Fabrication and Applications of Multifunctional Superhydrophobic Surfaces Based on Surface Chemistry and Morphology

Yang Liu

The Graduate Center, City University of New York

How does access to this work benefit you? Let us know!

Follow this and additional works at: http://academicworks.cuny.edu/gc_etds

Part of the Polymer Chemistry Commons

Recommended Citation

Liu, Yang, "Fabrication and Applications of Multifunctional Superhydrophobic Surfaces Based on Surface Chemistry and Morphology" (2017). CUNY Academic Works.
http://academicworks.cuny.edu/gc_etds/2021

This Dissertation is brought to you by CUNY Academic Works. It has been accepted for inclusion in All Graduate Works by Year: Dissertations, Theses, and Capstone Projects by an authorized administrator of CUNY Academic Works. For more information, please contact deposit@gc.cuny.edu.
FABRICATION AND APPLICATIONS OF MULTIFUNCTIONAL SUPERHYDROPHOBIC SURFACES BASED ON SURFACE CHEMISTRY AND MORPHOLOGY

by

YANG LIU

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2017
Fabrication and Applications of Multifunctional Superhydrophobic Surfaces Based on Surface Chemistry and Morphology

by

Yang Liu

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

__________________________  __________________________
Date                      Alan M. Lyons
                          Chair of Examining Committee

__________________________  __________________________
Date                      Brian R. Gibney
                          Executive Officer

Supervisory Committee:
Professor Alan M. Lyons
Professor Alexander Greer
Professor Sebastien Poget

THE CITY UNIVERSITY OF NEW YORK
ABSTRACT

Fabrication and Applications of Multifunctional Superhydrophobic Surfaces Based on Surface Chemistry and Morphology

by

Yang Liu

Advisor: Professor Alan M. Lyons

Superhydrophobic surfaces are gaining great interests in both fundamental researches and technological applications, because of their unique non-wetting and self-cleaning properties. By mimicking the hierarchical surface structure of the natural superhydrophobic surface, i.e. lotus leaf, numerous artificial superhydrophobic surfaces were developed. However, the challenge is how to fabricate superhydrophobic surfaces by a scalable and economical method. To address this challenge, our group has developed methodologies that enable the fabrication of superhydrophobic surfaces in inexpensive and potentially scalable ways, such as lamination and 3-D printing. To expand on applications, we also combined other desired functionalities into the superhydrophobic surfaces.

The transparent superhydrophobic surface has a great advantage of highly visible light transmittance, which make it have potential applications for solar-cell panels, optical lens, and automobile windshields, etc. Superhydrophobicity can be achieved by constructing hierarchical roughness on the surface of low surface energy material. However, the roughness may increase light scattering and lower the transparency. To minimize the affection on transparency, roughness at small scales, i.e. nanometers, is required. In Chapter 2, I discuss the fabrication of a transparent superhydrophobic surface by dip-coating and lamination method. The polymer substrate is first coated with a layer
of silica nanoparticles; the following lamination process makes the nanoparticles partially embedded into polymer substrate which increases the mechanical stability. The transparency was measured by UV-Vis spectroscopy. The surface morphology was characterized by scanning electron microscope and atomic force microscope. The mechanical stability of fabricated transparent superhydrophobic surface was evaluated by using a water flushing method.

Photocatalytic properties can also be integrated into superhydrophobic surfaces, which will enhance the self-cleaning property by removing the contaminations through photo-oxidation reactions. Photocatalytic superhydrophobic surfaces also have potential applications in water disinfection, treatment of organic waste solutions, and photodynamic therapy. In Chapter 3, two different methods were developed to fabricate photocatalytic superhydrophobic surfaces: 1. The nanocomposite of TiO$_2$ and polymer was created by a lamination method. The surface roughness was controlled by templating during the lamination. Surfaces also fabricated without the templating process. All the surfaces exhibited reversible wettability and photocatalytic properties; 2. Photocatalytic particles (TiO$_2$ or silicon-phthalocyanine) were immobilized on the surfaces of printed polydimethylsiloxane cone shape posts. The triple-level roughness (posts, particle aggregates, and individual particles) make the fabricated surface superhydrophobic and maintaining stable Cassie state during photo-reactions. In a specially designed three-phase photo-reactor, photocatalytic reactions such as photooxidation of Rhodamine B and bovine serum albumin, and singlet oxygen trapping were studied as a function of gas phase composition. The effect of bubbling through the liquid phase, which facilitates the transmission of reactive species were also discussed in Chapter 3. Base on the
photocatalytic TiO\textsubscript{2}/Polymer nanocomposite film we have made, we demonstrated an application of this film in photodegrading waste organic dye solution generated in biology teaching laboratories. Furthermore, we developed a laboratory module for an undergraduate analytical chemistry lab course. In this course, students will learn about the TiO\textsubscript{2} photocatalytic mechanism; degrade waste solutions collected from laboratories using sunlight and the TiO\textsubscript{2}/PE catalytic bags and investigate the degradation efficiency using UV-Vis absorption spectroscopy measurements.

