Controllable assembly and disassembly of high aspect ratio micropillar arrays and their applications

Wang, Yu

2017

Wang, Y. (2017). Controllable assembly and disassembly of high aspect ratio micropillar arrays and their applications. Doctoral thesis, Nanyang Technological University, Singapore.

<http://hdl.handle.net/10356/70840>

<https://doi.org/10.32657/10356/70840>

NANYANG
TECHNOLOGICAL
UNIVERSITY

**CONTROLLABLE ASSEMBLY AND DISASSEMBLY OF HIGH
ASPECT RATIO MICROPILLAR ARRAYS AND THEIR
APPLICATIONS**

WANG YU

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

2016

**CONTROLLABLE ASSEMBLY AND DISASSEMBLY OF HIGH
ASPECT RATIO MICROPILLAR ARRAYS AND THEIR
APPLICATIONS**

WANG YU

SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

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

2016

Statement of Originality

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

2016/08/19

.....
Date

.....
Wang Yu

Abstract

Flexible materials PDMS has been fabricated into different kinds of structures and contributed to the materials science and engineering area. Due to its easy-to-fabricate nature, many shapes had been designed to fit the need of applications. Among them, micropillar arrays are important for many applications, including cellular mechanosensory and actuators, dry adhesives mimicking gecko's foot structures and optical applications such as ultrathin paper whitening membranes. And as the surface area is relatively large and aspect ratio is high, the micropillars will deform under minuscule external forces such as capillary force when liquids evaporated from the micropillar surfaces. Previously researchers had developed many applications from this unique property and studied the mechanism of micropillar assembly under capillary force. However, it lacked in-depth understanding in the deformation process.

In this thesis, we had systematically studied the controlling factors of micropillar array assembly, including pattern geometry, and surface chemistry. Besides, mathematic models had been built to predict the pillar assembly patterns by calculation. We studied the micropillar deformation under capillary force both theoretically and experimentally. And we created a series of assembled micropillar patterns that can trap bacteria and microparticles based on their size. Moreover, we successfully developed a hydrogel coated PDMS micropillar array structure. It was responsive to dual stimuli, mechanical stretch, and pH changes and controllably release the trapped microparticles. These devices had potential to be applied in live bacteria capture for drug screening devices, and mechanical force triggered pH-responsive controlled drug release devices.

Acknowledgements

First of all, I would like to express my sincere gratitude to my supervisor, Prof. Chen Xiaodong. For the past four years as a graduate student, without his guidance and help, this thesis cannot be built. Prof. Chen always like to say that teaching someone to fish is much better than giving him fish. Thank you very much for teaching me how to fish, and it will be a lifetime treasure for me.

I gratefully thank Prof. Tan Lay Poh and Prof. Ng Kee Woei as my TAC members, for their valuable time, suggestions and comments during the past four years.

And I would also like to thank all my group members, especially Dr. Hu Benhui, Dr. Cai Pingqiang, Miss Zhang Xiaoqian, Miss Li Zhuyun from Bio-subgroup. And I also would like to thank my colleagues' help in material preparation, Dr. Qi Dianpeng and Mr. Liu Zhiyuan for their discussion and help when I was struggling with the difficulties during materials fabrication progress. And also thank all my group members, including former group members, Dr. Du Jianjun, Dr. Lang Xianjun, Dr Li Yongqiang, Dr. Li Yuangang, Dr. Liu Yaqing, Dr. Meng Fanben, Dr. Niu Zhiqiang, Dr. Peng Tai, Dr. Shao Qi, Dr. Tang Yuxin, Dr. Wang Hong, Dr. Wang Hua, Dr. Wang Wei, Dr. Wang Xiaotian, Dr. Wu Yunlong, Dr. Yang Hui, Dr. Yin Shengyan, Dr. Yu Jiancan, Dr. Zhang Chenguang, Dr. Zhang Yanyan, Dr. Zheng Kaihong, Dr. Zheng Liyan, Mr. Deng Jiyang, Mr. Xu Cai, Mr. Lv Zhisheng, Dr. Bu Jing, Dr. Zhu Bowen, Ms. Chen Geng, Ms. Leow Wan Ru, Dr. Liu Yuanjun, Ms. Jiangying and Ms. Guo Xintong et al. I had the honor to enjoy their presentation during group meetings, and that was very inspiring. I would also like to thank their kind support for the four wonderful years.

Also, I would like to express sincere gratefulness to Mr. Chen Xianglong from School of Civil and Environmental Engineering in National University of Singapore for his kind help in mathematical modeling.

I would like to thank Mr. Shamsul from School of Electrical and Electronic Engineering for his kind help in performing deep RIE.

Finally, I would like to thank all my friends and my family for their kind support and understanding during the four years. Their love had fueled me up all along the four years and gave me strength to face all the difficulties.

Table of Contents

Abstract	i
Acknowledgements	iii
Table of Contents	v
Table Captions	ix
Figure Captions	xi
Abbreviations	xxiii
 Chapter 1 Introduction.....	 1
1.1 Problem Statement	2
1.2 Objectives and Scope	5
1.3 Dissertation Overview.....	5
1.4 Findings and Outcomes/Originality	7
References	7
 Chapter 2 Literature Review	 9
2.1 Building Materials and Fabrication Methods of Micropillar array	10
2.1.1 Inorganic Materials	10
2.1.2 Organic Materials	11
2.2 Assembly and Disassembly of Micropillar Array	13
2.2.1 Gravity Induced Collapse of Micropillar Array	13
2.2.2 Menciaus Surface between Two Pillars	13
2.2.3 Capillary Action	13
2.3 Current Applications of High Aspect Ratio Micropillar Array	16

2.3.1	Dry Adhesives Mimicking Gecko's Foot	16
2.3.2	Cell Traction Force Sensors	18
2.3.3	Microparticle and Salt Microcrystal Trapping	22
2.3.4	Molecule Trapping	22
2.3.5	Optical Applications	24
2.4	Material-bio Interface	25
2.5	Questions to Answer Based on Literature	27
2.6	Ph.D. in Contest of Literature	28
	References	29
Chapter 3	Experimental Methodology	33
3.1	Rationale for selection	34
3.2	Preparation of PDMS Micropillar Arrays with Different Surface Chemistry	35
3.2.1	Materials	35
3.2.2	Lithography Process	36
3.2.3	Mold Fabrication Method	38
3.2.4	Surface Functionalization and Treatment of PDMS Micropillar Arrays	37
3.3	Characterization Methods	38
3.3.1	Scanning Electronic Microscopy Measurement	38
3.3.2	Water Contact Angle Measurement	39
3.3.3	Water Contact Angle Measurement	40
3.4	Bacteria Culture and Imaging	40
3.4.1	Morphology Study of Bacteria	41
3.4.2	LIVE/DEAD Bacterial Assay	41
	References	41
Chapter 4	Systematic Study of Micropillar Array Assembly and Disassembly	43
4.1	Introduction	44
4.2	Materials and Methods	46
4.2.1	Surface Treatment of PDMS Micropillar Arrays	47
4.2.2	Evaporation Induced Micropillar Array Assembly	48

4.3	Mathematic Model	49
4.3.1	Evaporation Process Analysis	49
4.3.2	Capillary Force Calculation	51
4.3.3	Elastic Resistance Calculation	53
4.4	Experiment Result	57
4.5	Conclusion	58
	References	58
Chapter 5 Live Bacteria Extraction and Controllable Releasing Platform		61
5.1	Introduction	62
5.1.1	Bacteria Surface Adhesion	62
5.1.2	Microfluidic Separation Method	66
5.1.3	Size Based Filtration Method	70
5.1.4	Microparticle-Based Capturing Method	71
5.1.5	Conclusion	72
5.2	Materials and Experiment	72
5.3	Results and Discussion	73
5.3.1	Bacterial Swimming within Confined Space between Pillar Arrays	73
5.3.2	Bacteria Capturing	75
5.3.3	Size Dependent Bacteria/Cell Selectivity	77
5.3.4	Stretching Induced pH-Dependent Bacteria Releasing	79
5.4	Microbial Drug Sensitivity Test	80
5.5	Conclusion	82
	References	82
Chapter 6 Surface Functionalized Micropillar Array for Stretch-Induced pH-Responsive Controlled Release of Microspheres		85
6.1	Introduction	86
6.2	Materials and Methods	89
6.2.1	Micropillar Surface Modification	90
6.2.2	FluoSphere Loading	90

6.2.3	Stretching Induced Releasing of Microparticles	91
6.3	Results and Discussion	91
6.3.1	PDMS Surface Modification	91
6.3.2	Microparticle Loading and Stretch-induced Releasing	94
6.4	Conclusion	96
	References.....	97
Chapter 7	Discussion and Future Work	99
7.1	General Discussion.....	100
7.2	Reconnaissance Work for Future Work	101
7.2.1	Micropillar Array Optical Sensor	101
7.2.2	Auxetic Substrate for High-Efficiency Drug Release.....	103
7.2.3	3D Printed Gas Sensor	105
7.3	Conclusion	108
	References.....	108

Table Captions

Table 1.1 Current applications of micropillar array system.

Table 5.1 Comparison of bacterial adhesive molecules.

Figure Captions

Figure 1.1 Illustration of micropillar array structure.

Figure 1.2 Different kinds of micropillar deformation under mechanical force with the pillar substrates omitted. Top left to right: (a) original shape of a single micropillar and its deformation under tension (b), compression (c) and bending (d). Bottom left to right: deformation of a single micropillar under shear force (e), tortuosity (f) and combined loadings (g).

Figure 1.3 Applications of micropillar array structures. (a) Microparticle trapping. (b) cell traction force sensor. (c) Insects and geckos can walk on ceiling thanks to the micropillars on their feet. (d) A robotic gecko can adhere to walls due to the micropillar array structure. By adhering-peeling cycles, the robot can walk on walls. (e) the paper whitening effect from ordered arrays (transparent) to clustered and disordered arrays (whitening). (f) single molecule trapping on gold modified pillar surfaces.

Figure 2.1 Schematic figure of top-down lithography method to fabricate inorganic micropillar arrays (for hard substrates such as silica).

Figure 2.2 Shape memory polymer fabricated micropillar arrays. And their shape recovery under heat. (a) Deformed and recovered shape memory micropillars. (a) Shape memory micropillars (micropillar diameter: 10 μm , spacing: 30 μm , height: 30 μm .) deformed along x-axis. (b) deformed diagonally to x-axis. (c) Recovery of shape memory polymer micropillars. (d) The recovery process of shape memory polymer micropillars of hexagonal array pattern, diameter: 10 μm , spacing: 10 μm , height: 30 μm).

Figure 2.3 Schematic figure of micropillar system immersed in liquid. Pillars are partially immersed in water, and the tips are in the air. The schematic figure explained the definition of characters in Equation (2-1) (2-2) and (2-3).

Figure 2.4 A model brush scheme figure. In a simplified brush model system, it consists of flexible hydrophilic fibers that are end-grafted to the surface. When the model brush is dipped in water and later slowly lifted above the water surface, water fills up the region that was enclosed by the fibers. (Reprinted with permission from reference.)

Figure 2.5 Gecko's foot hair structure. (images from Google Image) a. A gecko. b. Enlarged gecko's foot, it is fully covered with hair. c. Gecko's foot hair under SEM. Scale bar: 50 μm . It shows rows of setae d. Scheme figure of gecko's foot hair. It is a hierarchical structure with thinner and shorter pillar structure on top of a single Seta.

Figure 2.6 Gecko's foot mimic dry adhesive structure. (a-b). Single layer fibrillar adhesives. c-d. hierarchical fibrillar adhesive structure. a. polyurethane micro-pillar arrays. Pillar diameter is 4.5 μm , tip diameter 9 μm and length 20 μm . b1. Polystyrene nano-fiber dry adhesive. Pillar diameter: 330 nm, length: 1.5 μm . (c) Polyurethane hierarchical pillars with flat mushroom-like tops. The bottom pillars have 50 μm base and 100 μm top plate. And the secondary pillars have 3 μm base and 5 μm top plates. (am8002439) (d) PDMS pillar array with a diameter of 50 μm and height of 200 μm . Secondary pillar on top of larger pillars has diameter of 10 μm and length of 10 μm . (Adv. Mater. 2009, 21, 479–482)

Figure 2.7 Cell Traction Force sensors based on micropillar arrays. (A) Scheme figure of this design. The bending of micropillars can be transformed into shear force. (B) SEM image of micropillar array for cell traction force sensor. (C) Stained cell on micropillar cell traction force sensors. It can be clearly seen that the focal adhesion formed on the tips of micropillars. (D to F) Scanning electron micrographs. (D) Closely spaced micropillar array fabricated by PDMS molding method. (E) An individual cell lying on micropillar array based force sensor (1 μm diameter and 2 μm distance from pillar center-to-center). (F) A cell monolayer (2 μm diameter and 3 μm distance from pillar center-to-center). (F Inset) Magnified view of the area in the black square. It is the enlarged view of lamellipodia formed on micropillar array tips. (E and F) Cells spread only on the top of pillars. (G) Graphical depiction of finite-element method analysis of micropillars. The length of each pillar in the response to shear traction force (20 nN) on tips of pillars. (H) Micropillar

deflection δ and force F plot data and normal spring constant K to length L relationship calculation from Euler-Bernoulli beam theory. K is a measurement of micropillar rigidity.

Figure 2.8 Micropillar array for particle and salt crystal trapping. (A) Scheme figure of this trapping system design. (B) SEM image of micropillar array for selective microparticle trapping. Scale bar: 2 μm . (C) 3.8 μm particle trapping. Scale bar: 4 μm . (D) The relationship between particle diameter and intracellular distance. (E-H) Particle transportation of micropillar array system. Liquid evaporation will induce pillar assembly and soak in liquid again will induce disassembly of pillars. (I-L) Optical and Electron Microscopy images of microstructures and microparticles at different stages of particle trapping and releasing. Scale bar: 3 μm . (M) Multiple microparticle trapping during microparticle colloid evaporation process. (N) Patterned salt crystal trapped in micropillar units. Pillar spacing is 2.5 μm , and cell periods are 5 μm (N1, N3), 7.5 μm (N2, N4). The concentration of salt solution: 0.5 M/L. Scale bar: (N1-2) 5 μm , (N3-4) 1 μm .

Figure 2.9 Small molecule trapping devices. (a, b) Schematic figure of gold capped micropillar array self-assembly under capillary force. (c, d) SEM images of free-standing micropillars and assembled micropillars. (e) Schematic figure of BPE molecule trapping location. (f) Distribution of electric field intensity at 750 nm. Au pillar tip diameter 136 nm. (g, h) Pre-assembled and free-standing pillars have different distributions of trapped molecules.

Figure 2.10 Optical applications of (A) Scheme of capillary force driven collapse of carbon nanofiber arrays. (B) SEM image of carbon nanopillar array. (C) SEM image of assembled carbon nanopillar array. Scale bar in (B, C) is 3 μm . (D) Percentage of TiO_2 reflection is significantly reduced when carbon nanopillars assemble.

Figure 2.11 *P. aeruginosa* adhesion on pillar array structure substrates and flat substrates. (A Upper-B) Fluorescent image and SEM image of microbial adhesion on flat surfaces. (A Lower-C) Microbial patterns on structured surfaces with epoxy pillars. Scale bar: (A) 10 μm , (B-C) 1 μm .

Figure 2.12 *X. fastidiosa* 11399 single bacterium cell on pillar arrays. SEM image of bacterium cell on (A) flat InP surface and (B) pillar arrays. (C) Fluorescent image of bacterial cells. (D) Deformation of pillar arrays by microbial cells. (D-G) Deformation-based force sensing of a single bacterial cell. (F) Spatial distribution of deformation force mapping. (G) Distribution of forces from body and polar regions within a single bacterium. (H-J) The extracellular polymeric substrate produced by bacterial cells deformed substrates. The box plots in (G) and (J) shows the statistical difference. $P=0.0001$.

Figure 3.1 Assembled and free-standing pillar images under SEM. The geometry of these two pillar patterns is different.

Figure 3.2 PMAA hydrogel microparticle coated PDMS micropillar array under SEM. The geometry of these two pillar patterns is identically the same. a. After baking in 70 °C, the hydrogel layer appeared smooth. b. Scheme figure of smooth hydrogel layer composite. c. Room temperature coated PMAA hydrogel layer. It appeared rough as the PMAA hydrogel microparticle morphology remained when linked to the PDMS surface.

Figure 4.1 Force analysis of one single micropillar.

Figure 4.2 Schematic figure of controlling factors: pattern geometry, material elasticity, and surface chemistry

Figure 4.2 Schematic figure of controlling factors: pattern geometry, material elasticity, and surface chemistry

Figure 4.3 Scheme Figure of PDMS pillar array fabrication process.

Figure 4.4 Contact angle change after plasma treatment. (a) Non-treated PDMS surface exhibited hydrophobic property. (b) PDMS after plasma treatment exhibited hydrophilic property.

Figure 4.6 SEM images of free-standing micropillar arrays and assembled micropillar arrays. (a-b) (c-d) (e-f) three kinds of geometry, (a, c, e) freestanding state; (b, d, f) assembled state.

Figure 4.4 Evaporation process force analysis. (a) Optical microscope images of the four-pillar unit assembly during water evaporation. The time interval between each image was 5 seconds. (b) Top view scheme figure of water contact area during evaporation. Left: pillar totally immersed in water. Middle: during asymmetric evaporation, pillars only partially immersed in water, and the pillar tips were exposed to air. The capillary force will induce assembly of micropillars. Left: when water fully evaporated from the pillar surface, the capillary force was reduced to 0, and elastic resistance made micropillars free-standing. (c) Side view of micropillar deformation.

Figure 4.5 Scheme figure of force analysis: one single pillar in a four-pillar unit during the evaporation process. Up: top view; middle: side view; bottom: enlarged one pillar force analysis.

Figure 4.6 Cantilever beam model for a single micropillar under surface tension.

Figure 4.7 Dot patterned micropillars under surface tension. (a) Free-standing four-pillar unit. The distance between neighboring pillars is a . Pillar diameter is d . (b) Assembled four-pillar unit. (c) Deformation illustration: when pillars assemble from free-standing state to assembled state, the maximum deformation is δ_{max} . (d) The side view schematic figure of assembled pillars within four-pillar units. h is the pillar height. x is the height of water contact area.

Figure 4.8 Two four-pillar units of dot patterned micropillars under surface tension. (a) Top-view. (b) Side-view.

Figure 4.9 The windmill pattern assembly under capillary force induced by water evaporation.

Figure 4.10 SEM images of assembled micropillar arrays. Scale bar: 10 μm

Figure 5.1 Molecules used in bacteria capture to enhance surface affinity to bacteria.

Figure 5.2 Scheme figure of different (a) Biotinylated antibody on neutravidin functionalized surface for bacterial cell capture. (b) Aptamer coated carbon nanotubes for bacteria capturing. (c) Antimicrobial peptide coated microelectrode array for bacterial capture and detection. (d) Antibody and antibiotic sandwich structure for bacteria capture and killing.

Figure 5.3 Performance of bacteria capturing with Si nanowires and flat Si surfaces. (a) Bacterial yields on three different substrates at different incubation time points: Si nanowires (black squares), flat Si coated with ConA coating (red circles), and Si nanowires with ConA coating (blue triangles). (b) Fluorescence and SEM images of Si nanowires and flat Si surfaces, both coated by ConA. Red arrows pointed out the bacteria and nanowires.

Figure 5.4 Microfluidic-based droplet trapping device. (A-C) The design of a single trap. The fluid flow direction controlled the trapping and released of droplets. When a droplet took up a trap, there would be no space to fit in more droplets thus they will move away from the side of the trap. (D) Uniform distributed droplets trapped in this device. Scale bar: 75 μm . (E-G) Droplet releasing process. When flow went along the direction as indicated by the white arrows, the droplets will be released from the traps. Scale bar: 75 μm .

