2 Acitivition of CRAC Channel ····· 55
2.3.3 Biphasic effect of CRAC channel inhibitor 2-APB ····· 60
2.4 CONLUSION ····· 65

CHAPTER 3 USING INTEGRATED MICROFLUIDICS TO STUDY INTRACELLULAR CALCIUM RESPONSE TO MECHANICAL 66

3.1 INTRODUCTION ····· 67
3.2 MATERIAL AND METHODS ····· 69
3.2.1 Cell culture and reagents ····· 69
3.2.2 Microfluidic fabrication and actuation ····· 69
3.2.3 Individual cell entrapment, compression and reagent exchange ····· 71
3.2.4 Fluorescence image and data acquisition ····· 71
3.3 RESULT AND DISCUSSION ····· 76
3.3.1 Individual cell entrapment and exertion of mechanical stimulation ····· 76
3.3.2 Monitoring HL60 cell responses to mechanical stimulation ····· 76
3.3.3 Tracing the source of $[Ca^{2+}]_i$ increase in HL60 cells ····· 77
3.3.4 Influence of cytoskeleton on mechanosensitive channel ····· 79
3.3.5 Paralle studies of mechanosensation in HL60 and Jurkat cells ····· 84
3.4 CONCLUSION ····· 85

CHAPTER 4 REAL-TIME MONITORING OF SUSPENSION CELL-CELL COMMUNICATION USING AN INTEGRATED MICROFLUIDICS 87

4.1 INTRODUCTION ····· 87
4.2 MATERIAL AND METHODS ····· 88
4.2.1 Cell culture and reagents ····· 88
4.2.2 Microfluidic fabrication and actuation ····· 88

4.2.3 Microfluidic operation ····· 90
4.2.4 Fluorescence image and data acquisition ····· 90
4.3 RESULT AND DISCUSSION ····· 92
4.3.1 Design principle ····· 92
4.3.2 Trapping and mechanical stimulation on suspension cells by membrane deformation ····· 95
4.3.3 Immobilization of recipient cells in sandbag structures ····· 96
4.3.4 Monitoring cell-cell communication of suspension cells ····· 96
4.3.5 Effects of single mechanical stimulation on cell-cell communication in static environments ····· 101
4.3.6 Effects of persistent mechanical stimulation on cell-cell communication in static environments ····· 102
4.3.7 Effects of single mechanical stimulation on cell-cell communication in fluid flowing environment ····· 103
4.4 CONCLUSION ····· 106
CHAPTER 5 SUMMAY AND FUTURE EXPERIMENT 107
REFERENCES ..... ERROR! BOOKMARK NOT DEFINED.

LIST OF ABBREVIATIONS

Ab	Anti-insulin antibody
2-APB	2-aminoethyldiphenyl borate
APC	Antigen-presenting cells
CRAC	calcium-release activated Ca^{2+}
Cyto D	Cytochalasin D
DEP	Dielectrophoresis
EOF	Electroosmotic flow
FITC-insulin	Fluorescein isothio-cyanate-labeled insulin
IS	Immune synapses
μ-TAS	Micro-total-analysis-system
Noc	Nocodazole
PCB	Print circuit board
PDMS	Polydimethylsiloxane
TG	thapsigargin

LIST OF FIGURES

Fig. 1.1 Schematic diagram of T-shaped channel device for concentration gradient generation.

10

Fig. 1.2 ‘Christmas tree’ concentration generator.

17

Fig. 1.3 Molecule delivery and temperature step generation using laminar flows.

18

Fig. 1.4 The microfluidics for screening.

21

Fig. 1.5 Bioassays in dam and V-shaped microfluidic chips.

22

Fig. 1.6 The interaction of cells and substrates.

25

Fig. 1.7 On the basis of pressure-driven flows, three main microfluidic geometries for droplet generation.

27

Fig. 1.8 Single manipulation and stimulation microdevices.

31

Fig. 1.9 Integration of patch clamps in microfluidics.

35

Fig. 1.10 The ways of intercellular communication among suspension cells.

40

Fig. 2.1 Microfluidic fabrication and operation.

50

Fig. 2.2 Microfluidic chip for the formation of a single cell array.

53

Fig. 2.3 The evalulation of the microfluidic chip.

54

Fig. 2.4 The activation of CRAC channels in single cells for 10 min.

58

Fig. 2.5 CRAC activation curves in single cells.

59

Fig. 2.6 Fluorescence image of the cell array under the treatment of 8 different conditions in 8 independent channels.

63

Fig. 2.7 Fluorescent intensitie of CRAC channel responses to different stimulus in single cells.

64

Fig. 3.1 The assembly of sandwiched microdevice.

73

Fig. 3.2 Schematica diagram of the microfluidic chip.

74

Fig. 3.3 The manipulation for individual cell entrapment.

75

Fig. 3.4 The reseach of HL60 mechanosensation.

81

Fig. 3.5 Parallel monitoring the responses of HL60 and Jurkat cells.

83

Fig. 4.1 Schematic diagrams of the microfluidic chip and its design.

91

Fig. 4.2 Images of the mcirofluidic chip and its structures.

94

Fig. 4.3 Compression of trapped cells.

98

Fig. 4.4 Calcium wave propagation evoked by intercellular communication.

100

Fig. 4.5 Effects of mechanical stimulation modes and flow environment on cell-cell communication.

104

Fig. 4.6 Different patterns of intracalcium response traces.

105

LIST OF TABLES

Table 3.1 Reagents in 8 independent channels. 63