On a superhydrophobic surface, an aqueous droplet (<100 µL) can maintain a nearly spherical shape without wetting the surface. This geometry creates a unique environment in which chemical reactions at the solid-liquid-vapor interphase can be studied. Two types of superhydrophobic surfaces were fabricated using modified 3-D printing methods. In one case, which is discussed in Chapter 4, functionalized superhydrophobic surfaces were fabricated in which reactive particles are partially embedded into the printed PDMS posts. On this surface, interactions between the solid surface and solute molecules were studied as a function of convection within the droplet. In the second case, which is discussed in Chapter 5, glass pedestals were attached to the top of each PDMS post in the array. These glass pedestals enable the precise dispensing of nanoliter (25.0 nL ± 0.5 nL) droplets. This surface can also support larger (> 1µL) droplets while exhibiting contact angles >150°. Evaporation of droplets promotes the concentration of dilute solute molecules into a well-defined region that facilitates the identification of biopolymers in quantities as low as 5 attomoles, by MALDI-TOF mass spectrometry. In addition, this surface can be functionalized to selectively bind specific
biomolecules that can be subsequently identified by MALDI-TOF. This type of surface is especially useful for working with precious fluids such as venom from snakes and spiders.

With the advantage of precise dispensing of nanoliter droplets, we further improved the dispensing system by printing only PDMS post arrays of a special morphology structure on a glass slide to form a nano-Droplet Array Plate (nDAP). By using the nDAP dispensing system, I was able to study the effect of surfactant chemistry on the distribution of hydrophobic microbeads (35 µm) in the aqueous droplet and the dispensing properties. The number of microbeads dispensed was controlled by tuning the relative concentration of microbeads and surfactant. Multiple single-bead dispensing was achieved at optimized conditions. This work is discussed in Chapter 6.
# Table of Contents

Chapter 1. Introduction ........................................................................................................... 1

1.1 Wetting theories ................................................................................................................. 2

1.2 Multifunctional superhydrophobic surfaces .................................................................... 5

   1.2.1 Transparent Superhydrophobic surface .................................................................... 5

   1.2.2 Photocatalytic Superhydrophobic surface ................................................................. 6

   1.2.3 Reactions in droplets: microreactors on multifunctional superhydrophobic surfaces 11

1.3 Precise dispensing system: Nanoliter virtual well microplate (nVMP) and nanodroplet array plate (nDAP) ................................................................................................................. 13

1.4 Surfactants for dispersed system ...................................................................................... 16

   1.4.1 Polymeric nonionic surfactants ................................................................................. 18

   1.4.2 Adsorption of polymeric nonionic surfactant at solid hydrophobic surfaces 19

   1.4.3 Stabilization of suspensions with surfactants ........................................................... 20

Chapter 2. Fabrication and characterization of transparent superhydrophobic surface . . . . . 22

2.1 Introduction ......................................................................................................................... 22

2.2 Experimental ....................................................................................................................... 22

   2.2.1 Fabrication of transparent superhydrophobic surface ............................................. 22

   2.2.2 Characterizations ......................................................................................................... 28

   2.2.3 Surface durability ......................................................................................................... 29

2.3 Results and discussions ...................................................................................................... 30

   2.3.1 Effect of dip-coating cycles ......................................................................................... 30

   2.3.2 Effect of lamination pressure ....................................................................................... 33

   2.3.3 Effect of lamination temperature ................................................................................ 36

   2.3.4 Effect of nano-particle type on mechanical stability in water tunnel test ............. 37

2.4 Conclusions ......................................................................................................................... 43

Chapter 3. Fabrication and characterization of polymer nanocomposites with
superhydrophobic and catalytic properties .............................................................................. 44

3.1 Introduction ......................................................................................................................... 44

   3.2.1 Experimental ............................................................................................................... 46