Figure 5.5 A microorganism trap. (A-C) The design of microfluidic microorganism trap. (A) Optical image of the whole device. Scale bar: 10 mm. (B) Trapping channels and flow direction of this device. Scale bar: 1 mm. (C) SEM image of trapping arrays. Scale bar: 50 μm . (D) Schematic figure of trapping process. (E) Single yeast cell trapping performance. Scale bar: 10 μm .

Figure 5.6 Bacterial “jail” schematic figure and SEM images of the trapping device structure. Scale bar: 20 μm .^[1]

Figure 5.7 Nanopore arrays for microfluidic-based bacteria capture and release. (a) single bacteria capture and release. (b) fluorescent images of a single bacterium loaded and released from a nanopore.

Figure 5.8 Schematic figure of graphene-based membrane filtering system for bacteria

Figure 5.7 Schematic figure of antibody-functionalized magnetic beads for colorimetric immunomagnetic assay.

Figure 5.8 Alignment coefficient (C) vs. threshold (k) curves at different density.

Figure 5.9 Bacteria capture dynamics. Time interval: 5 sec. Scale bar: 5 μm .

Figure 5.10 Bright field and fluorescent images of SYPO 9 stained E. coli captured by the micropillar arrays. Scale bar: 25 μm .

Figure 5.11 Size distribution of captured bacteria, counted from the fluorescence image.

Figure 5.12 Localization of captured bacteria.

Figure 5.13 3D fluorescence image of micropillar array and trapped bacteria.

Figure 5.14 Bacteria capture from bacteria/mammalian cell mixture. (a) Optical image of bacteria and Hela cell mixture. Scale bar: 100 μm . (b) Schematic figure of the “dip-lift” process. (c) Bright field image of micropillar array for bacteria capturing. (d) SYTO 9 stained live E. coli, (e) PI stained dead E. coli, (f) DAPI stained Hela cell nucleus. Scale bar: 100 μm

Figure 5.15 Merged fluorescence and bright-field images of captured bacteria (stained by SYTO 9, green color) and micropillar arrays. (a) Capturing state; (b) releasing state. Scale bar: 50 μm .

Figure 5.16 The bacteria covered area changes at two states: capturing state and releasing state. Data calculated from fluorescence images.

Figure 5.17 Merged fluorescence and bright-field images of captured bacteria (stained by SYTO 9, green color) and micropillar arrays. (a) Capturing state; (b) releasing state. Scale bar: 50 μm .

Figure 5.18 The bacteria covered area changes at two states: capturing state and releasing state. Data calculated from fluorescence images.

Figure 5.19 Live/Dead staining of bacteria arrays. Green: live bacterial cells stained by SYTO 9; red: dead bacterial cells stained by PI. (a) Live *E. coli* arrays before IR irradiation. (b) Dead *E. coli* arrays after 5 min exposure to SA-17 AuNS and 800 nm IR irradiation. (c) Dead *E. coli* arrays after 10 min exposure to SA-17 AuNS and 800 nm IR irradiation. Scale bar: 100 μm . (d) Live *S. aureus* arrays before IR irradiation. (b) Dead *S. aureus* arrays after 5 min exposure to SA-17 AuNS and 800 nm IR irradiation. (c) Dead *S. aureus* arrays after 10 min exposure to SA-17 AuNS and 800 nm IR irradiation. Scale bar: 25 μm .

Figure 5.20 Microbial photo-thermal sensitivity quantification.

Figure 6.1 Force triggered drug release platforms. Alginate hydrogel platform for mechanical stimuli triggered VEGF release. (a) Mechanical stimulation of compression (2 min) and relaxation (8 min). (b) VEGF release rate under compression stimuli. Open circles: 10% strain amplitude; open squares: 25% strain amplitude; filled circles: control.

Figure 6.2 Tensile strain-triggered drug delivery device. (a) The microgel will be compressed thanks to the deformation of substrate and release nanoparticles. (b) Drug molecules will slowly diffuse into the microgel from the microparticle in a reversible manner.

Figure 6.3 Wrinkle based stretch-induced drug release system. Schematic figure of the fabrication procedure.

Figure 6.4 Crack based stretch-induced drug release system. Schematic figure of the scheme design. (a) The chemical composite of the hydrophobic coating. (b) & (c) Optical and SEM images of the drug release system. (d) Two kinds of surface coating with different contact angle and chemical composite. Upper image: pure PCL. Bottom image: PCL: PGC- C_{18} 1:1.

Figure 6.5 PDMS surface modification procedure. (a) Modification process on PDMS surface. The first step was to use O_2 plasma to activate the PDMS surface. Then use APTES to functionalize the PDMS surface. Finally, use EDC/NHS crosslinking method to link PMAA molecules to the surface of PDMS. (b) The chemical composition of the cross-linked PDMS surfaces at each modification step.

Figure 6.6 Contact angle measurement. (a) Flat PDMS without any treatment. (b) APTES functionalized flat PDMS surface. (c) PMAA coated PDMS surface.

Figure 6.7 Statistical analysis of contact angle change after surface functionalization.

Figure 6.8 FTIR spectrum of PDMS surface coated with PMAA.

Figure 6.9 FESEM image of PDMS surface coated with PMAA. Scale bar: 5 μm .

Figure 6.10 Alex 488 FluoSpheres loaded micropillar array. Scale bar: 100 μm .

Figure 6.11 SEM image of microparticle loaded micropillar array. Scale bar: 1 μm .

Figure 6.12 Quantification of FluoSphere numbers after stretching/washing cycle treatments.

Figure 6.13 SEM images of hydrogel matrix damage induced by stretching.

Figure 6.14 Scheme figure of negatively charged pillar surface. The adjacent pillars have the same surface charge thus the adhesion will be reduced.

Figure 7.1 Scheme figure of closed micropillars. Left: front view; right: top view.

Figure 7.2 Simulation result of metal film coated free-standing micropillars. Period length: $540 \times 540 \text{ nm}^2$, distance between neighboring pillars: 230 nm, radius of the pillar: 45 nm. The tops of pillars were coated with a silver film. R: Reflectance, T: Transmittance.

Figure 7.3 Schematic figure of micropillar assembly. Left: Model a, right: Model b.

Figure 7.4 Optical properties of different assembly patterns. Model a and b are shown in Figure 7.3.

Figure 7.5 Auxetic material schematic figure. Single blue arrows; stretching direction. Triple blue arrows: elongation direction.

Figure 7.6 Location of microsphere trapped by the PDMS micropillars. The particles are trapped in the confined space between assembled pillars along two directions.

Figure 7.7 Simulation and experiment results[3] of mechanics associated flat/deformed of microlens system. (a) (b) (c) XCT images of the microlens system at different deformations. (d) The relationship of deformed geometries and curvature of microlenses.

Figure 7.8 Demonstration of air pressure deformed ultra-thin PDMS micropillar array membrane.

Abbreviations

PDMS	Polydimethylsiloxane
APTES	(3-Aminopropyl)triethoxysilane
PMAA	Poly(methacrylic acid)
PS	polystyrene
E.coli	Escherichia coli
S.aureus	Staphylococcus aureus
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy

Chapter 1

Introduction

Among all the flexible materials, the structure of the material has a substantial impact on its properties. With unique properties, high aspect ratio micropillar arrays had been widely used in different applications these years. It has large surface area, lift-up discontinuous surface topography that separated the underneath substrate while maintained comparable mechanical properties. Inspired by nature gecko's foot structure, the micropillar array system has been adapted by the researchers and applied into materials design and fabrication; developing varies kinds of applications, such as paper whitening, small molecule trapping, and particle trapping. However, because of the nature of the micropillar array structure, with its small dimensions and high aspect ratio, these pillar arrays exhibited shortcomings such as easy to collapse under capillary force when wetted and dried in liquid. Moreover, the assembled hierarchical structures lacked controllable disassemble and systematic study of the assemble-disassemble process. Here in this thesis, we not only designed and fabricated a library of micropillar arrays with a variety of morphology, but also studied the assemble-disassemble process. By controlling the pillar size, i.e. diameter and height, stiffness and surface chemistry, we realized controlled assembly and disassembly of the micropillar arrays. Then we applied this highly controllable system into live bacteria extraction in a liquid environment for microbial drug sensitivity tests and controlled drug particle release under stretch.

1.1 Hypothesis/Problem Statement

Currently, the flexible micropillar arrays, its structure shown in **Figure 1.1** have been intensely studied. It has great potential in varies of applications thanks to its properties. Firstly, the feature size of each component, single micropillars, is reduced to micron scale and brought it large surface to volume ratio. It significantly enhanced its contact area when adhering to another object.

Micropillar Array

Figure 1.1 Illustration of micropillar array structure.

Secondly, thanks to its high aspect ratio structure, each building block in micropillar arrays can deform in varies ways under different external force. As shown in **Figure 1.2**, these deformations include elongation under tension along the longitudinal axis, contraction under compression, flexion under bending force, as well as shear deformation and distortion under shear stress and tortuosity^[1]. By tailoring the external force and its internal properties by adjusting the building material of micropillars, the deformation of micropillar arrays has the potential to be precisely controlled.

Thirdly, the large surface area of micropillar array has potential to be customized according to application requirements, including adjustments of surface hydrophobicity, surface charge, and chemical surface functionalization^[2]. Reactive groups can be introduced to the surface of micropillar arrays to achieve smart surfaces with responsiveness to stimuli^[3], such as pH, light, temperature, enzymes et al. And by controlling the surface properties of the micropillar arrays, smart platforms can be built to suit the expanding demands in functional devices.

Finally, the micropillar array has ordered structure, which brought it potential to be applied in the optical area.

Figure 1.2 Different kinds of micropillar deformation under mechanical force with the pillar substrates omitted. Top left to right: (a) original shape of a single micropillar and its deformation under tension (b), compression (c) and bending (d). Bottom left to right: deformation of a single micropillar under shear force (e), tortuosity (f) and combined loadings (g).

With the above properties, especially its flexibility to deform under mechanical force shown in **Figure 1.2**, the micropillar array system has been utilized in various applications, including cellular traction force sensors and actuators^[4], superhydrophobic and superhydrophilic surfaces^[5], micro- and nanoparticle capturing^[6] and molecule trapping^[7], paper whitening^[8] and dry adhesives^[9]. **Figure 1.3** gives a few examples of different applications of micropillar arrays. And in **Table 1.1** we concluded the current existing micropillar array based devices. From **Table 1.1** we can notice that most reversible assembly and disassembly of pillar arrays are in organic solvents. Controllable assembly and disassembly of micropillar arrays in water have not been successfully achieved. It limited the application in the biological field. Thus, it requires in-depth study of micropillar array assembly and design micropillar array based devices with controllable assembly and disassembly properties.

Figure 1.3 Applications of micropillar array structures. (a) microparticle trapping^[10]. (b) cell traction force sensor^[4d]. (c) insects and geckos can walk on ceiling thanks to the micropillars on their feet^[9d]. (d) A robotic gecko that can adhere to walls due to the micropillar array structure^[9e]. By adhering-peeling cycles, the robot can walk on walls. (e) the paper whitening effect from ordered arrays (transparent) to clustered and disordered arrays (whitening)^[8]. (f) single molecule trapping on gold modified pillar surfaces^[7].

Table 1.1 Current applications of micropillar array system.

Applications	Device	Micropillar Property	Driving force	Solvent	Reference
Mechanical sensor and actuator	Cell traction force sensor	deformation	External force (cell generated)	water	[4d]

Microparticle trapping	Trapping devices	Assembly and disassembly	Capillary force	Organic solvents	[10]
Molecule trapping	SERS sensor	Assembly and disassembly	Capillary force	Organic solvents	[11]
Dry adhesive	Robotic gecko	Large surface area	Van der Waals force	-	[9a, 9e]
Paper whitening	Ultrathin whitening film	Assembly and disassembly	Capillary force	Organic solvents	[8]

In this thesis, not only the mechanism of the micropillar arrays had been systemically studied, but a reversible capture and release micropillar system had been developed for applications in live bacteria extraction and stretch-induced controllable microparticle release platform.

With this development, the applications of micropillar array system had been pushed to a new level, and a bright future is awaiting to be discovered.

1.2 Objectives and Scope

The thesis addresses the systematic study of the controllable factors of the elastic micropillar array assembly and disassembly, including the geometry of the micropillar arrays, the elasticity of the building material, as well as the surface chemistry and adhesive force between neighboring pillars. And two applications of a platform of controllable micropillar arrays are developed. Firstly, it is applied to live bacteria extraction from the aquatic environment. Secondly, the micropillar array is used to hold the polymeric drug particles and controllably released by stretching.

1.3 Dissertation Overview

The thesis can be attributed to the following six chapters. And here are the details of the contents of each chapter.

Chapter 1 provided a rationale for the research. It explained why the micropillar array system is unique and excellent in its properties. And the goals and scope of this project were to understand the assembly and disassembly process of the micropillar arrays in a systematical manner. Then, two applications were demonstrated to prove the importance of this structure.

Chapter 2 reviewed the background mechanism of the physics behind micropillar array mechanical properties. One single pillar in the micropillar array system was under the influence of four forces, gravity, adhesive force, capillary force and elastic resistance due to its nature as an elastic material. And the bending, clustering and freestanding state of the pillar array system were studied. Finally, the current applications of this system were discussed.

Chapter 3 illustrated the experimental methodology of this project. The fabrication techniques to make micropillar arrays with different geometry was introduced, followed by the rationale of surface functionalization and modification on these pillar arrays. Finally, it explained how to culture the bacteria and how to realize live bacteria extraction in the aquatic environment. And the experimental set up for performing stretching and 3D printed air pressure device was covered.

Chapter 4 provided systematic study result of all the influencing factors on the mechanical behavior of the micropillar arrays. In this chapter, pattern height, a 2D organization of the pillar arrays, pillar shape and size were discussed as the first controlling factor. And pillar elasticity and material were analyzed as the second controlling factor. Finally, the influence of different surface chemistry was studied as the third controlling factor.

Chapter 5 demonstrated the micropillar array based platform and its performance in capturing live bacteria from water. A series of parameters were optimized to achieve the best performance, and this platform can be used as a drug sensitivity testing platform and drug screening diagnostic device.

Chapter 6 explained a new drug delivery system based on micropillar array system. Polymer-drug microparticles were loaded into the assembled pillar units simply by an evaporation process, and the assembled pillar arrays will disassemble to release these drug particles under stretch. In this chapter fluorescent, PS FluoSpheres were used as a demonstration of the efficiency.

Chapter 7 drew together the threads of this thesis. Moreover, perspectives and recommendation of future applications and study direction in micropillar array system were provided.

1.4 Findings and Outcomes

This research of controllable assembly and disassembly in micropillar array systems led to several novel outcomes by:

1. It established a new force analyzing model for the micropillar array and systematically studied the influence factors based on the force analyzing model. This study outcome provided an explanation for the mechanism behind this physical process.
2. A new bacteria capture device was developed. It is very easy to operate, and the bacteria were patterned into ordered clusters with uniform size for microbial drug sensitivity tests, drug screening and other applications.
3. A new approach of controlled drug release system is built up, and the controllable release was based on adding tensile stress to a thin membrane of micropillar array. This drug release system has the potential to be applied in new medication development for arthritis patients.

References:

- [1] Jiang, W.; Wang, L.; Liu, H.; Ma, H.; Tian, H.; Chen, B.; Shi, Y.; Yin, L.; Ding, Y., *RSC Adv.* **2014**, 4, 42002-42008.
- [2] (a) Chen, B.; Seidel, S.; Hori, H.; Gupta, M., *ACS Appl Mater Interfaces.* **2011**, 3, 4201-4205; (b) Liu, J.-L.; Feng, X.-Q., *Acta Mechanica Sinica.* **2012**, 28, 928-940.
- [3] Lee, E.; Yang, S., *Mrs Communications.* **2015**, 5, 97-114.
- [4] (a) Fu, J.; Wang, Y. K.; Yang, M. T.; Desai, R. A.; Yu, X.; Liu, Z.; Chen, C. S., *Nat Methods.* **2010**, 7, 733-736; (b) du Roure, O.; Saez, A.; Buguin, A.; Austin, R. H.; Chavrier, P.; Silberzan, P.; Ladoux, B., *Proc Natl Acad Sci U S A.* **2005**, 102, 2390-2395; (c) Sniadecki, N. J.; Anguelouch, A.; Yang, M. T.; Lamb, C. M.; Liu, Z.; Kirschner, S. B.; Liu, Y.; Reich, D. H.; Chen, C. S., *Proc Natl Acad Sci U S A.* **2007**, 104, 14553-

- 14558; (d) Tan, J. L.; Tien, J.; Pirone, D. M.; Gray, D. S.; Bhadriraju, K.; Chen, C. S., *Proc Natl Acad Sci U S A*. **2003**, *100*, 1484-1489.
- [5] Chen, C. M.; Yang, S., *Adv Mater*. **2014**, *26*, 1283-1288.
- [6] (a) Gruenberger, A.; Probst, C.; Binder, S.; Ziaee, R.; Eggeling, L.; Wiechert, W.; Kohlhey, D. In *Single Cell Trapping And Analysis Of Prokaryotic Production Strains In Sub- μ m Fluidic Structures*, 2011; (b) Di Carlo, D.; Wu, L. Y.; Lee, L. P., *Lab Chip*. **2006**, *6*, 1445-1449; (c) Huebner, A.; Bratton, D.; Whyte, G.; Yang, M.; Demello, A. J.; Abell, C.; Hollfelder, F., *Lab Chip*. **2009**, *9*, 692-698; (d) Kim, M. C.; Klapperich, C., *Lab Chip*. **2010**, *10*, 2464-2471; (e) Connell, J. L.; Wessel, A. K.; Parsek, M. R.; Ellington, A. D.; Whiteley, M.; Shear, J. B., *MBio*. **2010**, *1*, ; (f) Probst, C.; Grünberger, A.; Wiechert, W.; Kohlhey, D., *Micromachines*. **2013**, *4*, 357-369; (g) Drescher, K.; Shen, Y.; Bassler, B. L.; Stone, H. A., *Proc Natl Acad Sci U S A*. **2013**, *110*, 4345-4350; (h) Jo, M. C.; Liu, W.; Gu, L.; Dang, W. W.; Qin, L. D., *P Natl Acad Sci USA*. **2015**, *112*, 9364-9369; (i) Ryley, J.; Pereira-Smith, O. M., *Yeast*. **2006**, *23*, 1065-1073.
- [7] Yang, J.; Palla, M.; Bosco, F. G.; Rindzevicius, T.; Alstrom, T. S.; Schmidt, M. S.; Boisen, A.; Ju, J.; Lin, Q., *ACS Nano*. **2013**, *7*, 5350-5359.
- [8] Chandra, D.; Yang, S.; Soshinsky, A. A.; Gambogi, R. J., *ACS Appl Mater Interfaces*. **2009**, *1*, 1698-1704.
- [9] (a) Murphy, M. P.; Kim, S.; Sitti, M., *ACS Appl Mater Interfaces*. **2009**, *1*, 849-855; (b) Greiner, C.; Arzt, E.; del Campo, A., *Advanced Materials*. **2009**, *21*, 479-482; (c) Kim, S.; Sitti, M., *Applied Physics Letters*. **2006**, *89*, 261911; (d) Gorb, S. N.; Sinha, M.; Peressadko, A.; Daltorio, K. A.; Quinn, R. D., *Bioinspir Biomim*. **2007**, *2*, S117-125; (e) Li, Y.; Krahn, J.; Menon, C., *Journal of Bionic Engineering*. **2016**, *13*, 181-199; (f) Xue, L.; Kovalev, A.; Thole, F.; Rengarajan, G. T.; Steinhart, M.; Gorb, S. N., *Langmuir*. **2012**, *28*, 10781-10788; (g) Autumn, K.; Liang, Y. A.; Hsieh, S. T.; Zesch, W.; Chan, W. P.; Kenny, T. W.; Fearing, R.; Full, R. J., *Nature*. **2000**, *405*, 681-685; (h) Lee, D. Y.; Lee, D. H.; Lee, S. G.; Cho, K., *Soft Matter*. **2012**, *8*, 4905.
- [10] Segawa, H.; Yamazaki, Y.; Tachiki, S.; Yano, T.; Shibata, S., *J Colloid Interface Sci*. **2008**, *323*, 187-190.
- [11] Hu, M.; Ou, F. S.; Wu, W.; Naumov, I.; Li, X.; Bratkovsky, A. M.; Williams, R. S.; Li, Z., *J Am Chem Soc*. **2010**, *132*, 12820-12822.

