

DOCTORAL THESIS

Novel microfluidic platform for bioassays

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Abstract

Microfluidics have been created to acquire, operate, and process complex fluids in extremely tiny volumes with high efficiency and high speed, and without the requirement for an experienced operator. In addition, microfluidic systems also enable miniaturization and incorporation of different complex functions, which can help bring intricate diagnostic tools out of the laboratories. Ideally, these systems should be inexpensive, precise, reliable, robust, and well-suited to the medical diagnostic systems. Most of the microfluidic devices reported previously were based on devices made of polydimethylsiloxane (PDMS). PDMS is a material that dissolves in many common organic solvents. Meanwhile, it is also prone to absorb small molecules like the proteins, which is detrimental to a stable and reliable result. Current work focuses on bioassays that are badly needed in our life and these bioassays are addressed based on microfluidic platform with different materials. The translation of microfluidic technology into large scale implementations highly relies on new materials that address the limitations of PDMS.

Firstly, we fabricated two different microfluidic platforms for rapid antimicrobial susceptibility testing (AST). One was made of hydrogel, and the bacterial cells were cultured on the top of the device; the other was of polypropylene (PP), and bacterial cells were cultured inside the microchannels. Meanwhile, we developed a novel “barcode” sensor, a microscope-free method for cell accumulation and cell counting, as the downstream of the PP-based chips. As a result, AST can be accomplished

simply through an application on a mobile phone rather than using an expensive and sophisticated microscope.

Secondly, we presented a self-contained paper-based system for lead(II) ion detection based on G-quadruplex-based luminescence switch-on assay, comprising a novel type of paper-based chip and a matching portable device. Different from the reported paper-based devices, the paper substrate we chose was art paper, which is used for printing magazines. This type of paper could prevent the absorption of liquid into the paper matrix and hold the liquid in place for a period of time; and it could also be used for temporary liquid containing like a plastic substrate (such as polypropylene (PP) and polystyrene (PS)), but the surface of the paper is inherently hydrophilic. In such a design, liquid drops are suspended on the surface of the device in designed reservoirs, rather than absorbed into the paper; when the chip is tilted, the liquid drops will move to other reservoirs according to the guidance of channels defined on the surface. To differentiate it from reported μ PAD devices that are fabricated with water-permeable paper, we name this new type of paper-based devices suspending-droplet mode paper-based microfluidic devices (SD- μ PAD). Different from the conventional μ PADs that use capillary force to drive liquid, our SD- μ PADs uses wetting and gravity as driving force. To fabricate the superhydrophobic pattern on the paper device, we developed a new microcontact printing-based method to produce inexpensive and precisely patterned superhydrophobic coating on paper. The coating material is poly(dimethylsiloxane)

(PDMS), a hydrophobic and transparent silicone that has long been used for fabricating microfluidic devices. Importantly, the negative-relief stamp we used is made of Teflon, a non-stick polymer, so that the PDMS-coated paper could be peeled from the stamp flawlessly. After such fabrication process, the stamped area of the paper is coated with a textured PDMS layer that is decorated with arrays of micropillars, which could provide superhydrophobic effect and most effectively hold the droplets in place; the remaining area of the paper is still hydrophilic. As a demonstration of this new design, we developed a method using the reaction characteristics of iridium(III) complex for rapid, onsite detection of lead(II) ions in liquid samples. As the reagents have already been loaded onto the paper device during fabrication, the only reagent the users need to add is water. Because of the large Stokes shift of the iridium(III) complex probe, inexpensive optical filters can be employed, and we were able to make an inexpensive, battery-powered compact device for routine portable detection using a smartphone as a detector, allowing the rapid analysis and interpretation of results on site as well as the automatic dissemination of data to professional institutes, including tests even in poor rural areas in developing countries.

Thirdly, we upgraded our suspending-droplet mode paper-based microfluidic device (SD- μ PAD), which is used for the detection of lead(II) ions in liquid solution. The reason is that our paper-based SD chips are not suitable for long reaction process (> 20 min) detection of biomolecules due to the potential permeation and

contaminating problems of art papers. Hence, we chose polypropylene (PP), a hydrophobic, cheap, and thermal stable material ($< 110^{\circ}\text{C}$), as the material for the fabrication of the SD microfluidic chip. We established a convenient, low-cost, portable and reliable platform for monitoring VEGF₁₆₅ accurately, which can be applied for point-of-care (POC) testing. In this project, we also employed the label-free oligonucleotide-based luminescence switch-on assay on the microfluidic platform, which possesses the advantages of high sensitivity and high selectivity. Based on the detection of VEGF₁₆₅ in a three-step reaction process, we adopted a new design for the droplet transfer throughout the channels. This design could migrate the droplet through the chambers via controlling the orientation of the chip, which systematically combined the superhydrophobic force of the coating, the gravity of the droplet and the surface tension between PP and droplet. Therefore, traditional micro pump could be avoided and the total cost for the device could be substantially reduced. In addition, we developed an automatic, matched and portable device for the detection of VEGF₁₆₅, which assembled by a rotatable chip holder, a UV lamp, a filter, and a camera.