   3.2.1.1 Materials .................................................................................................................. 46

   3.2.1.2 Fabrication of TiO2-PE nanocomposite films ......................................................... 46

   3.2.1.3 UV illumination experiments .................................................................................. 47
### Chapter 3: Fabrication of TiO$_2$-Polymer Nanocomposite Surfaces

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.1.4 Characterization</td>
<td>48</td>
</tr>
<tr>
<td>3.2.1.5 Photodegradation experiment</td>
<td>48</td>
</tr>
<tr>
<td>3.2.2 Results and discussion</td>
<td>49</td>
</tr>
<tr>
<td>3.2.2.1 Fabrication of TiO$_2$-polymer nanocomposite surface</td>
<td>49</td>
</tr>
<tr>
<td>3.2.2.2 Superhydrophobic properties</td>
<td>52</td>
</tr>
<tr>
<td>3.2.2.3 UV-induced reversible wettability</td>
<td>52</td>
</tr>
<tr>
<td>3.2.2.4 Photodegradation of RhB</td>
<td>56</td>
</tr>
<tr>
<td>3.2.3 Conclusions</td>
<td>58</td>
</tr>
</tbody>
</table>

### Chapter 3.3: Photocatalytic TiO$_2$-Nanocomposite Films for Organic Dyes Degradation

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.1 Experimental</td>
<td>58</td>
</tr>
<tr>
<td>3.3.1.1 Fabrication of TiO$_2$-polyethylene nanocomposite films</td>
<td>59</td>
</tr>
<tr>
<td>3.3.1.2 Degradation of organic dyes used in undergraduate instructional laboratories</td>
<td>60</td>
</tr>
<tr>
<td>3.3.1.3 Bacteria inactivation experiment</td>
<td>60</td>
</tr>
<tr>
<td>3.3.2 Results and discussions</td>
<td>62</td>
</tr>
<tr>
<td>3.3.2.1 Degradation of organic dyes used in undergraduate instructional laboratories</td>
<td>62</td>
</tr>
<tr>
<td>3.3.2.2 Reusability test of plastic TiO$_2$ bags</td>
<td>66</td>
</tr>
<tr>
<td>3.3.2.3 Bacteria inactivation experiment</td>
<td>67</td>
</tr>
<tr>
<td>3.3.3 Conclusion</td>
<td>68</td>
</tr>
</tbody>
</table>

### Chapter 3.4: Photocatalytic Superhydrophobic Surfaces

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4.1 TiO$_2$-PDMS self-cleaning superhydrophobic surface</td>
<td>69</td>
</tr>
<tr>
<td>3.4.1.1 Experimental</td>
<td>69</td>
</tr>
<tr>
<td>3.4.1.2 Results and discussions</td>
<td>71</td>
</tr>
<tr>
<td>3.4.1.3 Conclusions</td>
<td>83</td>
</tr>
<tr>
<td>3.4.2 Silicon phthalocyanine-PDMS superhydrophobic surface on porous membrane: generation of singlet oxygen, effect of gas flow and sensitizer wetting on trapping efficiency</td>
<td>84</td>
</tr>
<tr>
<td>3.4.2.1 Experimental</td>
<td>85</td>
</tr>
<tr>
<td>3.4.2.2 Results and discussions</td>
<td>91</td>
</tr>
<tr>
<td>3.4.2.3 Conclusions</td>
<td>102</td>
</tr>
</tbody>
</table>