Chapter 2

Literature Review

In this chapter, the current fabrication techniques, mechanism study and applications of micropillar arrays were discussed. The methods to fabricate high aspect ratio micropillar array were reviewed. The materials to build micropillar arrays came in a variety of both inorganic and organic materials. And methods of fabrication included top-down fabrication, self-assembly, in-situ seeding and growth, and replica molding. When immersed in liquid and liquid starts to evaporate, the lateral capillary meniscus interaction force would be the key driving force of pillar assembly. And finally, the existing applications of micropillar arrays were discussed, including mechanical force sensor in cellular systems, actuators, paper whitening and so on. However, among all of these findings, it lacked systematically study of the micropillar array assembly and disassembly mechanism and precise control of assembled pattern formation had not been achieved yet. Moreover, all the existing methods to control micropillar array assembly and disassembly are in organic solvents. Thus, it lacked a method for controlled assembly and disassembly of micropillar arrays in water. Last but not least, new controlled disassembly strategies for micropillar array system needed to be discovered to fulfill controlled release applications.

2.1 Building Materials and Fabrication Methods of Micropillar array

Micropillar array is a unique structure that has been found throughout the nature world. And this structure combined mechanical and surface properties, which made it possible to bend, collapse, twist into a series of the complex hierarchical structure. And various kinds of materials had been used to fabricate micropillar arrays, including inorganic and organic materials. There are many fabrication methods, including bottom-up fabrication techniques and top-down approaches^[1]. Top-down approaches, self-assembly, and replica molding methods are the most common and widely used fabrication techniques. It is an important soft lithography technique to get ordered arrays of microstructures. This method is firstly developed thirty years ago by George Whitesides^[2] and widely used by researchers.

2.1.1 Inorganic Materials

The micropillar arrays fabricated in inorganic materials were typically made of silicon, quartz, and metals. And usually fabricated in top-down approaches. It involves photolithography to define desired 2D pattern and then selectively remove excessive parts by deep reactive ion etching method or chemical etching methods. Here in **Figure 2.1** shows the fabrication process^[3]. The fabrication steps are as follows:

- (1) Patterning of photoresists on substrates. In this step, many approaches can be applied. The most widely used methods are UV light photolithography and e-beam lithography. UV light photolithography utilizes UV light sensitive photoresists. And the photoresists can be categorized as positive photoresists, such as AZ 1518, and negative photoresists, such as SU-8 from Microchem company. When exposed to UV light, positive photoresists will become soluble in photoresist developer and can be washed off the substrates. On the contrary, negative photoresists will cross-link under UV light and become insoluble.
- (2) Etching. Followed by patterning, deep Ionic Reactive Etching methods can be applied to get high-aspect-ratio structures on silicon wafers.

Figure 2.1 Schematic figure of top-down lithography method to fabricate inorganic micropillar arrays (for hard substrates such as silica).

This fabrication technique is limited by certain challenges when producing high aspect-ratio micropillar arrays. Firstly, to achieve straight vertical sidewalls for each pillar requires highly anisotropic material etching to considerable depth. Secondly, when the aspect ratio is very high, the pillars tend to collapse after the etching process^[4]. This deformation is due to the capillary force pushing neighbor pillars towards each other. And the applications of these pillars made by stiff material are limited.

Thus, to make these pillars more useful, soft materials, especially elastic materials are used to fabricate flexible micropillar arrays.

2.1.2 Organic Materials

Polymers, such as PDMS^[5], epoxy^[6], polyimide^[7], hydrogels^[8] and shape memory

polymer^[9] PU have all been used to fabricate soft micropillar arrays. Micropillar arrays fabricated by polymer materials exhibit unique properties, contributing to their performance in different applications. For example, PDMS fabricated micropillar arrays has a Poisson's ratio of 0.5. It is an elastic material and can deform under external forces. And the transparency of the material makes a real-time observation of micropillar deformation under optical microscopy possible. Shape memory polymer^[9-10], such as polyurethane, has a special shape memory property. When deformed at a temperature above glass transition temperature and reheated above T_g , the deformation could be recovered as shown in **Figure 2.2**.

Figure 2.2 Shape memory polymer fabricated micropillar arrays^[10b]. And their shape recovery under heat. (a) Deformed and recovered shape memory micropillars. (a) Shape memory micropillars (micropillar diameter: 10 μm, spacing: 30 μm, height: 30 μm.) deformed along the x -axis. (b) deformed diagonally to the x -axis. (c) Recovery of shape memory polymer micropillars. (d) The recovery process of shape memory polymer micropillars of hexagonal array pattern, diameter: 10 μm, spacing: 10 μm, height: 30 μm).

This smart material has been applied to biomedical devices, temperature responsible wettability surfaces and adhesives, color switching devices et al..

2.2 Assembly and Disassembly of Micropillar Array

The nature of micropillar array system has several properties, including low bending modulus, large surface area, and high aspect ratio lead to instability of the system^[7]. Under relatively small external force, the micropillars tend to deform compared to rigid pillars with the same shape but in a larger scale. The force driving the assembly and disassembly of micropillar array are gravity force, an adhesive force that is controlled by the surface chemistry, elastic resistance and capillary force appeared when gas/liquid/air phase meets. And we will discuss the theoretical background of the mechanical instability of micropillar array system.

2.2.1 Gravity Induced Collapse of Micropillar Array

Soft micropillar array system with high aspect ratio can easily buckle under gravity. When the height of pillars is above the critical level, they become unstable against gravity and will collapse. The critical height is calculated as^[3, 7-8, 11]

$$h_c = \left(\frac{0.49Ed^2}{\rho g} \right)^{\frac{1}{3}} \quad (2-1)$$

d is the diameter of the micropillar, E is the Young's modulus and ρ is the density of the building material.

2.2.2 Menciaus Surface between Two Pillars

When two solid pillars are partially immersed in the liquid environment, i.e. during the liquid evaporation process, the tips of the pillars are in the air while the ends of the pillars

are immersed in liquid, the side of the pillars will experience a lateral force and surface tension force. The previous study had shown that the clustering of the micropillars when a liquid evaporates from their surface results from capillary menisci interaction.

Based on the equation created by Kralchevsky et al.^[12], the capillary interaction energy, W_c of two pillars when partially immersed in liquid, can be expressed as

$$W_c = 2\pi\gamma R^2 \cos^2\theta \ln\left(\frac{x + \sqrt{x^2 - 4R^2}}{l_c}\right) \quad (2-1)$$

In which l_c is the capillary length of the liquid, R is the radius of the pillar, x is the distance from the center-to-center of the neighbor pillars. γ is the surface tension, θ is the water contact angle.

And the capillary force can be calculated as

$$F_c = -\frac{\partial W_c}{\partial x} = -\frac{\pi\gamma R^2 \cos^2\theta}{\sqrt{\left(\frac{x}{2}\right)^2 - R^2}} \quad (2-2)$$

And the torque τ_c equals^[8, 13]

$$\tau_c = \frac{\pi\gamma R^2 h \cos^2\theta}{\sqrt{\left(\frac{x}{2}\right)^2 - R^2}} \quad (2-3)$$

Figure 2.3 Schematic figure of micropillar system immersed in liquid. Pillars are partially immersed in water, and the tips are in the air. The schematic figure explained the definition of characters in Equation (2-1) (2-2) and (2-3).

2.2.3 Capillary Action

Capillary action, defined as the ability of a liquid to run through narrow spaces, is the result of adhesion and surface tension. It occurs when the adhesion of liquid molecules to the side walls is stronger than the cohesive forces within liquid, as shown in **Figure**.

2.4^[14] a. This phenomenon is very common in nature, and it has been the driving force of many nature macroscopic and microscopic phenomenon, such as water ascending narrow tubes, even against gravitational forces, or water transportation from plant roots to leaves. Ancient people observed this process and invented useful tools based on this natural phenomenon, such as Chinese paint brush. It is made of bundles of animal hair, and can hold ink between each fiber and make it capable of writing.

Modern science and technologies also learn from ancient inventions. Inspired by the Chinese paint brush, a liquid transfer device that counts on the capillary action was fabricated.

Figure 2.4 A model brush scheme figure^[14]. In a simplified brush model system, it consists of flexible hydrophilic fibers that are end-grafted to the surface. When the model brush is dipped in

water and later slowly lifted above the water surface, water fills up the region that was enclosed by the fibers. (Reprinted with permission from reference.)

2.3 Current Applications of High Aspect Ratio Micropillar Array

In the nature world, the structure of micro- and nano-scale pillars exhibit unique mechanical properties while their large surface area brought them highly adjustable flexibility to fit the need for all kinds of applications. In this part of this thesis, we will discuss the current discoveries in applications for these micropillar array structures.

It can be widely seen in nature that important functional devices, structures, even human cells have this kind of pillar array structure. For example, we can hear the sound by sound wave induced bending on bundles of cilia^[7]; and beetles and geckos can walk upside-down even on ceilings, thanks to the clustering and un-clustering reversible process of micropillar arrays on their feet. The flexibility of assembly and disassembly, high surface area to volume ratio, and mechanical flexibility to bend and twist makes them possible to be tuned and fabricated.

2.3.1 Dry Adhesives Mimicking Gecko's Foot

When people spotted that the geckos have a special ability to run up and down even with the head downwards or walk elegantly on the ceiling, this small animal inspired people to study the structure of its feet. After years of study, people finally unveiled this myth, and interdisciplinary research had been conducted to mimic this structure to achieve adhesiveness on all kinds of surfaces. As shown in **Figure 2.5**^[15], the gecko's foot consists of hierarchical structures that are the key to this unique feature. The fibril structure on its skin is named seta, and one single seta size is around 110 μm in length and 4.2 μm in diameter. Setae appear on gecko's feet in an ordered pattern, and their tips are branched into hundreds and thousands of even smaller fibril structures, known as the spatula. The size of one single spatula is around 0.2 μm both in length and diameter. Different gecko species have different size of spatula and seta, but all in similar structures.

Figure 2.5 Gecko's foot hair structure^[15b]. (images from Google Image) (a) A gecko. (b) Enlarged gecko's foot, it is fully covered with hair. (c) Gecko's foot hair under SEM. Scale bar: 50 μm . It shows rows of seae. (d) Scheme figure of gecko's foot hair. It is a hierarchical structure with thinner and shorter pillar structure on top of a single Seta.

Publications concerning the mechanical mechanism and morphology brought functionality had been rapidly increasing these years. The gecko-mimic dry adhesive systems had been developed in millimeter, micron, and even nanometer scales. A variety of different materials and structure had been developed. Here are some examples of the designed pillar structure and the building materials as shown in **Figure 2.6**.

Figure 2.6 Gecko's foot mimic dry adhesive structure. (a-b). Single layer fibrillar adhesives. c-d. hierarchical fibrillar adhesive structure. a. polyurethane micro-pillar arrays^[16]. Pillar diameter is $4.5\ \mu\text{m}$, tip diameter $9\ \mu\text{m}$ and length $20\ \mu\text{m}$. b1. Polystyrene nano-fiber dry adhesive. Pillar diameter: $330\ \text{nm}$, length: $1.5\ \mu\text{m}$. (c) Polyurethane hierarchical pillars with flat mushroom-like tops. The bottom pillars have $50\ \mu\text{m}$ base and $100\ \mu\text{m}$ top plate. And the secondary pillars have $3\ \mu\text{m}$ base and $5\ \mu\text{m}$ top plates.^[17] (d) PDMS pillar array with a diameter of $50\ \mu\text{m}$ and height of $200\ \mu\text{m}$. The secondary pillar on top of larger pillars has a diameter of $10\ \mu\text{m}$ and length of $10\ \mu\text{m}$.^[18]

2.3.2 Cell Traction Force Sensors

Cells can generate internal forces or called cellular contraction, which result from action-myosin interactions and could be transmitted to the substrate as well as generate forces on the substrate or cell traction force. Cell traction force^[19] is the mechanical force applied by cells for communicating with their neighboring ones or extracellular matrix (ECM). Cell traction force plays an important role in regulating cell cytoskeleton, structure, adhesion, migration, proliferation, differentiation, and survival. Traction force generated by cells usually comes with biological signals, which contribute to various diseases and pathological situations.

There are many forms of cell traction force sensors. Among all of them, the most important one is using a bed of flexible micropillar arrays, as shown below in **Figure 2.x**.

Figure 2.7 Cell Traction Force sensors based on micropillar arrays. ^[19a, 19d, 20] (A) Scheme figure of this design. The bending of micropillars can be transformed into shear force. (B) SEM image of micropillar array for cell traction force sensor. (C) Stained cell on micropillar cell traction force sensor. It can be clearly seen that the focal adhesion formed on the tips of micropillars. (D to F) Scanning electron micrographs. (D) Closely spaced micropillar array fabricated by PDMS molding method. (E) An individual cell lying on micropillar array based force sensor (1 μm diameter and 2 μm distance from pillar center-to-center). (F) A cell monolayer (2 μm diameter and 3 μm distance from pillar center-to-center). (F Inset) Magnified view of the area in the black square. It is the enlarged view of lamellipodia formed on micropillar array tips. (E and F) Cells spread only on the top of pillars. (G) Graphical depiction of finite-element method analysis of micropillars. The length of each pillar in the response to shear traction force (20 nN) on tips of pillars. (H) Micropillar deflection δ and force F plot data and normal spring constant K to length L relationship calculation from Euler-Bernoulli beam theory. K is a measurement of micropillar rigidity.

The cell traction force sensors are typically made of thousands of micropillars, and the local traction force generated by cell cultured on top of the pillars can be measured along all directions thanks to the flexibility of multidirectional bending of elastic pillars.

2.3.3 Microparticle and Salt Microcrystal Trapping

During the microparticle assembly and disassembly process, the space between the pillar units will be confined and relaxed in a reversible manner. It made the system a great option for micro-object capturing and releasing applications. And these applications are highly demanded in selective cell capture, chemical analysis, particle patterning and other areas. Many capturing devices have been developed based on the micropillar array system, as shown in **Figure 2.8**. The selective trapping is realized by using a suitable size of micropillars to limit the space of each assembled unit. With suitable design, smaller particles will flow freely through the assembled space while larger particles cannot enter the confined space, thus remain either on top of the pillars and washed away by liquid or rest in the open area between assembled micropillar units as shown in **Figure 2.8 (B)**. For uniform particle trapping results, the excessive particles are usually removed by external force, such as air flow or water flow. The trapping efficiency and selectivity are optimized

by alternating the inter-unit and intra-unit size. However, all the existing methods to reverse the trapping process required organic solvents thus limit the application of trapping live microorganisms.

Figure 2.9 Micropillar array for particle and salt crystal trapping^[12, 21]. (A) Scheme figure of this trapping system design. (B) SEM image of micropillar array for selective microparticle trapping. Scale bar: 2 μm. (C) 3.8 μm particle trapping. Scale bar: 4 μm. (D) The relationship between particle diameter and intracellular distance. (E-H) Particle transportation of micropillar array system. Liquid evaporation will induce pillar assembly and soak in liquid again will induce disassembly of pillars. (I-L) Optical and Electron Microscopy images of microstructures and microparticles at different stages of particle trapping and releasing. Scale bar: 3 μm. (M) Multiple microparticle trapping during microparticle colloid evaporation process. (N) Patterned salt crystal trapped in micropillar units. Pillar spacing is 2.5 μm, and cell periods are 5 μm (N1, N3), 7.5 μm (N2, N4). The concentration of salt solution: 0.5 M/L. Scale bar: (N1-2) 5 μm, (N3-4) 1 μm.

And not only single microparticle trapping can be realized, by drying of colloidal particles on the assembled micropillar array system, multi-number microparticle trapping can also be realized (**Figure 2.9 M**). It is due to convective force and the capillary force among the microparticles. The particles tend to gather around the assembled pillar units, and the driving force is faster evaporation speeds between confined spaces and open spaces. Although this process is in the aquatic environment, it is an irreversible process, trapping without releasing.

The trapping of salt microcrystals is another important application of micropillar array system. Compared to conventional way to prepare microparticles, solution-evaporation induced microcrystal formation will be environmental-friendly and the formed an ordered pattern, as shown in **Figure 2.9 N**. The concentration of mother solution is the controlling factor of salt crystal size.

2.3.4 Molecule Trapping

To making the micropillar array system more functional, the tips of the pillars can be further modified by coating a layer of gold using nanoimprint lithography. A design of molecular trap based on top-gathering pillars is demonstrated in the following **Figure 2.10**^[22]. The size of pillar array is much smaller and even further reduced to nanometer scale. When aggregated, the gold-coated nanopillars formed hot spots for surface-enhanced Raman spectroscopy (SERS) and X-ray photoelectron spectroscopy (XPS). This approach was demonstrated by trapping and identification of trans-1,2-bis(4-pyridyl)ethylene (BPE) molecules (**Figure 2.10 g, h**).^[23]

BPE molecules have active pyridyl nitrogen atoms. Thus, they can bind to metal surfaces. This molecule is a probe for SERS substrates. As it has two pyridine rings, BPE molecules can either bind to gold surfaces with one or two nitrogen elements. Thus the SERS signal will be different.

And this design of gold nano-finger molecule trapping device provided a potential platform for quantitative detection of biomolecules. Firstly, pillars with gold tips were fabricated, and the gold surface was functionalized with adhesive molecules. When target

biomolecules adhered to the gold tips, then the free-standing pillars will assemble and form a SERS hotspot for detection.

Figure 2.10 Small molecule trapping devices^[23]. (a, b) Schematic figure of gold capped micropillar array self-assembly under capillary force. (c, d) SEM images of free-standing micropillars and assembled micropillars. (e) Schematic figure of BPE molecules trapping location. (f) Distribution of electric field intensity at 750 nm. Au pillar tip diameter 136 nm. (g, h) Pre-assembled and free-standing pillars have different distributions of trapped molecules.

2.3.5 Optical Applications

Ordered geometry and small feature size brought micropillar array system potential to be applied to optical field. Here two examples are shown in **Figure 2.x**.

Figure 2.11 Optical applications^[24] of (A) Scheme of capillary force driven collapse of carbon nanofiber arrays. (B) SEM image of carbon nanopillar array. (C) SEM image of assembled carbon

nanopillar array. Scale bar in (B, C) is 3 μm . (D) Percentage of TiO_2 reflection is significantly reduced when carbon nanopillars assemble.

In **Figure 2.11 A**, it is a schematic design of collapsed nanometer scale carbon nanopillar array system. Under capillary force, the nanopillar arrays tend to collapse and assemble randomly as the aspect ratio of the pillars is beyond the stable critical limit. (**Figure 2.11 B, C**) Thus, the system will have a significant increase in ability to absorb sunlight, and when working as the back-contact for a solar cell, it will enhance the efficiency of solar cell system (**Figure 2.11 D**). Thanks to Bragg diffraction of light in between its ordered structure, the free-standing micropillar arrays had a colorful appearance. However, it appeared white when the pillars assemble and collapse in a disordered way due to random light scattering. (**Figure 2.11 E**)

2.4 Material-bio Interface

In nature state, bacteria usually form biofilms that are closely attached to surfaces. The biofilms are composed of a polymeric matrix with bacteria cells embedded in it. The biofilms are resistant to environmental damages such as heat, antibiotic attack to a certain level and making it hard to be eliminated. Thus, it is vital to understand the microbial-material interfaces and find an effective way to anti-biofilm growth.

Biofilms form spatial patterns at different scales. And the development of biofilms is closely related to the environment. The surface topographical features are important to the biofilm adhesion as it limited cellular interactions between microbial cells in the biofilm matrix.