Finally, we developed a new whole Teflon membrane-based chip for the aptamer screening. Our article “Whole-Teflon microfluidic chips” introduced the fabrication of a microfluidic device entirely using Teflon materials, one group of the most inert materials in the world. It was a successful and representative introduction of new materials into the fabrication of microfluidic devices, which show dramatically

greater anti-fouling performance. However, even such device was inadequate for current purpose, as it is rigid and lacks convenient valve control functions for particle suspensions used in systematic evolution of ligands by exponential enrichment (SELEX). For this project, we propose a SMART screening strategy based on a highly integrated microfluidic chip. This new type of whole-Teflon devices, which are made of flexible Teflon membranes, offering convenient valving control for the whole SELEX process to be performed on chip and fulfilling the anti-fouling requirement in the meantime. The SELEX cycles including positive and negative selections could be automatically performed inside tiny-size microchambers on a microchip, and the enrichment is real-time monitored. The selection cycles would be ended after the resulted signal of the aptamers with high specificity reached a plateau, or no target aptamer is captured after a number of cycles of enrichment. Owing to the antifouling property of the chip materials, the loss of the sample is tremendously reduced. The SMART platform therefore is not only free of complicated manual operations, but also high-yield and well reproducible over conventional methods.

Table of Contents

DECLARATION	i
Abstract	ii
Acknowledgements	vii
Table of Contents	ix
List of Abbreviation	xv
1.1. Background of microfluidics	1
1.2. Materials for the generation of microfluidics	2
1.2.1. Inorganic Materials	2
1.2.2. Elastomers and Plastics	3
1.2.3. Hydrogels	6
1.2.4. Paper	7
1.3. Applications of Microfluidic Technologies	7
1.3.1. Cell Biology	8
1.3.2. Droplet-based Analysis	9
1.3.3. DNA Assays	10
1.3.4. Point-of-Care (POC) Testing	11
1.3.5. Drug Administering	13
1.4. Research motivation	14
Chapter 2. Cell-on-hydrogel platform made of agar and alginate for rapid,	

low-cost, multidimensional test of antimicrobial susceptibility	16
2.1. Introduction	16
2.2. Experimental sections	20
2.2.1. Materials and equipments	20
2.2.2. Fabrication of agar-alginate mixed gel device	20
2.2.3. Investigation of the on-chip diffusion process.....	21
2.2.4. Antimicrobial sensitivity testing.....	21
2.3. Results and Discussion	23
2.3.1. Fabrication process of whole hydrogel microdevice.....	23
2.3.2. Diffusion test of the whole hydrogel device.....	26
2.3.3. AST on the whole hydrogel device	29
2.4. Conclusions	42
 Chapter 3. Reliable and reusable whole-polypropylene plastic microfluidic devices for rapid, low-cost antimicrobial susceptibility test	 43
3.1. Introduction	43
3.2. Experimental sections	48
3.2.1. Materials and equipments	48
3.2.2. Computational fluid dynamics (CFD) simulation	48
3.2.3. Fabrication of the microfluidic devices	49
3.2.4. Concentration gradient test.....	50
3.2.5. AST experiment.....	50
3.2.6. AST experiment of clinical samples	50

3.3. Results and discussion	52
3.3.1. Overall design of the system	52
3.3.2. Fabrication process of the whole PP chip	54
3.3.3. CFD simulation of shear stress for optimizing channel designs	60
3.3.4. Antimicrobial susceptibility test and reusability performance on PP chip	63
Chapter 4. “Barcode” sensor for rapid, convenient and resource- independent antimicrobial susceptibility testing	72
4.1. Introduction	72
4.2. Experimental sections	75
4.2.1. Materials and equipments	75
4.2.2. Computational fluid dynamics (CFD) simulation	75
4.2.3. Fabrication of the microfluidic devices	76
4.2.4. AST experiment	77
4.3. Results and discussion	78
4.3.1. Overall design of the system	78
4.3.2. Fabrication process of the “barcode” sensor	79
4.3.3. Computational fluid dynamics (CFD) simulation of the principle of cell accumulation inside the “barcode” sensor.	81
4.3.4. “Barcode” sensor for rapid and resource-independent AST.	83

Chapter 5. A suspending-droplet mode paper-based microfluidic platform for low-cost, rapid, and convenient detection of lead(II) ions in liquid solution	85
5.1. Introduction	85
5.2. Experimental sections	90
5.2.1. Materials and Chemicals	90
5.2.2. Fabrication of paper-based chip	90
5.2.3. Fabrication of the portable detection device	91
5.2.4. Detection of the lead(II) ion	92
5.2.5. Procedures of two control experiments	93
5.3. Results and discussion	95
5.3.1. Principle of the SD-μPAD	95
5.3.2. Fabrication of the SD-μPAD	97
5.3.3. Investigation of the droplet manipulation on the SD-μPAD	100
5.3.4. Design of the SD-μPAD device for Lead Ion Detection	105
5.3.5. Design of the portable device	108
5.3.6. Lead(II) ion assay on the Fully Portable System	110
5.4 Conclusion	114
Chapter 6. A suspending-droplet mode plastic-based microfluidic platform for low-cost, convenient, and multi-step detection of VEGF₁₆₅	116
6.1. Introduction	116

6.2. Experimental sections	118
6.2.1. Materials and Chemicals	118
6.2.2. Fabrication of plastic-based chip	118
6.2.3. Fabrication of the portable detection device	119
6.2.4. Detection of the VEGF₁₆₅	119
6.3. Results and discussion	121
6.3.1. Principle of the PP-based suspending-droplet microfluidic chips	121
6.3.2. VEGF₁₆₅ detection assay on the portable system	126
Chapter 7. The next generation aptamer screening system: a SMART, high yield and high throughput microfluidic platform	129
7.1. Introduction	129
7.2. Experimental sections	133
7.2.1. Materials and equipments	133
7.2.2. Fabrication of the microfluidic devices	133
7.2.3. Antifouling experiment	134
7.2.4. Functional test	134
7.3. Results and discussion	135
7.3.1. Overall design of the system	135
Chapter 8. Overall Conclusions and Future Work	140
8.1. Overall conclusions	140
8.2. Future work	145

List of references	146
List of publications	170
CURRICULUM VITAE	172