### Chapter 4: Reaction in Individual Droplets on a Superhydrophobic Surface

<table>
<thead>
<tr>
<th>Subsection</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Introduction</td>
<td>104</td>
</tr>
</tbody>
</table>
4.2 Experimental.................................................................................................................. 106
  4.2.1 Design of environmental chamber ........................................................................ 106
  4.2.2 Fabrication of multifunctional superhydrophobic surfaces.................................... 107
  4.2.3 Generation of singlet oxygen on a photocatalytic superhydrophobic surface . 107
  4.2.4 Pretreatment of glass particles.................................................................................. 108
  4.2.5 NeutrAvidin binding with biotin modified superhydrophobic surface .............. 109
4.3 Results and discussion .................................................................................................. 111
  4.3.1 Convection within droplet on a superhydrophobic surface..................................... 111
  4.3.2 Effect of convection on trapping of singlet oxygen .................................................. 113
  4.3.3 Effect of convection on interaction between NeutrAvidin and biotin ................. 117
4.4 Conclusion ...................................................................................................................... 125
Chapter 5. Controlled precise dispensing of nanoliter droplets for detecting biomolecules with high sensitivity.................................................................................................................. 126
  5.1 Introduction.................................................................................................................... 126
  5.2 Experimental............................................................................................................... 126
      5.2.1 Fabrication of the nVWP ..................................................................................... 126
      5.2.2 Glass pedestal preparation ................................................................................... 128
      5.2.3 Dispensing of nanoliter droplets onto the nVWP .............................................. 128
      5.2.4 MALDI-TOF detection of proteins on nVWP surfaces deposited from nanoliter droplets ............................................................................................................................ 131
      5.2.5 Detection of selectively adsorbed peptides on nikel-chelated treated glass nVWP surfaces ............................................................................................................................ 132
  5.3 Results and discussions................................................................................................. 133
      5.3.1 nVWP fabrication ............................................................................................... 133
      5.3.2 Dispensing Process ............................................................................................... 134
      5.3.3 Detection sensitivity of proteins on nVWP by MALDI-TOF ......................... 135
      5.3.4 Selective binding of a snake venom peptide by an ion-channel protein anchored on nVWP ............................................................................................................................ 136
  5.4 Conclusion ...................................................................................................................... 138
Chapter 6. Single particle dispensing, effect of surfactant chemistry on the distribution of hydrophobic microbeads ............................................................................................................ 140
  6.1 Introduction.................................................................................................................... 140
  6.2 Experimental............................................................................................................... 146
      6.2.1 Fabrication of nDAP ......................................................................................... 146
6.2.2 Preparation of microbeads dispersion in surfactant solution ....................... 147
6.2.3 Dispensing of a nanoliter droplet onto nDAP ........................................ 147
6.3 Results and discussions .............................................................................. 149
  6.3.1 Effect of ethylene oxide/propylene oxide chain length on dispersion quality 149
  6.3.2 Effect of surfactant concentration .......................................................... 160
  6.3.3 Universal curve of microbeads dispensing .............................................. 161
  6.3.4 Multiple dispensing ............................................................................... 163
  6.3.5 Single microbeads dispensing ................................................................. 167
6.4 Conclusion .................................................................................................. 170
Bibliography ...................................................................................................... 172
Chapter 1 .......................................................................................................... 172
Chapter 2 .......................................................................................................... 176
Chapter 3 .......................................................................................................... 177
Chapter 4 .......................................................................................................... 179
Chapter 5 .......................................................................................................... 180
Chapter 6 .......................................................................................................... 180
List of Figures