Pillar arrays were used as an important tool to study the effect of substrate topography on microbial adhesion and biofilm development. Here in **Figure 2.12**^[25], the pillar arrays substrates with the same order of dimensions of bacterial cells were fabricated. The parameters of pillar diameter, height and spacing were varied systematically to study the bacterial growth. The result of this study indicated that the bacteria exhibited a preferential association with substrates to maximize the contact area.

Figure 2.12 *P. aeruginosa* adhesion on pillar array structure substrates and flat substrates^[25]. (A Upper-B) Fluorescent image and SEM image of microbial adhesion on flat surfaces. (A Lower-C) Microbial patterns on structured surfaces with epoxy pillars. Scale bar: (A) 10 μm , (B-C) 1 μm .

To forming a biofilm, the very first step is single bacterium adhesion to the surface^[26]. The extracellular polymeric substances, including proteins, lipids, and lipopolysaccharides, are very important to bacterial adhesion. Thus it is necessary to study the bacterial cell adhesion. An InP pillar array^[25b] was fabricated to evaluate the single cell adhesion forces and the influence of chemical modification on adhesion. As shown in **Figure 2.13**, when a single bacterial cell adhered to the surface with pillars scaled smaller than the bacterium, it will deform the pillar just like mammalian cells acted on cellular traction force sensors introduced in **2.3.2**. And the extracellular polymeric substrate produced by microbial cells were strongly attached to the pillar surface. This result suggested that bacterial cell attachments at early adhesion stage of biofilm formation are based on substrate mediated mechanism. The adhesion force at polar region of bacterial cells was stronger than the body region, and when bacterial cells were removed, the substrate remained in strong adhesion with the pillar structures. These indicated the biofilm formation was strongly associated with substrate adhesion and the cells attached to surfaces in a polar region adhesive manner.

Figure 2.13 *X. fastidiosa* 11399 single bacterium cell on pillar arrays^[25b]. SEM image of bacterium cell on (A) flat InP surface and (B) pillar arrays. (C) Fluorescent image of bacterial cells. (D) Deformation of pillar arrays by microbial cells. (D-G) Deformation-based force sensing of the single bacterial cell. (F) Spatial distribution of deformation force mapping. (G) Distribution of forces from the body and polar regions within a single bacterium. (H-J) The extracellular polymeric substrate produced by bacterial cells deformed substrates. The box plots in (G) and (J) shows the statistical difference. $P=0.0001$.

2.5 Questions to Answer Based on Literature

As discussed in the literature review above, many devices using the micropillar array system had been developed based on its flexibility to deform under external forces, predictable mechanical behavior, large surface area, and tunable surface chemistry.

However, there are still questions remain unanswered and applications yet developed.

Firstly, the deformation of micropillar array lacked systematic study for the design of ordered assembled patterns. Only a critical dimension of deformability had been studied but controllable confined space between collapsed pillars has not yet achieved. What are the mathematical model of pillar assembly and disassembly? How to build a micropillar array with homogeneous 2-pillar, 3-pillar, 4-pillar units or multiple collapsed patterns?

Secondly, the controlled disassembly of micropillar arrays in water based system had not been successfully achieved. Current devices only can realize pillar disassembly in organic solvents such as hexane, isopropanol, acetone et al. This limited its applications in biomedical fields as cells and microbial cannot survive in organic solvents. A controllable micropillar array in water needs to be developed for biological applications.

2.6 Ph.D. in Contest of Literature

In my thesis, I systematically studied the assembly and disassembly of micropillar arrays and achieved stretch-induced pH-responsive micropillar array disassembly. And this thesis mainly contributed in the following areas:

Firstly, I built up a mathematic model based on the evaporation induced micropillar assembly. And by controlling the surface chemistry, geometry, and elasticity of micropillar arrays, I successfully controlled the assembly and disassembly of the micropillar arrays and fabricated highly ordered 2-pillar, 3-pillar, 4-pillar and multi-pillar assembled units for trapping small objects such as bacteria and microparticles. The confined space between the assembled pillars was effectively controlled for size-based sorting of small objects.

Secondly, current bacteria capture devices are mainly based on filtering, immunoassay based microbial surface recognition and microfluidic devices. To achieve ordered microbial patterns for drug screening is very time-consuming and required special devices such as flow-cytometry. And the captured microbial cells cannot be released efficiently. It lacked an easy-to-operate device that can pattern bacterial cells in a short time. By designing the micropillar array geometry, I successfully realized highly-ordered microbial patterning. This device can extract live bacteria patterns from water in less than an hour. And after usage, the bacteria can be released from the substrates, making the micropillar

arrays recyclable.

And the application of this device was demonstrated as a microbial sensitivity testing platform.

Thirdly, I filled in the gap of mechanical force stimulated pH-responsive microparticle controlled release using surface modified micropillar array system. I proved the dual stimuli responsive platform was efficient in microparticle controlled release and this platform had great potential to be applied in controlled drug release area.

References:

- [1] Grinthal, A.; Kang, S. H.; Epstein, A. K.; Aizenberg, M.; Khan, M.; Aizenberg, J., *Nano Today*. **2012**, 7, 35-52.
- [2] Xia, Y. N.; Whitesides, G. M., *Annu. Rev. Mater. Sci.* **1998**, 28,
- [3] Chandra, D. Capillary Force in High Aspect-Ratio Micropillar Arrays. University of Pennsylvania, Publicly accessible Penn Dissertations, 2009.
- [4] De Volder, M.; Hart, A. J., *Angew Chem Int Ed Engl.* **2013**, 52, 2412-2425.
- [5] Sun, L.; Luo, Y.; Gao, Z.; Zhao, W.; Lin, B., *Electrophoresis*. **2015**, 36, 889-892.
- [6] Wilhelm, E.; Neumann, C.; Sachsenheimer, K.; Schmitt, T.; Lange, K.; Rapp, B. E., *Lab Chip*. **2013**, 13, 2268-2271.
- [7] Wei, Z.; Schneider, T. M.; Kim, J.; Kim, H. Y.; Aizenberg, J.; Mahadevan, L., *Proc Math Phys Eng Sci.* **2015**, 471, 20140593.
- [8] Chandra, D.; Yang, S., *Langmuir*. **2009**, 25, 10430-10434.
- [9] Zheng, Y.; Li, J.; Lee, E.; Yang, S., *RSC Adv.* **2015**, 5, 30495-30499.
- [10] (a) Lv, T.; Cheng, Z.; Zhang, E.; Kang, H.; Liu, Y.; Jiang, L., *Small*. **2016**, ; (b) Li, J.; Shim, J.; Deng, J.; Overvelde, J. T. B.; Zhu, X.; Bertoldi, K.; Yang, S., *Soft Matter*. **2012**, 8, 10322; (c) Lee, W. L.; Low, H. Y., *Sci Rep.* **2016**, 6, 23686; (d) Chen, C. M.; Yang, S., *Adv Mater.* **2014**, 26, 1283-1288.
- [11] Akbari, A.; Hill, R. J.; van de Ven, T. G. M., *SIAM Journal on Applied Mathematics*. **2016**, 76, 87-109.
- [12] Kralchevsky, P. A.; Paunov, V. N.; Ivanov, I. B.; Nagayama, K., *J Colloid Interf Sci.* **1992**, 151, 79-94.
- [13] Lee, E.; Yang, S., *Mrs Communications*. **2015**, 5, 97-114.

- [14] (a) Yamamoto, T.; Meng, Q. a.; Wang, Q.; Liu, H.; Jiang, L.; Doi, M., *NPG Asia Materials*. **2016**, *8*, e241; (b) Wang, Q.; Su, B.; Liu, H.; Jiang, L., *Adv Mater*. **2014**, *26*, 4889-4894.
- [15] (a) Arzt, E.; Gorb, S.; Spolenak, R., *Proc Natl Acad Sci U S A*. **2003**, *100*, 10603-10606; (b) Autumn, K.; Niewiarowski, P. H.; Puthoff, J. B., *Annual Review of Ecology, Evolution, and Systematics*. **2014**, *45*, 445-470.
- [16] Lee, D. Y.; Lee, D. H.; Lee, S. G.; Cho, K., *Soft Matter*. **2012**, *8*, 4905.
- [17] Reddy, S.; Arzt, E.; del Campo, A., *Advanced Materials*. **2007**, *19*, 3833-3837.
- [18] Greiner, C.; Arzt, E.; del Campo, A., *Advanced Materials*. **2009**, *21*, 479-482.
- [19] (a) du Roure, O.; Saez, A.; Buguin, A.; Austin, R. H.; Chavrier, P.; Silberzan, P.; Ladoux, B., *Proc Natl Acad Sci U S A*. **2005**, *102*, 2390-2395; (b) Fu, J.; Wang, Y. K.; Yang, M. T.; Desai, R. A.; Yu, X.; Liu, Z.; Chen, C. S., *Nat Methods*. **2010**, *7*, 733-736; (c) Sniadecki, N. J.; Anguelouch, A.; Yang, M. T.; Lamb, C. M.; Liu, Z.; Kirschner, S. B.; Liu, Y.; Reich, D. H.; Chen, C. S., *Proc Natl Acad Sci U S A*. **2007**, *104*, 14553-14558; (d) Tan, J. L.; Tien, J.; Pirone, D. M.; Gray, D. S.; Bhadriraju, K.; Chen, C. S., *Proc Natl Acad Sci U S A*. **2003**, *100*, 1484-1489.
- [20] (a) Sun, Z.; Wang, D.; Xiang, J., *ACS Nano*. **2014**, *8*, 11261-11267; (b) Li, Z.; Song, J.; Mantini, G.; Lu, M. Y.; Fang, H.; Falconi, C.; Chen, L. J.; Wang, Z. L., *Nano Lett*. **2009**, *9*, 3575-3580.
- [21] (a) Lao, Z.; Hu, Y.; Zhang, C.; Yang, L.; Li, J.; Chu, J.; Wu, D., *ACS Nano*. **2015**, *9*, 12060-12069; (b) Segawa, H.; Yamazaki, Y.; Tachiki, S.; Yano, T.; Shibata, S., *J Colloid Interface Sci*. **2008**, *323*, 187-190; (c) Wu, D.; Wu, S. Z.; Zhao, S.; Yao, J.; Wang, J. N.; Chen, Q. D.; Sun, H. B., *Small*. **2013**, *9*, 760-767.
- [22] Hu, M.; Ou, F. S.; Wu, W.; Naumov, I.; Li, X.; Bratkovsky, A. M.; Williams, R. S.; Li, Z., *J Am Chem Soc*. **2010**, *132*, 12820-12822.
- [23] Kim, A.; Ou, F. S.; Ohlberg, D. A.; Hu, M.; Williams, R. S.; Li, Z., *J Am Chem Soc*. **2011**, *133*, 8234-8239.
- [24] (a) Chandra, D.; Yang, S.; Soshinsky, A. A.; Gambogi, R. J., *ACS Appl Mater Interfaces*. **2009**, *1*, 1698-1704; (b) Pint, C. L.; Takei, K.; Kapadia, R.; Zheng, M.; Ford, A. C.; Zhang, J.; Jamshidi, A.; Bardhan, R.; Urban, J. J.; Wu, M.; Ager, J. W.; Oye, M. M.; Javey, A., *Advanced Energy Materials*. **2011**, *1*, 1040-1045.

- [25] (a) Hochbaum, A. I.; Aizenberg, J., *Nano Lett.* **2010**, *10*, 3717-3721; (b) Sahoo, P. K.; Janissen, R.; Monteiro, M. P.; Cavalli, A.; Murillo, D. M.; Merfa, M. V.; Cesar, C. L.; Carvalho, H. F.; de Souza, A. A.; Bakkers, E. P.; Cotta, M. A., *Nano Lett.* **2016**, *16*, 4656-4664.
- [26] (a) Jeong, H. E.; Kim, I.; Karam, P.; Choi, H. J.; Yang, P., *Nano Lett.* **2013**, *13*, 2864-2869; (b) Sakimoto, K. K.; Liu, C.; Lim, J.; Yang, P., *Nano Lett.* **2014**, *14*, 5471-5476.

Chapter 3

Experimental Methodology

This chapter introduces the design, fabrication and characterization method of micropillar arrays. The micropillar arrays were fabricated by replica molding method. Firstly, the silicon masters were fabricated by photolithography and deep reactive ion etching method. Then, the precursor of the polymer was poured into the mold and cured under certain condition. Finally, a free-standing polymer micropillar array was surface functionalized by silane treatment or grafting a layer of responsive hydrogel. The performance and morphology were characterized by SEM and optical microscopies. Last but not least the bacteria culture and staining methods were illustrated.

3.1 Rationale for selection

Polydimethylsiloxane (PDMS) is an important elastomeric polymer for various applications. Thanks to its nature of excellent mechanical properties, easy modeling nature, chemically stable in the aquatic environment and biocompatibility^[1], it is a great material for biomedical applications. Also, the surface of PDMS can be easily functionalized using silane and cross-linked to other functional molecules, polymer or small molecules. This process can be designed accordingly.

In chapter 2 we had introduced that micropillar arrays fabricated by the flexible material can bend under capillary force during the evaporation process, thus assemble into a highly ordered patterns and this process is easy to control. Fabrication and mechanical mechanism analysis of micropillar arrays had been widely studied, and different model systems had been built. However, it lacked a systematic study of pillar assembly and disassembly process.

Figure 3.1 Assembled and free-standing pillar images under SEM. The geometry of these two pillar patterns is different.

There are three approaches to consider to control the assembly and disassembly of the micropillar arrays. As introduced in Chapter 2, there are four forces act as the control factor, capillary force, adhesive force, gravity and elastic resistance. Capillary force is dependent on the surface energy of PDMS and the adhesive force is influenced by the surface chemistry of pillar surface. Gravity and elastic resistance of PDMS pillars are highly influenced by the geometry, i.e. height, shape and size of the pillars. And to adhere to each

other, the neighbor pillars need to be close enough. And when we try to disassemble the pillar arrays, firstly we have to make sure the adhesion force between neighbor pillars is not too large. Secondly, we need to add force to increase the space of neighbor pillars thus separate them.

To achieve controllable assembly and disassembly of micropillar arrays, we will introduce two methods, firstly, controlling the geometry with replica molding method, and the silica replica is fabricated by photolithography and deep reactive ion etching method. Secondly, we controlled the surface of PDMS pillar arrays with plasma treatment, silane modification and hydrogel hybridization on PDMS surface. After introducing liquid (water, organic solvent) to the pillar surface and evaporate the liquid, the pillars will automatically assemble into arrays. And we used stretching force and adjusted pH values to separate the assembled pillars to induce disassembly.

Micropillars have been widely used in device design and fabrications. There are many materials had been applied to fabricate micropillar arrays, such as epoxy^[2], hydrogel^[3], and PDMS^[4].

In this study, we chose PDMS as a model system to study the impact of the geometry and surface chemistry of PDMS pillar arrays on their ability to trap items.

3.2 Preparation of PDMS Micropillar Arrays with Different Surface Chemistry

To achieve the different performance of micropillar array assembly and disassembly, we functionalized the PDMS surface with different methods to get different surface properties. And in this chapter, we will introduce the methods in details.

3.2.1 Materials

AZ-1518 photoresist and AZ 300 MIF developer were purchased from AZ Electronic Materials.

PDMS elastomer Sylgard 184 base and curing agent were purchased from Dow Corning Corporation.

(3-Aminopropyl)Triethoxysilane (APTES) was purchased from Sigma-Aldrich and used without further purification.

Poly(methacrylic acid) (Molecular Weight: ~100,000) was purchased from Polysciences Inc.

N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and an N-Hydroxysulfosuccinimide sodium salt (NHS) were purchased from Sigma-Aldrich.

3.2.2 Lithography Process

Bare Si wafers were used as substrates for spin coating a thin layer of AZ-1518 films for lithography. Before spin coating the photoresist, they were freshly cleaned by oxygen plasma at 70% O₂ 300 W for 10 minutes and baked at 200 °C for 10 min to remove moisture. Then after cooled to room temperature, around 5 mL AZ-1518 photoresist (choose different type accordingly) was deposited on the Si wafer. A thin layer of AZ-1518 film was spin-coated at 3000 rpm for 60 s on the substrate and prebaked at 95 °C on a hotplate for 1-3 min. The AZ-1518 coated wafer went through photolithography through a mask aligner (SUSS MJB4 Mask Aligner, Garching, Germany) with a chrome mask with different patterns carefully aligned on top of it and exposed to UV light. Then the substrate was developed in the MIF 300 developer for 15-60 s and immersed in DI water for 5-10 s. After that, the substrate was thoroughly washed with DI water. Finally, the substrate was blow-dried by N₂ flow and stored avoiding light.

The excessive photoresist was removed by O₂ plasma treatment, and the wafer was etched by deep Reactive Ion Etching method to reach the designed height. After dRIE etching, the photoresist was removed by acetone bath.

3.2.3 Mold fabrication method

The Si master (fabricated by RIE) were treated by Trichloro(1H,1H,2H,2H-perfluorooctyl)silane for 1 h before replication. The Si master was firstly thoroughly cleaned by O₂ plasma cleaner at 300 W, 50% O₂ atmosphere for 300 s. Then 10 µL Trichloro(1H,1H,2H,2H-perfluorooctyl)silane was dropped onto a glass slide, then

together with the Si master, they were placed in a vacuum desiccator and vacuumed for 1 h. Subsequently, the master patterns were replicated to a polydimethylsiloxane (PDMS, Dow Corning Sylgard 184, 10:1 Wt/Wt precursor/crosslinker) mold. The mold was degassed under vacuum for 2 hours to remove the air bubbles and crosslinked at 45 °C overnight or 60 °C for 4 hours.

The PDMS membrane was carefully removed from the master by tweezers, and the thickness of the membrane was controlled by the amount of PDMS per square cm.

3.2.4 Surface Functionalization and Treatment of PDMS Micropillar Arrays.

The surface chemistry plays an important role in controlling the assembly and disassembly process of PDMS micropillar arrays. For different surface chemistry generation, the PDMS surface was treated by three different methods: O₂ plasma cleaning, hydrogel grafting and without treatment (remained hydrophobic).

The none-treated PDMS micropillar arrays were used directly after peeling off from the silica mold.

And O₂ plasma treated PDMS micropillar array were got by the following procedures. After crosslinking as introduced in 3.2.1, the PDMS membrane was carefully removed from the mold and placed in a glass petri dish, followed by O₂ plasma treatment at 300 W, 50% O₂ atmosphere for 300 s. And used directly after plasma treatment to avoid loss of hydrophilicity.

The hydrogel coated PDMS micropillar array was fabricated by carbodiimide crosslinking chemistry. Firstly the PDMS micropillar array was pre-treated by O₂ plasma treatment at 300 W, 50% O₂ atmosphere for 300 s to generate a layer of silanol group (SiOH). Then the activated PDMS was soaked in APTES solution (3%~5% APTES, 5% water, and 90% Ethanol) for 15 min then thoroughly washed with fresh water and dried under N₂ flow. Then the PDMS was placed in the 70 °C oven and baked for 2 hours. After that, the PDMS was cooled to room temperature. The APTES modified PDMS surface was prepared and ready for hydrogel crosslinking.

Before use, 500 mg of poly(methacrylic acid) was grounded in a mortar and then soaked in 10 mL of deionized water and sonicated until fully dissolved. Then the solution was

filtered through grade 413 filter paper (VWR). The resultant solution was then freeze-dried and redissolved in deionized water to form 2 g/L solution. The pH of 9 mL PMAA solution was adjusted to 5.0 by adding 1 mL 3 M sodium acetic buffer. And 80 mg NHS and 110 mg EDC were added into the solution to activate carboxylic acid groups. The mixture was shaken for 20 min before used to soak the PDMS surface. The PDMS micropillar array was fully immersed in the activated PMAA solution for 2 hours and then washed with fresh water.

3.3 Characterization Methods

3.3.1 Scanning Electronic Microscopy Measurement

The SEM images of the samples were taken by Zeiss SUPRA 55 SEM system. The PDMS micropillar arrays without surface coating were directly sputter coated a layer of Pt and imaged at 5 kV or 10 kV. And the samples with hydrogel coatings were prepared as follows:

1. Room dried sample

The sample was placed in a petri dish and stored at room temperature and humidity around 25 °C overnight and then sputter coated with a layer of Pt at 20 mA for 60 sec before imaging.

2. Oven dried sample

The sample was placed in a petri dish and placed in a 70 °C oven for 2 hours and cooled to room temperature then sputter coated with a layer of Pt at 20 mA for 60 sec before imaging.

3. Freeze dried sample

The sample was placed in a petri dish with water and placed in a -70 °C fridge overnight to freeze and placed in a freeze dryer for 24 hours. After completely dried, the sample was taken out and sputter coated with a layer of Pt at 20 mA for 60 sec prior to imaging.

Figure 3.1 shows the result of hydrogel coated PDMS micropillar arrays. The samples were acquired by different procedures to change the morphology. Sample 1 (**Figure 3.1 a**) was dried in 70 °C oven for 2 hours. Sample 2 (**Figure 3.1 c**) was dried at room temperature. It can be spotted from the SEM images that the morphologies of hydrogel coatings were

different under different experiment conditions. The PMAA hydrogel formed particle morphology at slower drying rate at room temperature, while densely packed into a smooth layer on the PDMS pillars at faster drying rate in the oven. This method was used to control the roughness of the PDMS surface.

Figure 3.1 PMAA hydrogel microparticle coated PDMS micropillar array under SEM. The geometry of these two pillar patterns is identically the same. a. After baking in 70 °C, the hydrogel layer appeared smooth. b. Scheme figure of smooth hydrogel layer composite. c. Room temperature coated PMAA hydrogel layer. It appeared rough as the PMAA hydrogel microparticle morphology remained when linked to the PDMS surface.

3.3.2 Water Contact Angle Measurement

The water contact angles on flat PDMS were measured using DatePhysics OCA 15Pro contact angle measurement instrument based on the electronically controlled syringe dispensing a liquid onto the substrate surface. And by recording the substrate and liquid

drop with a digital video camera, the contact angle information was got and analyzed by the SCA software.

For each sample, 6 measurements of contact angles were taken at different spots. Water drop were 6 μL in volume.

3.3.3 3D Imaging of Assembled Micropillar Arrays

The 3D structures of micropillar arrays in hydrated environment were imaged in the following procedure. 1 mL 2% Fluosphere (Invitrogen, diameter of 100 nm) solution was freeze-dried and 10 mg Fluospheres powder were mixed with PDMS (10:1 Wt/Wt precursor/crosslinker). PDMS micropillar arrays were fabricated replica molding method using the Fluosphere mixed PDMS. 3D images were acquired by Leica SP8 confocal microscopy.

3.4 Bacteria Culture and Imaging

The bacteria species used were *Escherichia coli* (E. coli) (ATCC 8739) and *Staphylococcus aureus* (S. aureus) (ATCC 6538). Prior to experiments, the bacteria were grown overnight in Luria-Bertani broth medium (LB, Sigma-Aldrich) and 1 μL bacteria mixture was re-suspended in 10 mL fresh LB broth medium after overnight incubation. Then the bacteria was harvested at the exponential growth phase S2 via centrifugation. The supernatant was then discarded, and the cell pellet was resuspended in normal saline (NS). The bacteria concentration in NS was monitored photometrically by measuring the optical density (OD) at a wavelength of 600 nm.

Before performing the bacteria capture experiments, the OD600 values^[5] of bacteria stock solutions were re-adjusted to 0.1, which corresponded to the concentrations, obtained based on colony counting method, 10^8 CFU/mL for E. coli and 4×10^8 CFU/mL for S. aureus, respectively.

To observe the localization of captured bacteria, the micropillar array with captured bacteria was first rinsed with fresh NS to remove excessive bacteria attached on the micropillar surface but not effectively captured. Then the micropillar array was incubated

with 100× diluted SYTO 9 dye (Invitrogen) for 30 min at room temperature, and rinsed with 1 mL of NS for three times before microscopy imaging. And the bacteria capturing process was observed by fluorescence microscopy (Leica DMI 6100) in a time-lapse way.

3.4.1 Morphology Study of bacteria

The morphology changes of bacteria captured substrate were examined by a field emission scanning electron microscopy (FESEM). After capture, the substrate with bacteria was treated with 2% glutaraldehyde (Sigma-Aldrich) fixation for 2 h at room temperature and gradient dehydration by a series of ethanol solutions (50%, 70%, 90%, and 100%) for 10 min with each step. Then, the micropillar array was kept at room temperature and air dried to maintain the morphologies of the bacteria captured. After complete drying, the substrate was sputter coated with Pt and imaged with FESEM (Zeiss, SUPRA 55).

3.4.2 LIVE/DEAD Bacterial Assay

LIVE/DEAD bacterial assay was performed to examine the viability of bacteria captured by the micropillar array. After capture, the substrate with bacteria was firstly gently rinsed by 1 mL fresh NS for three times to remove excessive bacteria attached to the pillars but not effectively captured. Then the micropillar array was stained with 0.5 mL of LIVE/DEAD dye solution containing 1 μ M SYTO 9 and 5 μ M propidium iodide (Invitrogen) for 15 min at room temperature. The green colored SYTO 9 dye enters both alive and membrane-collapsed bacterial cells, while the red colored propidium iodide dye can only enter membrane-damaged cells. The substrate was then imaged using a confocal microscopy (TCS SP8, Leica) or fluorescent microscopy (DMI6100, Leica).

References:

- [1] Sun, L.; Luo, Y.; Gao, Z.; Zhao, W.; Lin, B., *Electrophoresis*. **2015**, *36*, 889-892.

- [2] (a) Chen, C. M.; Yang, S., *Adv Mater.* **2014**, 26, 1283-1288; (b) Zheng, Y.; Li, J.; Lee, E.; Yang, S., *RSC Adv.* **2015**, 5, 30495-30499; (c) Lee, W. L.; Low, H. Y., *Sci Rep.* **2016**, 6, 23686; (d) Lv, T.; Cheng, Z.; Zhang, E.; Kang, H.; Liu, Y.; Jiang, L., *Small.* **2016**,
- [3] (a) Chandra, D.; Yang, S., *Langmuir.* **2009**, 25, 10430-10434; (b) Lee, E.; Yang, S., *Mrs Communications.* **2015**, 5, 97-114.
- [4] Bodas, D.; Khan-Malek, C., *Microelectronic Engineering.* **2006**, 83, 1277-1279.
- [5] Li, Y. Q.; Zhu, B.; Li, Y.; Leow, W. R.; Goh, R.; Ma, B.; Fong, E.; Tang, M.; Chen, X., *Angew Chem Int Ed Engl.* **2014**, 53, 5837-5841.

Chapter 4

Systematic Study of Micropillar Array Assembly and Disassembly

In the literature review, we have observed in previous publications that geometry of micropillar arrays will influence the assembled pattern. For a typical micropillar array, the driving force of lateral collapse is a capillary force and the force that can keep the assembled state is the adhesive force from the surface chemistry of the pillar top while the elastic resistance from the nature of building material tends to maintain the micropillars in a free-standing state. Here in this chapter, we present experimental results and theoretical mechanism analysis to understand further how these influencing factors controlled the assembly and disassembly of the micropillar array.

4.1 Introduction

The nature of micropillar arrays brought unique characters to this structure: small feature size, high aspect ratio, and large surface area. The micropillar array can be utilized in many areas, such as cell traction force sensor^[1], actuators^[2], gecko's foot inspired dry adhesives^[3] as well as photonic applications, i.e. paper whitening^[4]. Thanks to their susceptibility to deform under adhesive and capillary forces, micropillar arrays, with their self-assembled hierarchical structures, is making a great contribution to smart device design and fabrication.

PDMS, a most widely used silicon-based organic polymer material, has many unique properties including optical transparency, biocompatibility, and excellent mechanical properties. As a viscoelastic material, PDMS can flow to cover a surface and mold to any topography on the surface, and after crosslinking under heat, it will act as an elastic solid-like rubber and exhibits stretchability. The Poisson's ratio of PDMS is 0.5.

Crosslinked PDMS will present an hydrophobic external surface, but it can be altered by plasma oxidation, adding reactive silanol groups to its surface. After plasma treatment, the PDMS surface will be hydrophilic. And the oxidized surface can also be further functionalized by silanes and modified by crosslinking the reactive end groups with other polymers. It is a very flexible process providing possibilities of variable surface chemistry alteration on PDMS surface.

And crosslinked PDMS solids cannot be swelled by polar solvents, such as alcohols, glycerol, and water. However, it will swell to a different extent when soaked in organic solvents, such as relatively low swelling solvent, acetone, isopropanol and high swelling solvent, chloroform, THF. The swelling property of PDMS can be used to control its mechanical properties.

An elastic pillar in a liquid environment will bear different forces as shown in **Figure 4.1**. Including gravity (G), adhesiveness on the pillar side (F_A) and capillary force (T) when partially immersed in liquids. Also, the elasticity of the material gives it an internal elastic resistance (F_E) under an external force that makes it resistant to deformation to a certain extent and remains its original shape.

Figure 4.1 Scheme Figure of Force Analysis: a pillar in a liquid environment will bear three forces: gravity (G), adhesive forces when the distance of neighboring pillars reduced to a critical level (F_A), capillary force (T). Moreover, due to the nature of the elastic material, the pillars tend to obtain its original shape thanks to its elastic resistance (F_E).

As discussed in Chapter 2, the low bending modulus and large surface area of micropillar array result in great features but also lead to increased instability and deformability. The force on the surface dominates their mechanical properties. And we used three methods to control the mechanical properties as shown in **Figure 4.2**: Firstly, by controlling the geometry of the pillars, it can monitor the pillar number in each assembled unit; secondly, by controlling the elasticity, it can control the deformation of the micropillar array; through surface functionalization, we can make these pillars have variable surface stickiness. And by controlling surface liquid evaporation, we brought capillary force as a driving force of lateral collapse. In this chapter, these approaches will be discussed in details.

Figure 4.2 Schematic figure of controlling factors: pattern geometry, material elasticity, and surface chemistry (Scale bar: 20 μm).

And in this chapter, we introduced a series of mathematic models built to predict the assembly patterns of the micropillar arrays under capillary force. The capillary force was calculated from the mathematic model and matched with experiment results.

By these controlling methods, we successfully controlled the assembly patterns of micropillar arrays and formed 2-pillar, 4-pillar, multi-pillar units. The mechanism was explained by pillar deformation calculation and fluid physics models.

4.2 Materials and Methods

The model system of micropillar arrays was fabricated replica molding methods as shown in **Figure 4.3**. Firstly a series of patterns was designed, and a photomask was made for

photolithography. Then the Si master was fabricated by positive photoresist patterning followed by deep reactive ion etching method. Finally, the PDMS micropillar array was made by replica molding method. The assembly and disassembly of PDMS micropillar array were analyzed.

Figure 4.3 Scheme Figure of PDMS pillar array fabrication process.

4.2.1 Surface Treatment of PDMS Micropillar Arrays

The surface of non-treated PDMS micropillar arrays fabricated by replica molding method and used without any further treatments was hydrophobic. To get hydrophilic PDMS micropillar arrays, they went through plasma treatment at power 300 W, 50% O_2 atmosphere for 300 s. And used directly after plasma treatment to avoid loss of hydrophilicity.

Figure 4.4 Contact angle change after plasma treatment. (a) Non-treated PDMS surface exhibited hydrophobic property. (b) PDMS after plasma treatment exhibited hydrophilic property.

4.2.2 Evaporation Induced Micropillar Array Assembly

To generate assembled micropillar arrays, we used oxygen plasma treatment as described in 4.2.1. And then we dropped 100 μL of DI water on the pillar surface. After evaporation, the assembled pillar units were formed.

Figure 4.6 SEM images of free-standing micropillar arrays and assembled micropillar arrays. (a-b) (c-d) (e-f) three kinds of geometry, (a, c, e) freestanding state; (b, d, f) assembled state. (Scale bar: 10 μm)

In Figure 4.6, we compared the free-standing micropillar arrays and the assembled micropillar arrays.

4.3 Mathematic model

To further control the assembly and disassembly process, we built up a model system to predict the deformation of micropillar arrays. And a series of in-situ water evaporation experiment were conducted to provide solid proof for the mathematic model.

4.3.1 Evaporation Process Analysis

When the water evaporated from micropillar array surface, the dynamic process of capillary force induced assembly was imaged as shown in **Figure 4.5 (a)**. The micropillar array in From left to right, top-view of the micropillar array deformation was imaged. In the beginning, the micropillar arrays were fully immersed in water, and the surface tension on pillar surface at all directions was the same. And as water evaporated, parts of the micropillar were exposed in air and within a four-pillar unit, the inner side of the pillars was immersed in water, but outer parts were in the air. Thus the balance of surface tension on a single pillar no longer existed. The direction of surface tension was towards the center of the liquid immersed parts. Pillar started to deform under this external force as shown in **Figure 4.5 (a) Middle**. And assembled four-pillar units were formed. Finally, when the water fully evaporated, the surface tension generated by water no longer existed. The elastic resistance took over, and the micropillars would go back to the free-standing state as shown in **Figure 4.5 (a) Left**.

Here in this process, when the adhesiveness of pillar surface was strong, such as the pillar tips of plasma treated PDMS micropillar arrays, the deformed 4-pillar unit tips will adhere to each other. Moreover, if the elastic resistance cannot overcome the adhesive force, then

this assembly process cannot be reversed, and the pillar tips cannot be separated. In this condition, a permanently assembled pattern will appear.

Figure 4.4 Evaporation process force analysis. (a) Optical microscope images of the four-pillar unit assembly during water evaporation. The time interval between each image was 5 seconds. (b) Top view scheme figure of water contact area during evaporation. Left: pillar totally immersed in water. Middle: during asymmetric evaporation, pillars only partially immersed in water, and the pillar tips were exposed to air. The capillary force will induce assembly of micropillars. Left: when water fully evaporated from the pillar surface, the capillary force was reduced to 0, and elastic resistance made micropillars free-standing. (c) Side view of micropillar deformation.

And the deformation of micropillar array was shown in **Figure 4.4 (b)**. The pillar tips assembled towards the center until closely packed together, and disassembled when water fully evaporated. The calculation of forces exerted on the pillar will be discussed as follows.

4.3.2 Capillary force calculation

During the evaporation process, the deformation of micropillars was a dynamic process. And we took a moment at partial immersion state as an example to calculate the forces. The scheme figure of top and side view on one pillar in a four-pillar unit is shown in **Figure 4.3.3**

Top View:

Side View:

Force on single pillar

Figure 4.5 Scheme figure of force analysis: one single pillar in a four-pillar unit during the evaporation process. Up: top view; middle: side view; bottom: enlarged one pillar force analysis.

There are two assumptions must be met for the following equations to apply;

1. The water depth is too infinitesimal to be accounted for. Thus, the hydrostatic force can be neglected, and the liquid surface tension dominates.
2. We assume that the force equation of the surface tension can be applied to describe such process.

And the surface tension σ is the magnitude F_C of the capillary force exerted parallel to the surface of a liquid divided by the length l of the line as shown in **Figure 4.5 (c)** over which the force acts. Equation (1) describes F_C :

$$F_C = \sigma l = \int^{\sigma(\theta, x, y, z)} r d\alpha \quad (1)$$

And in equation (1), r is the radius of single pillar, θ is the contact angle, the cross section of a pillar is a circle and the water contact area is shown in **Figure 4.4** as a red arc, in which l is the length of arc. And the surface tension σ is defined as

$$\sigma = \left(\frac{\partial G}{\partial A} \right)_{T, P} \quad (2)$$

where G is the Gibbs free energy of water at the experiment temperature and pressure. A is the water contact area. This indicates that the surface tension σ is correlated with Gibbs free energy and

$$F_C = \int_{\Omega} \sigma d\Omega = \int_{\alpha_1}^{\alpha_2} \sigma r d\alpha \quad (3)$$

$$A = h\Omega = h \int_{\alpha_1}^{\alpha_2} r d\alpha \quad (4)$$

where h is the height of water level, the pillar cross section is a circle and the water contact part is an arc of the circle, Ω is the arc length.

4.3.3 Elastic resistance calculation

During the evaporation process, surface tension is the driving force of pillar deformation, thus, the elastic resistance F_e equals F_c . Based on the above equations, we can calculate the maximum deformation δ_{max} with the following equation.

Figure 4.6 Cantilever beam model for a single micropillar under surface tension.

The maximum deformation of one single micropillar δ_{max} under surface tension during the evaporation process can be considered as a model of cantilever beam under a concentrated point load.

$$\delta_{max} = \frac{\cos \theta F_c h^2}{6EI} (3h - x) \quad (5)$$

$F_c \cos \theta$ is the horizontal component of capillary force. d is the pillar diameter. And the elastic resistance F_e equals with $F_c \cos \theta$, we can calculate F_e :

$$F_e = F_c \cos \theta = \frac{6\delta_{max}EI}{h^2(3h - x)} \quad (6)$$

In Equation (5) and (6), E is the Young's modulus of PDMS, I is the moment of Inertia, h is the pillar height, x is the water contact area height.

To study the influence of pillar geometry on assembly and disassembly, we built a series of patterns with different pillar shapes, neighbor pillar distances and neighbor unit distances. And the patterns can be further categorized into two groups: dot patterns and windmill patterns. Here we will analyze the dot patterns first. The designs are shown in **Figure 4.7**.

Figure 4.7 Dot patterned micropillars under surface tension. (a) Free-standing four-pillar unit. The distance between neighboring pillars is a . Pillar diameter is d . (b) Assembled four-pillar unit. (c) Deformation illustration: when pillars assemble from free-standing state to assembled state, the maximum deformation is δ_{max} . (d) The side-view the schematic figure of assembled pillars within four-pillar units. h is the pillar height. x is the height of water contact area.

In **Figure 4.7 (a)**, the distance between neighboring pillars from center to center equals $(a + d)$. And the maximum deformation within a four-pillar unit equals

$$\delta_{max} = \sqrt{2(a + d)^2} \quad (7)$$

Thus, to return to free-standing state, the critical elastic resistance must be larger than the maximum capillary force at the largest deformation state. And the equation is

$$F_{emax} = \frac{6\sqrt{2(a + d)^2}EI}{h^2(3h - x)} \quad (8)$$

When F_c is bigger than F_{emax} then the capillary force is large enough to deform the micropillars to assembly state.

Homogeneous dot-pattern micropillar arrays with same inter-unit and intra-unit distance had the equal possibility to deform towards each direction to form assembled units. Thus the assembled patterns tend to be randomly distributed. The intra-unit distance should be increased for highly ordered assembled units. The mechanism is analyzed in the following equations.

It can be clearly observed in **Figure 4.8** that to form intra-unit assembly, the deformation distance between neighboring units equals

$$\delta'_{max} = \frac{1}{2}\sqrt{(a^2 + b^2)} \quad (9)$$

If δ'_{max} equals δ_{max} , which means the required capillary force of inter-unit bending equals intra-unit bending, and the critical b_{crit} size equals

$$b_{crit} = \sqrt{7a^2 + 16ad + 8d^2} \quad (10)$$

And when b is larger than b_{crit} , then the micropillars will form intra-unit assembly. And oppositely, when b is smaller than b_{crit} , then the micropillars will form an inter-unit assembly, respectively.

Figure 4.8 Two four-pillar units of dot patterned micropillars under surface tension. (a) Top-view. (b) Side-view.