Figure 1.1. Surface tension diagram of Young’s equation and associated contact angle. .......................... 2
Figure 1.2. Advancing and receding dynamic contact angles. ............................................................... 3
Figure 1.4. Schematic illustration of the formation of photogenerated charge carriers (holes and electrons) upon absorption of UV light. ................................................................. 7
Figure 1.5. Switchable wettability of TiO$_2$ surfaces. ................................................................. 9
Figure 1.6. Hydroxyl groups formation on TiO$_2$ during UV irradiation process. ................. 10
Figure 1.7. Schematic structure of a surfactant. ................................................................. 17
Figure 1.8. Surfactant classification according to the composition of head group. .......... 18
Figure 2.1. DSC plot of ZF16 polymer film. ................................................................. 25
Figure 2.2. TGA plot of ZF16 polymer film. ................................................................. 26
Figure 2.3. UV-Vis spectrum of ZF16 polymer film. ................................................................. 27
Figure 2.4. Lamination of dip-coated polymer film. ................................................................. 28
Figure 2.5. Illustration of water tunnel test. ................................................................. 30
Figure 2.6. SEM images of dip-coated surfaces. ................................................................. 32
Figure 2.7. Light transmittance spectra of fabricated superhydrophobic films and ZF16 without treating. ................................................................. 33
Figure 2.8. Transmittance plotted vs. lamination pressure. ................................................................. 34
Figure 2.9. Illustration of effect of pressure on lamination process. ................................................................. 35
Figure 2.10. The effect of lamination temperature on light transmittance. ................................................................. 36
Figure 2.11. Stability of superhydrophobic surface under water flushing test. ................. 39
Figure 2.12. XPS spectra of superhydrophobic surface A before and after water flushing test. ................................................................. 40
Figure 2.13. The change of light transmittance during water tunnel test. ................................................................. 42
Figure 2.14. Stability of superhydrophobic surfaces fabricated at different conditions... 42
Figure 3.1. Schematic of the formation of the microstructures during the processing.... 47
Figure 3.2. SEM images of the fabricated surfaces. ....................................................... 51
Figure 3.3. Static contact angle measurement on TiO₂-polymer surface. .................. 52
Figure 3.4. The change of CA as a function of illumination time. ......................... 55
Figure 3.5. UV-Vis spectra of RhB solution at different UV exposure time on TiO₂ photocatalytic film. ................................................................. 57
Figure 3.6. UV-Vis spectra of RhB solution on TiO₂ photocatalytic film without UV exposure. ........................................................................................................... 57
Figure 3.7. Plot of RhB concentration change during photodegradation. ............... 58
Figure 3.8. Outdoor exposure experiment. ................................................................. 64
Figure 3.10. Reusability of TiO₂-PE nanocomposite film........................................ 67
Figure 3.11. Bacteria survival ratio.................................................................................. 68
Figure 3.12. SEM images of a PDMS post printed on 0.5 mm pitch, partially embedded with TiO₂ nanoparticles and optical images of a 20 µL water drop on the surface.... 72
Figure 3.13. 20 µL water droplet residing on TiO₂-PDMS surface.............................. 73
Figure 3.14. Optical images of the liquid-air interface position relative to the PDMS post surface after UV irradiation on a TiO₂-PDMS surface................................. 74
Figure 3.15. Schematic drawing of the cuvette reactor with a gas bubble formed on the surface. ........................................................................................................... 75
Figure 3.16. Optical photographs of a superhydrophobic surface composed of TiO₂ particles in cuvette photocatalytic reactor. ......................................................... 75
Figure 3.17. UV-vis spectrum of Rhodamine B at different irradiation times on a TiO$_2$-PDMS surface. ................................................................. 76
Figure 3.18. Change in RhB concentration as a function of irradiation time. .............. 76
Figure 3.19. Degradation of RhB under different plastron gas conditions. ................. 78
Figure 3.20. Degradation of RhB on different surfaces at static ambient air conditions.. 79
Figure 3.21. Optical microscope images of RhB coated TiO$_2$-PDMS posts before and after UV irradiation................................................................. 80
Figure 3.22. Confocal images of protein coated TiO$_2$-PDMS posts before and after UV irradiation. .............................................................................. 82
Figure 3.23. Confocal images of protein coated SiO$_2$-PDMS posts before and after UV irradiation................................................................. 82
Figure 3.24. A schematic of the fabrication of surface B. .............................................. 86
Figure 3.25. A schematic of the fabrication of surface C. ............................................. 86
Figure 3.26. SEM images of PDMS posts coated with Pc particles at controlled locations. ......................................................................................... 87
Figure 3.27. Schematic images of PDMS posts coated with Pc particles at controlled locations. ......................................................................................... 88
Figure 3.28. Geometry of the $^1$O$_2$ photoreactor device.......................................... 89
Figure 3.29. Microstructure and optical images of Pc-PDMS surface. ......................... 91
Figure 3.30. Endoperoxide 2 yield in static experiments where D$_2$O solutions were presaturated with O$_2$ or N$_2$. ......................................................... 93
Figure 3.31. Optical images of surface A. ................................................................. 94
Figure 3.32. Endoperoxide yield in bubbling experiments where O$_2$ or N$_2$ gas was sparged through the plenum into the D$_2$O solution.......................................................... 95

Figure 3.33. Mechanism of singlet oxygen generation via O$_2$ flowing through the plastron of a superhydrophobic sensitizer surface.............................................................. 100

Figure 4.1 Schematic of environmental test chamber for studying the effect of convection on reactions in micro-droplets on a multifunctional superhydrophobic surface............. 106

Figure 4.2. Schematic of the binding reaction at liquid-solid interface......................... 111

Figure 4.3. A 10 µL water droplet on the superhydrophobic photocatalytic surface. ... 112

Figure 4.4. SEM image of the superhydrophobic photocatalytic surface..................... 112

Figure 4.5. Convective motion of particles in a droplet. .......................................... 113

Figure 4.6. Effect of convection and concentration on the decrease of (1) after 1 hour of reaction.................................................................................................................. 117

Figure 4.7. A 10 µL water droplet on the superhydrophobic surface fabricated with biotinylated glass particles................................................................. 118