As for the windmill patterns, the pillars have two directions for bending. And during the windmill pattern assembly, the system can be considered as a beam deflection under concentrated load, thus

$$\delta_{max} = \frac{F_c \cos \theta x^2}{6EI} (3h - x) \quad (11)$$

And according to **Figure 4.9**, the intra-unit maximum deformation δ_{max}

$$\delta_{max} = \sqrt{\left(\frac{a}{2} + \frac{l}{4} - \frac{w}{4}\right)^2 + \left(\frac{a}{2} + \frac{w}{4} - \frac{l}{4}\right)^2} \quad (12)$$

And suppose the distance between two neighboring units is b , then δ'_{max}

$$\delta'_{max} = \sqrt{\left(\frac{b}{2} + \frac{l}{4} - \frac{w}{4}\right)^2 + \left(\frac{b}{2} + \frac{w}{4} - \frac{l}{4}\right)^2} \quad (13)$$

Thus, critical b equals a , when b is larger than a then the pillar assembly will favor an

intra-unit assembly.

Figure 4.9 The windmill pattern assembly under capillary force induced by water evaporation.

4.4 Experiment Result

From the above calculation, the relationship of micropillar morphology can significantly influence the assembled patterns. Thus, a series of PDMS micropillar arrays with gradual changes in pillar diameter, pillar height, intra- and inter-unit distance were fabricated, and the assembly was induced by evaporating water on the micropillar surface.

And from the SEM images, we can clearly observe a trend of increasing intra-unit pillar numbers.

Figure 4.10 SEM images of assembled micropillar arrays. Scale bar: 10 μm

4.5 Conclusion

In this chapter, the influencing factors of micropillar array assembly were systematically studied, and a series of micropillar geometry was designed to form 2-pillar, 3-pillar, 4-pillar and multi-pillar assembled units. The process of micropillar array assembly and disassembly was systematically studied the geometry factors including pillar shape, size, height, as well as the surface chemistry and micropillar elasticity were studied. The geometry factors are the most important as it determined the homogeneity of the assembled patterns. The influence of geometry was as follows: the pillar size and height determined whether the pillar will exhibit large deformation. And the distance between neighboring pillars determined whether the pillars can adhere to each other. Based on the calculation and experiments, a series of assembled patterns were developed, including 2-pillar, 3-pillar, 4-pillar and multi-pillar units.

References:

- [1] (a) Fu, J.; Wang, Y. K.; Yang, M. T.; Desai, R. A.; Yu, X.; Liu, Z.; Chen, C. S., *Nat Methods*. **2010**, 7, 733-736; (b) du Roure, O.; Saez, A.; Buguin, A.; Austin, R. H.; Chavrier, P.; Silberzan, P.; Ladoux, B., *Proc Natl Acad Sci U S A*. **2005**, 102, 2390-2395; (c) Sniadecki, N. J.; Anguelouch, A.; Yang, M. T.; Lamb, C. M.; Liu, Z.; Kirschner, S. B.; Liu, Y.; Reich, D. H.; Chen, C. S., *Proc Natl Acad Sci U S A*. **2007**, 104, 14553-14558; (d) Tan, J. L.; Tien, J.; Pirone, D. M.; Gray, D. S.; Bhadriraju, K.; Chen, C. S., *Proc Natl Acad Sci U S A*. **2003**, 100, 1484-1489.
- [2] Reddy, S.; Arzt, E.; del Campo, A., *Advanced Materials*. **2007**, 19, 3833-3837.

- [3] (a) Murphy, M. P.; Kim, S.; Sitti, M., *ACS Appl Mater Interfaces*. **2009**, *1*, 849-855; (b) Greiner, C.; Arzt, E.; del Campo, A., *Advanced Materials*. **2009**, *21*, 479-482; (c) Kim, S.; Sitti, M., *Applied Physics Letters*. **2006**, *89*, 261911; (d) Gorb, S. N.; Sinha, M.; Peressadko, A.; Daltorio, K. A.; Quinn, R. D., *Bioinspir Biomim*. **2007**, *2*, S117-125; (e) Li, Y.; Krahn, J.; Menon, C., *Journal of Bionic Engineering*. **2016**, *13*, 181-199; (f) Xue, L.; Kovalev, A.; Thole, F.; Rengarajan, G. T.; Steinhart, M.; Gorb, S. N., *Langmuir*. **2012**, *28*, 10781-10788; (g) Autumn, K.; Liang, Y. A.; Hsieh, S. T.; Zesch, W.; Chan, W. P.; Kenny, T. W.; Fearing, R.; Full, R. J., *Nature*. **2000**, *405*, 681-685; (h) Lee, D. Y.; Lee, D. H.; Lee, S. G.; Cho, K., *Soft Matter*. **2012**, *8*, 4905.
- [4] Chandra, D.; Yang, S.; Soshinsky, A. A.; Gambogi, R. J., *ACS Appl Mater Interfaces*. **2009**, *1*, 1698-1704.

Chapter 5

Live Bacteria Extraction and Controllable Releasing Platform

*In this chapter, an application of micropillar array was demonstrated. When assembled, the pillar array tip will combine to form an assembled finger-like structure which can trap small objects in the confined space. Here we used bacterial species *E. coli* as a demonstration for the performance of micropillar array in capturing and releasing of bacteria in water.*

5.1 Introduction

The World Health Organization (WHO) reported that microbial infectious diseases are one of the leading causes of mortality worldwide. As the world has been challenged with multiple life-threatening microbial infection diseases including Methicillin-resistant *Staphylococcus aureus* infections that had taken lives and caused severe disabilities from raw fish in Singapore. It is necessary to develop platforms to detect live bacteria and develop new drugs to treat the infections. Growing drug resistance in microbial is making the battle of human beings against deadly pathogens more severe. Therefore, microbial capture, detection, and new anti-microbial drug development play critical roles in public health improvement.

The most widely used way to monitor fecal indicator bacteria is culture-based technologies, immunoassays, biochemical reaction tests, and polymerase chain reaction methods. However, these methods have many disadvantages, such as time-consuming, comprehensive operating procedures that require well-trained experts, or expensive reagent and instrument dependent. Moreover, many species of microbial are hard to culture. Thus, it requires efficient and rapid methods to capture microbial.

Usually, these methods involve two steps: firstly, bacteria are captured, and the target bacteria species are tagged or amplified; secondly, the target bacteria are detected using various of technologies. The mainstream method to capture bacteria can be categorized into four major groups:

- (1) bacteria surface adhesion methods.;
- (2) microfluidic-based methods;
- (3) size based filtration;
- (4) microparticle-based capturing;

Here we will discuss these methods in detail and analyze their advantages and disadvantages.

5.1.1 Bacteria Surface Adhesion

The main idea of this method is to use specific molecules to enhance the affinity of bacteria surface to the capturing devices. And there are two approaches: using adhesive molecules to improve the affinity of device surfaces and to increase the adhesive surface area of capturing devices.

Using whole cell capture devices does not demand pretreatment process to bacterial cells, such as cell structure disruption and bacterial proliferation. These methods exhibit numerous advantages, including easy manipulation, short processing time. Here we will introduce this approach in details.

Bacteria, the surface recognition method, is utilizing a surface recognition agent to bind the whole bacterial cell without cellular disruption. Various biological materials can be used as affinity enhancement molecules, such as antibodies, aptamers^[1], concanavalin A^[2], polypeptides, carbohydrates, bacterial phages^[3], and antibiotics^[4] as shown in **Figure 5.1**.^[5]

Figure 5.1 Molecules used in bacteria capture to enhance surface affinity to bacteria.^[5]

Antibodies are a family of well-developed target molecules for specific enrichment of bacterial species. Its high sensitivity, flexibility in chemical functionalization, and good selectivity had made it an important technique for bacteria enrichment. Here in **Figure 5.2 (a)**^[6] and **(d)**^[4] demonstrated an ELISA based bacteria capture and detection method. When coated on the substrates, the primary antibody can specifically bind with the target bacteria,

and by the specific binding of primary antibody and enzyme crosslinked secondary antibodies, a sandwich detection was performed. This design can be further tuned with different responsive strategies for customized detection platforms. Fluorescence, pH, colorimetric changes and other signals can all be used. However, antibody-based detection platforms are usually expensive and require strict storage environment as antibodies are sensitive to temperature and pH.

Shown in **Figure 5.2 (b)**, Rius et al.^[7] developed a platform that used aptamer immobilized carbon nanotubes as a potentiometric sensor that can capture and detect bacteria. It was highly sensitive and can be applied in real-time detection of living microbial. The advantages of aptamers include in vitro selection, relatively easy to synthesise than antibodies, relatively higher stability than antibodies, and flexible chemical modification. But it also has some disadvantages, such as sensitive to pH and high instrument requirements (need PCR instruments to produce a large quantity of aptamers).

Figure 5.2 Scheme figure of different (a) Biotinylated antibody on neutravidin functionalized surface for bacterial cell capture.^[6] (b) Aptamer coated carbon nanotubes for bacteria capturing^[7]. (c) Antimicrobial peptide coated microelectrode array for bacterial capture and detection.^[8] (d) Antibody and antibiotic sandwich structure for bacteria capture and killing.^[4]

Compared with antibody and DNA probes, another family of important adhesive molecules, antimicrobial peptides have advantages such as higher stability in harsh environments, easier synthesizing process, and affinities to broader affinity to a wide range of both Gram-positive and Gram-negative bacterial strains. As demonstrated in **Figure 5.2 (c)**, the antimicrobial peptide^[8] was coated on gold sensors, and when the bacteria were captured, an electrical signal will be detected. Despite the advantages, the antimicrobial peptide has its Achilles' heel that limited its application that microbial will develop antibiotic resistance.

And as these adhesive molecules have different advantages and disadvantages. Here we analyzed their properties in the table below.

Table 5.1 Comparison of bacterial adhesive molecules.

Adhesive molecule	Advantages	Disadvantages
Antibody	High selectivity and sensitivity, flexible surface functionalization	High cost, instable in harsh environment, hard to synthesis
Aptamer	High selectivity and sensitivity, flexible surface functionalization, in vitro synthesis	Relatively high cost, instable in harsh environment
Antimicrobial peptide	High stability, easier to synthesis, broad affinity	Microbial resistance

As bacterial cells can sense and respond to the extracellular environments, to increase the adhesion of bacteria on material-bio interfaces, another effective approach is to increase the contact area of the capturing devices. Many approaches had been made to achieve this goal. Instead of using flat surfaces, nanopillars, wrinkles, nanoparticle arrays, and hierarchical structures had been fabricated and applied in bacteria capture devices. The mechanism of these approaches is to introduce secondary features on the surfaces to connect bacterial subcellular structures. By combining these structures with adhesive molecules, better capture efficiency can be achieved. Here is an example. As shown in **Figure 5.3^[9]**, in the SEM image **(b)**, the bacterial cells formed protrusions on Si

nanowires. It indicated the surface topographical changes significantly enhanced the interaction between bacterial cells and the nanostructures.

Figure 5.3 Performance of bacteria captured with Si nanowires and flat Si surfaces.^[9] (a) Bacterial yields on three different substrates at different incubation time points: Si nanowires (black squares), flat Si coated with ConA coating (red circles), and Si nanowires with ConA coating (blue triangles). (b) Fluorescence and SEM images of Si nanowires and flat Si surfaces, both coated by ConA. Red arrows pointed out the bacteria and nanowires.

In conclusion, this molecular recognition method is very efficient, but it suffers from many disadvantages, for example, high cost for antibodies and proteins, poor stability of functional molecules in variable environments, such as pH changes and temperature. And the performances are not stable, varying among batches and sources. Besides, some of the molecules are not commercially available and require time and efforts for home preparation and screening. Moreover, antibiotic resistance in bacteria cannot be avoided. Last but not most important, most of the affinity molecules are target specific. Thus, these are species-specified methods and not effective for broadband capturing. Thus, other methods, such as microfluidic approaches are developed to fill this gap.

5.1.2 Microfluidic Separation Method

Microfluidic devices, fabricated by softlithography technique, have many unique properties thanks to the building material. Usually, they were made by inexpensive, biocompatible and transparent polymers such as PDMS, PMMA, and photoresists. Microfluidic devices are becoming an increasingly important tool in microbial studies for

two reasons: Firstly, the microfluidic devices created an environment that greatly increased the surface area to volume ratio. It is customized to handle small volume liquids. When running through constrained channels of microfluidic devices, the bacterial cells dispersed in water can be enriched by channel walls. Secondly, the transparency of building materials makes it capable of real-time observation of bacterial movement, interactions with channel walls, and viability. Thanks to these advantages, microfluidic devices are becoming an ideal platform for microbial capture and analyzation, significantly reduced time and cost compared to conventional systems.

Here are a few examples of microfluidic devices for bacteria capture. In **Figure 5.4**^[10], a design of trapping device was demonstrated. This device can trap water droplets in oil streams. Shown in **Figure 5.4 (A-C)**, droplets entered the traps from the large openings and blocked the exit. Thus remained in the trap until oil flowed from the opposite direction and released the droplets. When each droplet was filled with microbial solutions, microbial cell encapsulation was realized, and in each droplet microreactors, the genotype-phenotype study of microorganisms can be conducted without contamination threats.

Figure 5.4 Microfluidic-based droplet trapping device^[10]. (A-C) The design of a single trap. The fluid flow direction controlled the trapping and released of droplets. When a droplet took up a trap, there would be no space to fit in more droplets thus they will move away from the side of the trap. (D) Uniform distributed droplets trapped in this device. Scale bar: 75 μm . (E-G) Droplet releasing process. When flow went along the direction as indicated by the white arrows, the droplets will be released from the traps. Scale bar: 75 μm .

Another approach was based on a similar design strategy that used the same shape but smaller size trapping arrays that directly trapped bacterial cells, as shown in **Figure 5.5**^[11]. The strength of this design was single cell trapping and removal of daughter cells. It can track the entire lifespan of individual cells at once.

Figure 5.5 A microorganism trap.^[12] (A-C) The design of microfluidic microorganism trap. (A) Optical image of the whole device. Scale bar: 10 mm. (B) Trapping channels and flow direction of this device. Scale bar: 1 mm. (C) SEM image of trapping arrays. Scale bar: 50 μm. (D) Schematic figure of trapping process. (E) Single yeast cell trapping performance. Scale bar: 10 μm.

Another trapping design was shown in **Figure 5.6**^[13] and **Figure 5.7**. The key was to fabricate an individual microbial cell “jail” for single microbial cell micromanipulation. In **Figure 5.6**^[13], a PDMS cell “jail” was fabricated by replica molding method. And the structure was shown in the SEM images. The target microbial was cultured in the device to reach log phase for efficient cell loading. And when continuous flow was added to the

system, smaller daughter cells will be washed away, and single mother cells remained in the “jails” for further analysis. And in **Figure 5.7**^[14], nanopore arrays were used to capture bacteria, one bacterium per pore. A negative pressure was generated below the nanopore arrays to create a liquid flow running downwards, for loading microbial cells in the nanopore arrays. On the contrary, to release microbial cells from the nanopores, the buffer solution was injected from the bottom of the pores and induced upwards liquid flow that washed cells out of the nanopores.

Figure 5.6 Bacterial “jail” schematic figure and SEM images of the trapping device structure. Scale bar: 20 μm .^[13]

Figure 5.7 Nanopore arrays for microfluidic-based bacteria capture and release.^[14] (a) single bacteria capture and release. (b) fluorescent images of a single bacterium loaded and released from a nanopore.

In conclusion, the microfluidic devices had been widely used in microbial capture. And a series of pattern geometries were designed and fabricated to satisfy the increasing demand in single bacterium cell capture. As concluded at the beginning of this Chapter, the advantages for microfluidic devices includes precise control, low cost, and high efficiency capturing. Its disadvantages cannot be neglected. To use a microfluidic device, syringe controllers, and liquid pumps must be used for precise control of flow speed, direction, and liquid amount. Thus, portable devices and easy operating procedures required further development.

5.1.3 Size Based Filtration Method

The average bacterial cell size is around 1 to 2 μm . Using porous material with a pore size smaller than the bacterial cell size, then bacteria can be enriched by the porous material. The advantages of this design included high efficiency, tunable porous material to realize real-time elimination, and broadband bacterial enrichment. Here we provided an example of this approach. In **Figure 5.8**, a carbon nanotube membrane^[15] was fabricated with a pore size of 400 nm. PGLa antimicrobial peptide was conjugated on the porous material for bacterial elimination. When this carbon nanotube membrane filtered contaminated water, the pathogens will be killed and removed from the water.

The limitation of these devices was also analyzed. Firstly, it was hard to remove the bacteria cells from the porous materials, thus limited the recyclability of the device. It made the device costly. Secondly, the bacteria cells captured by the porous material were damaged and cannot be used for further analysis.

Figure 5.8 Schematic figure of graphene-based membrane filtering system for bacteria. ^[15]

5.1.4 Microparticle-Based Capturing Method

Usually, microparticle-based capturing approaches involve magnetic particles with surface functionalization of adhesive molecules. This method can also be named as immunomagnetic bacteria capturing approaches. The capturing process had two steps. Firstly, bacterial cells adhered to the magnetic particle surface by specifically binding with adhesive molecules, such as antibodies, aptamers, and peptides. Secondly, the bacteria enriched magnetic particles can be separated from the suspension by external magnetic field. Here in **Figure 5.9**, it demonstrated an example of this design^[16]. Basically, it was antibody-conjugated magnetic beads. Moreover, bacteria-magnetic beads complex was attracted and separated from the suspension by a magnet. A colorimetric assay was conducted for bacteria detection. Here in this system, bacterial intrinsic peroxidase activity was utilized to design this colorimetric detection technique.

This capture technique was based on the bacterial cell affinity to the functionalized particle surface. Thus, the limitations of this technique were similar with **5.1.1**. And the advantages included fast separation by a magnetic field and high efficiency as particles can disperse in water and significantly increased the contact area.

Figure 5.9 Schematic figure of antibody-functionalized magnetic beads for colorimetric immunomagnetic assay. ^[16]

5.1.4 Conclusion

From the above-introduced approaches of bacteria capturing devices, we can conclude the future of this area required the following properties:

Firstly, a portable capturing device with easy operation procedure and low instrument requirements.

Secondly, it lacked methods for live bacteria extraction from water for whole cell analysis. To fill these gaps, we developed our micropillar array based live bacteria capturing device.

5.2 Materials and Experiment

In this chapter, plasma treated hydrophilic PDMS micropillar arrays were used directly after treatment. The treatment procedures had been introduced in details in **Chapter 3**.

E. coli was used as a model bacterial species for this device. After harvesting *E. coli* cells at S2 phase, the cells were suspended in fresh NS, and the device was tested by dipping the device in the bacteria solution and air dried at room temperature. After bacteria capture, the bacteria cells were stained by LIVE/DEAD staining assay and observed under fluorescence microscopy (Leica DMI 6100). Before SEM imaging, the device with bacterial cells was air dried and coated with a layer of Pt. SEM images were taken by Zeiss SUPRA 55 FESEM.

For bacteria release, PMAA coated PDMS arrays were fabricated by EDC/NHS crosslinking method introduced in **Chapter 3**. The PDMS pillar arrays were stretched until reached 150% elongation and washed by pH=10 buffer solution for three times to release the bacteria cells captured by the device. Both capturing state and releasing state micropillar arrays were observed by fluorescence microscopy (Leica DMI 6100). The releasing efficiency was quantified by ImageJ software.

5.3 Results and Discussion

5.3.1 Bacterial Swimming within Confined Space between Pillar Arrays

In Chapter 4, we had discussed the assembly of micropillars induced by water evaporation on micropillar surfaces. Here we designed a series of micropillar arrays for microorganism capturing. Firstly, we observed the movement of *E. coli* cells during the evaporation process. Microbial have numerous motility strategies that guaranteed their movement in liquids and on surfaces. With its flagella or pili, suspended single bacterium can swim through liquids and swarm towards each other. It can also glide and twitch on wet surfaces that only has a limited amount of moisture. The bacterial flagella unfold, and rotation is the driving force for swimming.

The alignment of collective bacteria in limited-space liquids during water evaporation process can be considered as a model system of bacterial movement within the thin liquid film. During the evaporation process, the primary chemoattractant, oxygen, was expected to be highly uniform because of the thin-film geometry and mixing of fluid by the

bacteria. It is a pure hydrodynamic process for collective bacterial swimming, and the alignment of bacteria is randomly distributed.

In the previous study shown in **Figure 5.10**^[17], the bacteria concentrations in thin liquid membranes were adjusted, and the bacteria movement was tracked. In Figure 5.8, ρ was the filling fractions. ρ was between 0 to 1, and when ρ equaled 1, the surface was fully covered by bacteria. The alignment of bacteria cells was calculated by the alignment coefficient C .

Figure 5.10 Alignment coefficient (C) vs. threshold (k) curves at different density.^[17]

From **Figure 5.10** we can conclude that the alignment of bacteria was significantly decreased at higher concentration. Thus, during the water evaporation process, as the concentration of bacteria was increasing as the amount of solvent (water) was gradually reduced throughout the evaporation process. The bacteria were randomly swimming between the pillars without specific orientation. It was in the same trend as the time-lapse observation of water evaporation process in **Figure 5.10**.

Figure 5.11 Bacteria capture dynamics. Time interval: 5 sec. Scale bar: 5 μm .

Bacterial cells moved freely in a liquid membrane formed on the hydrophilic PDMS surfaces. And when water evaporated, asymmetric evaporation speeds of intra- and inter-units induced micropillar assembly and trapped bacterial cells within the confined space of assembled pillars. From the time lapse images, it can be clearly seen that the bacterial cells swam freely when the micropillars were fully immersed in water. Most bacterial cells were moving freely in the microchannels between pillar arrays randomly. And when water level started to decrease and exposed the pillar tips in the air, micropillars will assemble towards each other within 4-finger units. Thus the space for the bacterial movement was reduced. Most bacterial cells will be washed away with the shrinking water film, and only a few bacterial cells will be trapped in the 4-finger units. As evaporation continued, each 4-unit arrays will be isolated, with water and bacterial cells only in the central area of each unit. Finally, when water fully evaporated, the bacterial cells were enriched between closed 2-pillar units as shown in the last image of **Figure 5.11**.

5.3.2 Bacteria Capturing

We had discussed the bacteria enrichment dynamic process during water evaporation above, and this capturing phenomenon occurred in a large area as shown in **Figure 5.12**. *E. coli* was stained by SYPO 9, and each green dot was a cluster of captured bacteria. The water evaporation induced micropillar assembly was highly ordered, and the size distribution was narrow according to **Figure 5.13**.

From **Figure 5.12** and **Figure 5.13**, it can be concluded that the bacteria cluster formation after capturing was size-dependent. As the assembled pillar formed confined space, only certain numbers of trapped bacteria can fit in. Thus this size selectivity provided a uniform bacteria pattern. The size distribution of captured bacteria was quite narrow.

Figure 5.12 Bright field and fluorescent images of SYPO 9 stained *E. coli* captured by the micropillar arrays. Scale bar: 25 μm .

Figure 5.13 Size distribution of captured bacteria, counted from the fluorescence image.

Confirming the location of trapped bacteria, a series of imaging techniques were applied. In **Figure 5.14** and **Figure 5.15**, the micropillars with captured bacteria cells were imaged by SEM and 3D fluorescence imaging. As shown in **Figure 5.14**, the pillars were stained by 0.1 μm Alex 544 Fluorosphere (Invitrogen), and the bacterial cells were labeled by BacLight Green (Life Technologies).

From the SEM images, we can conclude that the pillar tips will adhere to assembled state and the bacteria were trapped in the confined space after pillar assembly. This conclusion was confirmed by fluorescence imaging.

Figure 5.14 Localization of captured bacteria. (a) and (b) Scale bar: 10 μm . (c) Scale bar: 1 μm .

Figure 5.15 3D fluorescence image of micropillar array and trapped bacteria. (Scale bar: 10 μm)

5.3.3 Size Dependent Bacteria/Cell Selectivity

To further investigate the performance of this size-based capturing device, we investigated

the selectivity of micropillars by conducting bacteria capturing in a mixture of bacteria and mammalian cells. Here Hela cells were used for demonstration.

Hela cells were trypsinized from the incubator, centrifuged to discard trypsin solution. And the re-suspended in fresh NS. Then bacteria were harvested and washed with NS. The bacteria were mixed with Hela cells, and a capturing experiment was conducted by the dipping-lifting method.

Figure 5.16 Bacteria capture from bacteria/mammalian cell mixture. (a) Optical image of bacteria and Hela cell mixture. Scale bar: 100 μm . (b) Schematic figure of the “dip-lift” process. (c) Bright field image of micropillar array for bacteria is capturing. (d) SYTO 9 stained live E. coli, (e) PI stained dead E. coli, (f) DAPI stained Hela cell nucleus. Scale bar: 100 μm

And then the micropillar array was stained by SYTO 9, PI, and DAPI for Live/Dead assay and cell nucleus. It can be confirmed from **Figure 5.16** that the size selectivity of micropillar array was exquisite that only bacteria cells were captured by the device but not the Hela cells. And the bacteria cells captured were still alive for further analysis.

The mechanism of selective trapping was size-dependent. The large Hela cells cannot fit in the confined space between assembled pillar arrays. And the dip-lift technique only required the short time of contact. And the Hela cells cannot grow mature focal adhesion to anchor on the pillar surface. Thus, only bacterial cells were selectively captured.

5.3.4 Stretching Induced pH-Dependent Bacteria Releasing

For controllable bacteria releasing after capturing, we used PMAA coated PDMS micropillar arrays. The fabrication procedures had been introduced in **Chapter 3**. Here the performance of releasing was tested.

Firstly, we used micropillar arrays to capture the bacteria. From **Figure 5.17 (a)**, the bacteria were trapped in each 4-pillar units and the occupation of units was very high. And after stretching for three cycles at 150% strain and washed by pH=8 silane buffer, the bacteria cells were released from the capturing device. The efficiency was calculated from the fluorescence images and shown in **Figure 5.18**.

Figure 5.17 Merged fluorescence and bright-field images of captured bacteria (stained by SYTO 9, green color) and micropillar arrays. (a) Capturing state; (b) releasing state. Scale bar: 50 μm .

Figure 5.18 The bacteria covered area changes at two states: capturing state and releasing state. Data calculated from fluorescence images.

The bacteria cells can be released from the micropillar arrays as micropillar arrays will disassemble under stretch. The bacterial cell wall has a negative charge^[18]. Gram-positive bacteria have teichoic acids linked to either peptidoglycan or the plasma membrane and have a net negative charge. Gram-negative bacteria surface charge is also negative with the contribution of surface phospholipids and lipopolysaccharides. When captured by the PMAA pH-responsive hydrogel coated PDMS micropillar arrays, the bacteria were directly in contact with the PMAA hydrogel. When pH was adjusted, the PMAA surface will exhibit negative charge, and the adhesiveness of PMAA to bacterial cell surfaces will be reduced. And stretching of micropillar array membranes will induce disassembly of micropillar arrays and the pillars no longer adhere to each other; thus the trapped bacteria would have freedom of movement, and they were washed away by the buffer solution.

5.4 Microbial Drug Sensitivity Test

The captured uniform bacteria arrays can be applied in microbial drug sensitivity tests and new drug screening. Here we demonstrated its application in anti-microbial drug sensitivity test.

The drug was a synthesized aptamer functionalized gold nanostar.^[19] The gold nanostars have a photo-thermal effect and can generate heat when exposed to 800 nm IR irradiation. Here we characterized the bacteria sensitivity to this photo-thermal material. *E. coli* and *S. aureus* were used for this study. Firstly, both bacterial species were harvested at S2 phase and washed by fresh NS for two times and stained with Live/Dead bacteria staining assay. Then, the bacterial cells were loaded to the micropillar array membranes and formed ordered microbial cell arrays. Finally, 0.001 nM SA-17 AuNS solution was introduced to the microbial cell arrays and exposed to 800 nm IR irradiation. At different time points, microbial viability was characterized by Live/Dead bacteria staining assay and fluorescence images were taken. The results of microbial viability were shown in **Figure 5.19**.

The viability of microbial was quantified and the results were shown in **Figure 5.20**. And it can be concluded that the *S. aureus* was more heat sensitive than *E. coli* and the gold

nanostars had strong photo-thermal microbial elimination effect with 100% killing rate in 15 min.

Figure 5.19 Live/Dead staining of bacteria arrays. Green: live bacterial cells stained by SYTO 9; red: dead bacterial cells stained by PI. (a) Live *E. coli* arrays before IR irradiation. (b) Dead *E. coli* arrays after 5 min exposure to SA-17 AuNS and 800 nm IR irradiation. (c) Dead *E. coli* arrays after 10 min exposure to SA-17 AuNS and 800 nm IR irradiation. Scale bar: 100 μm . (d) Live *S. aureus* arrays before IR irradiation. (e) Dead *S. aureus* arrays after 5 min exposure to SA-17 AuNS and 800 nm IR irradiation. (f) Dead *S. aureus* arrays after 10 min exposure to SA-17 AuNS and 800 nm IR irradiation. Scale bar: 25 μm .

Figure 5.20 Microbial photo-thermal sensitivity quantification.

5.5 Conclusion

In this chapter, we demonstrated an application of micropillar arrays for live bacteria capture. And from the results, we can clearly see the performance of capturing efficiency was high, and the captured bacteria clusters were uniformly distributed in each microarray units.

And by using the PMAA coated PDMS microarrays, we successfully achieved stretching induced pH-responsive bacteria releasing.

In conclusion, this microarray system has great potential for controllable trapping and releasing of microorganisms to a great area. The trapped microorganisms tend to form uniform clusters for high-throughput quantification of drug sensitivity test, drug efficiency tests, bacterial species sorting, and pathogen detections. And the controlled releasing property made it a recyclable platform that significantly reduced costs.

References:

- [1] Zhang, C.; Lv, X.; Han, X.; Man, Y.; Saeed, Y.; Qing, H.; Deng, Y., *Anal. Methods*. **2015**, 7, 6339-6345.
- [2] Jaipuri, F. A.; Collet, B. Y. M.; Pohl, N. L., *Angewandte Chemie*. **2008**, 120, 1731-1734.
- [3] (a) Singh, A.; Arutyunov, D.; Szymanski, C. M.; Evoy, S., *Analyst*. **2012**, 137, 3405-3421; (b) Singh, A.; Arya, S. K.; Glass, N.; Hanifi-Moghaddam, P.; Naidoo, R.; Szymanski, C. M.; Tanha, J.; Evoy, S., *Biosens Bioelectron*. **2010**, 26, 131-138.
- [4] Gao, H.; Yang, S.; Han, J.; Xiong, J.; Kong, W.; Li, C.; Liao, G.; Fu, Z., *Chem Commun (Camb)*. **2015**, 51, 12497-12500.
- [5] Templier, V.; Roux, A.; Roupioz, Y.; Livache, T., *TrAC Trends in Analytical Chemistry*. **2016**, 79, 71-79.
- [6] Laczka, O. F.; Labbate, M.; Seymour, J. R.; Bourne, D. G.; Fielder, S. S.; Doblin, M. A., *PLoS One*. **2014**, 9, e108387.

- [7] Zelada-Guillen, G. A.; Riu, J.; Duzgun, A.; Rius, F. X., *Angew Chem Int Ed Engl.* **2009**, *48*, 7334-7337.
- [8] Etayash, H.; Jiang, K.; Thundat, T.; Kaur, K., *Anal Chem.* **2014**, *86*, 1693-1700.
- [9] Li, Y. Q.; Zhu, B.; Li, Y.; Leow, W. R.; Goh, R.; Ma, B.; Fong, E.; Tang, M.; Chen, X., *Angew Chem Int Ed Engl.* **2014**, *53*, 5837-5841.
- [10] Huebner, A.; Bratton, D.; Whyte, G.; Yang, M.; Demello, A. J.; Abell, C.; Hollfelder, F., *Lab Chip.* **2009**, *9*, 692-698.
- [11] Jo, M.; Liu, W.; Gu, L.; Dang, W.; Qin, L., *PNAS.* **2015**, *112*,
- [12] Jo, M. C.; Liu, W.; Gu, L.; Dang, W. W.; Qin, L. D., *P Natl Acad Sci USA.* **2015**, *112*, 9364-9369.
- [13] Ryley, J.; Pereira-Smith, O. M., *Yeast.* **2006**, *23*, 1065-1073.
- [14] Guo, P.; Hall, E. W.; Schirhagl, R.; Mukaibo, H.; Martin, C. R.; Zare, R. N., *Lab Chip.* **2012**, *12*, 558-561.
- [15] Nellore, B. P.; Kanchanapally, R.; Pedraza, F.; Sinha, S. S.; Pramanik, A.; Hamme, A. T.; Arslan, Z.; Sardar, D.; Ray, P. C., *ACS Appl Mater Interfaces.* **2015**, *7*, 19210-19218.
- [16] Wen, J.; Zhou, S.; Chen, J., *Sci Rep.* **2014**, *4*, 5191.
- [17] Sokolov, A.; Aranson, I. S.; Kessler, J. O.; Goldstein, R. E., *Phys Rev Lett.* **2007**, *98*, 158102.
- [18] Tuson, H. H.; Weibel, D. B., *Soft Matter.* **2013**, *9*, 4368-4380.
- [19] (a) Dam, D. H.; Culver, K. S.; Kandela, I.; Lee, R. C.; Chandra, K.; Lee, H.; Mantis, C.; Ugolkov, A.; Mazar, A. P.; Odom, T. W., *Nanomedicine.* **2015**, *11*, 671-679; (b) Dam, D. H.; Culver, K. S.; Odom, T. W., *Mol Pharm.* **2014**, *11*, 580-587; (c) Dam, D. H. M.; Culver, K. S. B.; Sisco, P. N.; Odom, T. W., *Therapeutic Delivery.* **2012**, *3*, 1263-1267.

Chapter 6

Surface Functionalized Micropillar Array for Stretch-Induced pH-Responsive Controlled Release of Microspheres

In this chapter, another application of micropillar array was demonstrated. There are many existing platforms that utilized mechanical force as a trigger for responsive controllable drug release. And here we demonstrated another design using the micropillar array system. The surface of PDMS micropillar array system was coated with a layer of pH-responsive PMAA hydrogel. And we used fluorescent PS microspheres as a demonstration. We loaded the microspheres into the micropillar arrays through evaporation induced assembly. During the liquid evaporation process, the micropillar array will assemble and load microspheres in the confined space. It was dual stimuli responsive and only when the two triggers coexist; the loaded microsphere can be released.

6.1 Introduction

Nanomedicine had drawn extensive attention in the biomedical area as many therapeutic platforms had been developed these years. Moreover, nanoparticles and microparticles are a very important drug delivery vehicle. To realize on-demand and precise drug release, a series of methods to trigger drug release has been developed, including light, ultrasound, heat, pH, redox, and enzymes. Among all the existing triggers, a mechanical stimulus, like the macroscopic force is a promising strategy. Human body anatomy includes many movements, including tension and compression generated by bone joints, tendons, and muscles.^[1] Mechanical force stimuli can be achieved by these movements and controlled by the patient in a self-administrated manner without acquiring additional instruments or chemicals. Thus, mechanical force triggered controllable drug release platforms brought attention to researchers and inspired promising designs.

Mooney group^[2] reported the earliest design of the compression-responsive system to release vascular endothelial growth factor (VEGF), and the mechanism of this platform can be concluded as follows. A hydrogel-based drug carrier was fabricated and by compressive stimulation of the hydrogel matrix, the pressure of the carrier would be increased leading to deformation of the matrix. Moreover, the drug will be released. Further modification to this platform was performed to perfect the performance by reversibly bind the drug molecules to the hydrogel carrier. Thus, the drug can be released in a cyclic manner under periodic force triggers, as shown in **Figure 6.1**.

The main idea of this fabrication was to build a sponge-like platform that can absorb the drug molecules. And when compression was added to the platform, it deformed the matrix and induced drug release. There are many advantages of this platform. Firstly, these platforms are usually reversible. Secondly, the flexibility of the hydrogel platform allows chemical modification to increase the drug loading efficiency. Thirdly, the hydrogel material is biocompatible.

The disadvantages include slow uncontrollable diffusion of drug molecules. The drug molecules are usually absorbed into the hydrogel matrix, and when immersed in water, the smaller drug molecules can slowly diffuse. It limited the shelf-life of hydrogel absorbent based mechanical force triggered drug releasing platforms.

Figure 6.1 Force triggered drug release platforms. Alginate hydrogel platform for mechanical stimuli triggered VEGF release.^[2] (a) Mechanical stimulation of compression (2 min) and relaxation (8 min). (b) VEGF release rate under compression stimuli. Open circles: 10% strain amplitude; open squares: 25% strain amplitude; filled circles: control.

Another approach was developed by Gu group^[3] as shown in **Figure 6.2**. By combining a stretchable elastomer and microgel with drug nanoparticles. When a tensile strain was applied along x-axis to the elastomer substrate, the compressive force appeared along y-axis and triggered drug release from the microgels. The efficiency of drug release was tested on finger joints. When patients flexed and stretched the finger, then the drug will be disseminated. Moreover, by controlling the timing and stretching/releasing cycles, the drug dosage was controlled.

Figure 6.2 Tensile strain-triggered drug delivery device^[3]. (a) The microgel will be compressed thanks to the deformation of substrate and release nanoparticles. (b) Drug molecules will slowly diffuse into the microgel from the microparticle in a reversible manner.

Wrinkle and crack based mechanical force stimuli triggered drug release system were also developed. As shown in **Figure 6.3**^[4], a layer of polystyrene was coated on top of PDMS substrate. By heating and cooling the substrate, isotropic buckling of the PDMS substrate formed wrinkles. The drug capsules were patterned in the wrinkles with transfer printing method, and when the strain was applied to the substrate, microcapsules would release drug molecules.

Figure 6.3 Wrinkle based stretch-induced drug release system.^[4] Schematic figure of the fabrication procedure.

And in **Figure 6.4**^[5], this device consisted of a hydrophilic core loaded with drug and hydrophobic encapsulation layers. And when tension was applied, the crack would break

the weak shell and introduce water into the hydrophilic core to dissolve and release drug molecules.

Figure 6.4 Crack based stretch-induced drug release system^[5]. Schematic figure of the system design. (a) The chemical composite of the hydrophobic coating. (b) & (c) Optical and SEM images of the drug release system. (d) Two kinds of surface coating with different contact angle and chemical composite. Upper image: pure PCL. Bottom image: PCL: PGC-C₁₈ 1:1.

All existing methods are based on hydrogel and by introducing mechanical stress to deform the hydrogel matrix in a compressive manner, the drug molecules are “squeezed” out of a sponge-like structure.^[4, 6] There are many advantages to this kind of system. However, the disadvantages cannot be neglected. Firstly, the robustness of hydrogel is usually low, indicating that cycles of mechanical compression and will cause a fracture in the material. Then the performance under cyclic drug loading and releasing will be declined. Secondly, the diffusion of drug molecules from hydrated hydrogel system may limit the special and temporal control of drug release. To fill in this gaps, in this chapter, we will introduce a new design of stretch-induced drug release platform. Instead of wrinkles or cracks, the drug microparticles were loaded in the micropillar array system, and the release of drug microparticles was triggered by dual stimuli: suitable pH and stretching.

6.2 Materials and Methods

In this chapter, we successfully achieved dual stimuli triggered responsive drug release platform by modifying the surface of micropillar arrays with a layer of pH-responsive PMAA hydrogel. Here for a demonstration of the platform performance, we used negative

surface charged fluorescent polystyrene microsphere to evaluate the loading and releasing efficiency with this micropillar array-based device.

6.2.1 Micropillar Surface Modification

The surface of PDMS was functionalized by the following procedure as shown in **Figure 6.5**. It was a three-step functionalization process.

1. PDMS surface activation.

PDMS micropillar arrays were treated by O₂ plasma at 300 W, 50% O₂ atmosphere for 300 s. And used directly after plasma treatment to avoid reactivity loss of the silanol groups.

2. APTES surface grafting.

The activated PDMS was soaked in APTES solution (3%~5% APTES, 5% water, and 90% Ethanol) for 15 min then thoroughly washed with fresh water and dried under N₂ flow. Then the PDMS was placed in the 70 °C oven and baked for 2 hours. After that, the PDMS was cooled to room temperature. The APTES modified PDMS surface was prepared and ready for hydrogel crosslinking.

3. PMAA is crosslinking.

The hydrogel coated PDMS micropillar array was fabricated by carbodiimide crosslinking chemistry. Firstly, 2 g/L PMAA (MW ~100,000) solution in water was prepared. The pH of 9 mL PMAA solution was adjusted to 5.0 by adding activated by adding 1 mL 3 M sodium acetic buffer. And 80 mg NHS and 110 mg EDC were added into the solution to activate carboxylic acid groups. The mixture was shaken for 20 min before used to soak the PDMS surface. The PDMS micropillar array was fully immersed in the activated PMAA solution for 2 hours and then washed with fresh water.

6.2.2 FluoSphere Loading

The FluoSpheres were used in this system to test the microparticle trapping and releasing efficiency. The procedure of FluoSphere were as follows:

Firstly, the FluoSphere utilized in this experiment was 2 μm in diameter. They were diluted in ethanol at a concentration of 1: 100 from stock solution and suspended by sonication for 10 min.

Secondly, the FluoSphere solution was dropped on PMAA coated PDMS micropillar array and air dried at room temperature. FluoSphere loaded micropillar arrays were imaged under SEM and fluorescence microscopy.

Figure 6.5 PDMS surface modification procedure. (a) Modification process on PDMS surface. The first step was to use O_2 plasma to activate the PDMS surface. Then use APTES to functionalize the PDMS surface. Finally, use EDC/NHS crosslinking method to link PMAA molecules to the surface of PDMS. (b) The chemical composition of the cross-linked PDMS surfaces at each modification step.

6.2.3 Stretching Induced Releasing of Microparticles

After FluoSphere loading, the FluoSpheres were released from micropillar arrays by stretching the membrane to 150% strain and washing with water, pH=4 and pH=10 buffer solutions. And the results were analyzed by fluorescence microscopy imaging.

6.3 Results and Discussion

6.3.1 PDMS Surface Modification

After surface functionalization, the contact angle of the PDMS surface was measured to determine the surface hydrophilicity as a proof of successful chemical modification. Here in **Figure 6.6** showed the water contact angle of PDMS surface before and after a series of treatments.

Figure 6.6 Contact angle measurement. (a) Flat PDMS without any treatment. (b) APTES functionalized flat PDMS surface. (c) PMAA coated PDMS surface.

PDMS is a hydrophobic polymer. Thus untreated PDMS surface exhibited hydrophobicity and the contact angle was 116.7°. And when APTES was grafted on the PDMS surface, it reduced the hydrophobicity of PDMS surface to contact angle of 104.8°. Moreover, after successful grafting of PMAA hydrogel, with its hydrophilic surface character and ability to absorb water, the surface of PDMS changed to 60.8°.

Figure 6.7 Statistical analysis of contact angle change after surface functionalization.

The contact angles on flat PDMS before and after a series of surface functionalization were statistically concluded in the above **Figure 6.7**. The measurements were taken at more than four spots on each sample. From **Figure 6.7**, it can be concluded that the PDMS surface changed from hydrophobic to hydrophilic after grafting a layer of PMAA hydrogel.

To further prove the successful linking of PMAA on PDMS surface, FT-IR spectrum of PMAA functionalized PDMS was tested and the result was shown in **Figure 6.8**. The peak at 1720.02 cm^{-1} is assigning to the characteristic stretching vibration of the carbonyl groups ($\text{C}=\text{O}$) in PMAA segments. And this proved PMAA was successfully linked with PDMS.

Figure 6.8 FTIR spectrum of PDMS surface coated with PMAA.

And we used FESEM (Zeiss, SUPRA 55) to observe the morphology of the coated PMAA hydrogels. In **Figure 6.9**, the morphology of hydrogel on a flat surface were microparticles. Because the PMAA crosslinked on PDMS surface was polymerized, and the molecular weight was 100,000 g/mol. The long polymer chain will fold during dehydration process and form a microparticle morphology.

Figure 6.9 FESEM image of PDMS surface coated with PMAA. Scale bar: 5 μm .

6.3.2 Microparticle Loading and Stretch-induced Releasing

The first step to building a controlled release platform is to load the drug microparticles into the device. Here we used evaporation induced pillar assembly to trap the model particle FluoSphere (Invitrogen) as a demonstration. From the SEM and fluorescence images, we can see the particle localization in micropillar arrays.

Figure 6.10 Alex 488 FluoSpheres loaded micropillar array. Scale bar: 100 μm .

From **Figure 6.10** and **Figure 6.11**, we can conclude that the FluoSpheres trapped in the micropillar arrays were located at the 2-pillar unit centers and 4-pillar unit centers. It was corresponding to the assembly patterns described in **Chapter 4**. The assembly of micropillar arrays created confined space that held the microparticles inside.

Figure 6.11 SEM image of microparticle loaded micropillar array. Scale bar: 1 μm .

And the controlled releasing efficiency was tested by quantification of remaining FluoSphere counts after a stretching/washing cycle. Here we defined a stretching/washing cycle as stretching the micropillar array membrane to 150% strain and carefully rinse with 1 mL pH=10 buffer solution. Here in Figure 6.12, the amount of FluoSphere changes before and after certain numbers of stretching/washing cycles. It can be concluded that after 3 stretching/washing cycles, the total number of remaining FluoSpheres reduced to 50%. And after 6 stretching/washing cycles, the FluoSpheres were fully released from the micropillar arrays.

Figure 6.12 Quantification of FluoSphere numbers after stretching/washing cycle treatments.

And the PMAA hydrogel morphology before and after stretching was characterized by SEM images. From **Figure 6.13**, it can be observed that the PMAA matrix was

homogenous without cracks at the PDMS bottom layer. But after stretching, large cracks on the PMAA hydrogel matrix along the stretching direction can be observed (red arrows).

Figure 6.13 SEM images of hydrogel matrix damage induced by stretching.

6.4 Conclusion

In this chapter, the FluoSphere was used as a model particle system to study the controllable release of PMAA coated PDMS micropillar arrays. From the results listed above, it can be concluded that the hydrogel layer played a major role in the controlled release process. The layer of PMAA coated on PDMS decreased the adhesiveness between adjacent pillars since plasma-treated PDMS micropillars have relatively strong interactions but at basic pH, the hydrogel-coated micropillars have a charged surface thus reduced the adhesiveness.

Figure 6.14 Scheme figure of negatively charged pillar surface. The adjacent pillars have the same surface charge thus the adhesion will be reduced.

And during the stretching process, the strain will overcome the adhesive force and separate assembled pillars. The trapped microparticles can be released.

This stretching induced pH responsive microparticle controlled releasing platform has great potential to be applied in stretching induced drug release. Here negative surface charged FluoSphere was used as a model system to study the efficiency of this device and the performance was promising. Thus drug loaded microparticles with similar size and surface properties can be designed for a new drug delivery platform development.

References:

- [1] Janssen, M.; Mihov, G.; Welting, T.; Thies, J.; Emans, P., *Polymers*. **2014**, 6, 799-819.
- [2] Lee, K. Y.; Peters, M. C.; Anderson, K. W.; Mooney, D. J., *Nature*. **2000**, 408, 998-1000.
- [3] Di, J.; Yao, S.; Ye, Y.; Cui, Z.; Yu, J.; Ghosh, T. K.; Zhu, Y.; Gu, Z., *ACS Nano*. **2015**, 9, 9407-9415.
- [4] Hyun, D. C.; Moon, G. D.; Park, C. J.; Kim, B. S.; Xia, Y.; Jeong, U., *Angew Chem Int Ed Engl*. **2011**, 50, 724-727.
- [5] Wang, J.; Kaplan, J. A.; Colson, Y. L.; Grinstaff, M. W., *Angew Chem Int Ed Engl*. **2016**, 55, 2796-2800.
- [6] (a) Khang, D. Y.; Jiang, H.; Huang, Y.; Rogers, J. A., *Science*. **2006**, 311, 208-212; (b) Michinobu, T.; Shinoda, S.; Nakanishi, T.; Hill, J. P.; Fujii, K.; Player, T. N.; Tsukube, H.; Ariga, K., *J Am Chem Soc*. **2006**, 128, 14478-14479; (c) Kim, B.; Yoo, S.; Kim, Y.-J.; Park, J.; Kang, B.; Haam, S.; Kang, S.-W.; Kang, K.; Jeong, U., *Advanced Materials Interfaces*. **2016**, 3, 1500803; (d) Larsen, M. B.; Boydston, A. J., *J Am Chem Soc*. **2014**, 136, 1276-1279.

Chapter 7

Discussion and Future Work

In this chapter, a general discussion and conclusion of the whole thesis are provided. In this thesis, three achievements were discussed. Firstly, the mechanism of micropillar array assembly and disassembly was studied and precious control of micropillar assembly and disassembly were achieved. Secondly, a micropillar array platform for live bacteria capture from water was developed and applied in microbial drug sensitivity tests. Thirdly, a stretching-induced pH-responsive micropillar array system for controlled release of microparticles was demonstrated. Moreover, based on the current status of this research project, the future work that can be conducted is analyzed in details in this chapter.

7.1 General Discussion

In this thesis, the research of controllable assembly and disassembly in micropillar array systems led to several novel outcomes by:

Firstly, the process of micropillar array assembly and disassembly was systematically studied. The geometry factors including pillar shape, size, height, as well as the surface chemistry and micropillar elasticity have been investigated. The geometry factors are the most important as it determined the homogeneity of the assembled patterns. The influence of geometry was as follows: the pillar size and height determined whether the pillar will exhibit significant deformation. And the distance between neighboring pillars determined whether the pillars can adhere with each other. Based on the calculation and experiments, a series of assembled patterns were developed, including 2-pillar, 3-pillar, 4-pillar and multi-pillar units. By precisely controlling the micropillar array assembly, the ordered arrays can be applied in many fields.

Secondly, a new bacteria capturing device was developed. This device was designed and fabricated based on the micropillar array system and can be customized for bacterial cell size. The bacteria were patterned into uniformly distributed bacteria clusters with good viability. Moreover, through surface modification, we coated the surface of PDMS micropillar arrays with pH-responsive PMAA hydrogel. When the environmental pH value was adjusted to above 7, then the PMAA hydrogel layer will exhibit negative surface charge. As bacteria cell surface charge was negative, the adhesiveness of bacterial cell surface to the micropillar surface will be reduced, and the bacteria cells can be released from the micropillar arrays. These bacteria clusters had potential for drug screening, bacteria sorting, drug sensitivity tests and other applications. There are many advantages of this device, and here the benefits were listed:

- (1) It is very easy to operate by a simply dip in the bacteria solution and lift out and air dried at room temperature.
- (2) It reduced costs by recycling. The captured bacteria can be released from the capture device that allowed multiple usages with one piece of device.
- (3) It is a label-free capture device that can be used for capturing of broadband microorganisms with similar sizes. Thus it can be utilized for bacteria screening.

- (4) The captured bacteria exhibited high viability and can be applied in whole cell studies. With this device, the bacterial cells were patterned into ordered bacteria clusters for drug screening tests, drug sensitivity tests and other applications require uniformly distributed bacterial clusters.

Thirdly, a new approach of controlled drug release system was built up. The controllable microparticle releasing was triggered by a dual stimulus, mechanical force, and pH. By coating the PDMS micropillar arrays with a layer of PMAA hydrogel, the adhesiveness between micropillars was reduced. When tensile stress was added to the thin membrane of micropillar arrays, the elongation of substrates will separate the assembled micropillars, and the trapped microparticles will be released. Here in this thesis, a negative surface charged FluoSphere was used for demonstration of the controlled releasing efficiency.

In the future, we can design drug containing microparticles with negative surface charge, for example, drug-loaded PLGA microparticles. Thus, this controllable microparticle releasing system had the potential to be applied in new medication development for arthritis patients. The human joints have the ability to stretch and relax. And these movements can be the source of the mechanical force that triggers drug microparticle releasing from the micropillar array systems.

7.2 Reconnaissance Work for Future Work

In this thesis, we successfully controlled the assembly and disassembly of the micropillar arrays, and two applications were demonstrated in this promising field. Based on the current research, I will give an outlook for the future applications of micropillar array systems in different areas.

7.2.1 Micropillar Array Optical Sensors

As previously discussed in **Chapter 2**, with uniform size, shape and ordered structures, the micropillar arrays have many applications in the optical field such as ultrathin whitening membranes and light absorption for solar cells. The uniform size and shape can be customized for optical sensors and the flexibility of pillar surface for metal deposition made

it more useful. With SEM images, the actual pillar shape and size dimensions can be directly measured, and the optical properties can be predicted by electromagnetic simulation methods.

Here we demonstrated an example of electromagnetic simulation of the optical properties of subwavelength PDMS micropillars. The model of closed micropillars was shown in **Figure 7.1**.

Figure 7.1 Scheme figure of closed micropillars. Left: front view; right: top view.

The simulation results were as follows, shown in **Figure 7.2** and **7.3**. PDMS without metal film coating was transparent. Thus the transmittance T was around 1.0, and reflectance R was around 0. And when the pillar tops were coated with a layer of silver metal film, it exhibited a peak at 600 nm.

Figure 7.2 Simulation result of metal film coated free-standing micropillars. Period length: $540 \times 540 \text{ nm}^2$, distance between neighboring pillars: 230 nm, radius of the pillar: 45 nm. The tops of pillars were coated with a silver film. R: Reflectance, T: Transmittance.

The pillar arrays had two assembly shapes, as shown in **Figure 7.3**.

Figure 7.3 Schematic figure of micropillar assembly. Left: Model a, right: Model b.

The left assembled structure (Model a) only attached at the pillar tips with the very small contact area, while the right schematic figure (Model b) exhibited larger contact area. It can be tuned by adjusting the pillar height, neighboring pillar distance and the elasticity of micropillar arrays. From Figure 7.4, we can find that these two assembly states significantly changed the peak position and numbers.

Figure 7.4 Optical properties of different assembly patterns. Model a and b are shown in **Figure 7.3**.

This subwavelength PDMS pillar structure can be employed for optical sensors. From the simulation results, we can conclude that the factors that controlled the pillar assembly can influence its optical properties, thus provided a new platform for sensor development.

7.2.2 Auxetic Substrate for High-Efficiency Drug Release

Auxetic materials^[1] are a unique family of materials that have unique mechanical

properties. The Poisson's ratio of the material is negative, which means when adding a longitudinal tensile strain to the material, instead of exhibits lateral contractile strain, the material also undergoes lateral extension. When it is stretched on x-axis, the y-axis also elongates.

Figure 7.5 Auxetic material schematic figure. Single big blue arrows; stretching direction. Triple blue arrows: elongation direction.

In **Figure 7.5** the schematic figure of an auxetic structure is illustrated. The solid lines represent the building materials. The key components in an auxetic structure are stiff construction materials that have little deformation during the stretching process, and flexible joints that allow rotation to all directions.

From **Figure 7.6** we can see that the microparticles can be trapped between assembled pillars. Since PDMS is an elastic material with Poisson's ratio of 0.5, which means the longitudinal elongation equals the lateral compression. Thus, only assembled pillar units on longitudinal direction can be separated by stretching. It limited the releasing efficiency. If auxetic materials are used to build the micropillar arrays, when we stretch the device along an x-axis, instead of compression along the y-axis, it also elongates. It will significantly increase the efficiency of microparticle releasing from the micropillar arrays.

Figure 7.6 Location of microsphere trapped by the PDMS micropillars. The particles are trapped in the confined space between assembled pillars along two directions.

7.2.3 3D Printed Gas Sensor

It has been a major concern for human health that the changes in gastrointestinal microbiome are linked with diseases. To studying the human-microbial interface, many devices had been built for bacteria/cell co-culture and interface study. The microbiome in diabetes, obesity, inflammatory diseases, and cancer varies in composites, total number, microbial species and biochemical reactions^[2]. It is a heated topic to study the human microbiome.

Among all the devices for bacteria/cell co-culture, a real-time monitor/actuator device is the best choice of in-vitro gut model system. Here to realize the function of monitor/actuator system, the micropillar array structure is a good option.

In this thesis, the micropillar arrays were studied in details and precise control of micropillar assembly and disassembly were achieved. Conclusions had been drawn that the mechanical force can induce pillar disassembly. Stretching increased the distance between assembled pillars. As soon as stretching force overcame the adhesiveness of assembled pillars, they were separated.

As a flexible material, there are many ways to deform the PDMS membrane to induce pillar separation. And local curvature change is a one of the useful mechanical deformation method.

Here in Figure 7.7, it demonstrated the local curvature change of a microlens system under elastic deformation.

Figure 7.7 Simulation and experiment results^[3] of mechanics associated flat/deformed of microlens system. (a) (b) (c) XCT images of the microlens system at different deformations. (d) The relationship of deformed geometries and curvature of microlenses.

From **Figure 7.7**, we can find that the curvature change on substrates will lead to separation of pillars by increased neighboring pillar distance. Here this property of micropillar arrays can be used for device design in air pressure sensing and air pressure induced actuators.

In **Figure 7.8** is a demonstration of how air pressure can deform the ultrathin PDMS

micropillar array membrane.

Figure 7.8 Demonstration of air pressure deformed ultra-thin PDMS micropillar array membrane.

In **Figure 7.8**, a 3D printed device was fabricated with a reactor structure. The tested solution was injected into the reactor, and when the reaction started, gas was produced, and the air pressure in the reactor was increased. The air pressure then deformed the ultrathin PDMS micropillar array membrane and induced local curvature change.

The deformation of PDMS membrane can be the source of mechanical force for pillar disassembly. And by loading the micropillar arrays with microparticles with different functions, this device can act as gas sensors and actuators.

7.3 Conclusion

The micropillar array structure, with its unique properties, can be applied to smart device design and fabrication. Its large surface area, high-aspect-ratio, and mechanical properties can be previously controlled for microstructure trapping and controlled releasing.

For future research, the micropillar arrays can be designed for applications such as optical sensors, stretching induced drug releasing system for wound healing on joints, as well as gas sensors and actuators.

References:

- [1] Evans, K. E.; Alderson, A., *Advanced Materials*. **2000**, *12*, 617-628.
- [2] Shah, P.; Fritz, J. V.; Glaab, E.; Desai, M. S.; Greenhalgh, K.; Frachet, A.; Niegowska, M.; Estes, M.; Jager, C.; Seguin-Devaux, C.; Zenhausern, F.; Wilmes, P., *Nat Commun*. **2016**, *7*, 11535.
- [3] Song, Y. M.; Xie, Y.; Malyarchuk, V.; Xiao, J.; Jung, I.; Choi, K. J.; Liu, Z.; Park, H.; Lu, C.; Kim, R. H.; Li, R.; Crozier, K. B.; Huang, Y.; Rogers, J. A., *Nature*. **2013**, *497*, 95-99.

List of publication:

- [1] A New Bacteria Capture Device Based on High Aspect Ratio Micropillar Arrays, *under preparation*.
- [2] Mechanical Stimuli Triggered Drug Controlled Release Platform, *under preparation*.
- [3] Yin, S.; Wu, Y.-L.; Hu, B.; Wang, Y.; Cai, P.; Tan, C. K.; Qi, D.; Zheng, L.; Leow, W. R.; Tan, N. S.; Wang, S.; Chen, X. "Three-dimensional Graphene Composite Macroscopic Structures for Capture of Cancer Cells" *Adv. Mater. Inter.* **2014**, 1, 1300043.
- [4] Tijore, A.; Cai, P.; Nai, M. H.; Li, Z.; Wang, Y.; Tay, C. Y.; Lim, C. T.; Chen, X.; Tan, L. P.* "Role of Cytoskeletal Tension in the Induction of Cardiomyogenic Differentiation in Micropatterned Human Mesenchymal Stem Cell" *Adv. Healthcare Mater.* **2015**, 4, 1399-1407.