Figure 4.8. SEM image of the biotinylated superhydrophobic surface....................... 118

Figure 4.9 Confocal images of biotinylated superhydrophobic surface after binding with fluorescent tagged NeutrAvidin......................................................... 122

Figure 4.10 Fraction of biotin coverage (θ) plotted as a function of time at different conditions and comparison with reference. ......................................................... 123

Figure 4.11 Concentration profiles plotted as a function of height above the superhydrophobic surface at different times for a static droplet................................. 124

Figure 4.12 Concentration of NeutrAvidin at a height of 200 µm above the surface plotted as a function of time....................................................................................... 124
Figure 5.1. Process schematic for the fabrication of nVWPs........................................ 127
Figure 5.2. Schematic of the automated dispensing process......................................... 129
Figure 5.3. Optical images of the modified MALDI target plate.................................... 132
Figure 5.4. Schematic of selective adsorption of peptides on nickel-chelated treated glass pedestals................................................................. 133
Figure 5.5. Optical image of fabricated nVWP ............................................................ 134
Figure 5.6. Images taken high-speed camera illustrating the dispensing process.......... 135
Figure 5.7. MALDI-TOF results for NeutrAvidin-matrix solutions deposited as 30 nL droplets on nVWP substrate with 500 μm diameter glass pedestals.......................... 136
Figure 5.8. MALDI-TOF spectra of solutions deposited on Ni-chelate coated glass pedestals................................................................. 138
Figure 6.1. Schematic process of direct die placement.................................................. 143
Figure 6.2. A schematic illustration of die placement using LEAP................................ 144
Figure 6.1. Optical image of nDAP.............................................................................. 147
Figure 6.2 nDAP dispensing system........................................................................... 149
Figure 6.3. A 130 nL droplet containing 2 mg/mL microbeads was dispensed on an nDAP post........................................................................................................... 149
Figure 6.4. HLB grid of Pluronic surfactants.................................................................. 150
Figure 6.5. Images taken with a high-speed camera illustrating the dispensing process. 153
Figure 6.6. Effect of hydrophilic PO chains on microbeads dispensing........................ 157
Figure 6.7. Schematic illustration of surfactants on the surface of microbead.............. 160
Figure 6.8. Effect of surfactant concentration on the number of microbeads dispensed in a 132 nL droplet................................................................................. 161
Figure 6.9. Universal curve of dispensing property................................. 163
Figure 6.10. Number of microbeads dispensed in multiple dispensing experiments. .. 165
Figure 6.11. Number of microbeads dispensed in multiple dispensing experiments. .. 166
Figure 6.12. Number of microbeads dispensed in multiple dispensing experiments. .. 166
Figure 6.13. Probability of a single microbead dispensed plotted as a function of the measured average number of microbeads in each droplet. ................................. 169
List of Tables
Table 2.1. Properties of candidate polymers ................................................................. 24
Table 2.2. Physical properties of AEROSIL silica nanoparticles ........................................ 24
Table 2.3. Water contact angles and stability of prepared superhydrophobic surfaces with different dip-coating cycles .................................................................................. 32
Table 2.4. Water contact angles and stability of prepared superhydrophobic surfaces at different lamination pressure .......................................................................................... 35
Table 2.5. Water contact angles and stability of prepared superhydrophobic surfaces at different lamination temperatures .................................................................................. 37
Table 2.6. Transparent superhydrophobic surface prepared at different conditions .......... 38
Table 2.7. Element ratios on transparent superhydrophobic surfaces .............................. 41
Table 3.1. Dye solution concentration before and after 2.5h UV irradiation ...................... 64
Table 3.2. Singlet Oxygen Trapping Experiments ........................................................... 96
Table 3.3. Effect of Sensitizer Location on $^{1}$O₂ Trapped in D₂O Solution ...................... 98
Table 4.1. Effect convection rate on singlet oxygen trapping .............................................. 115
Table 4.2. Effect of initial concentration on singlet oxygen trapping ................................. 116
Table 6.1. Properties of Pluronic® surfactants used in this study ........................................ 151
Table 6.2. Measurements of dispensed volume .................................................................... 154
Table 6.3. Calculation of the number of beads in droplet assuming an ideal dispersion 156
Table 6.4. Comparison of experimental results with theoretical values ............................ 158
Table 6.5. Probability of dispensing a single microbead ..................................................... 168
List of Schemes

Scheme 3.1. Photodegradation of RhB on TiO$_2$-HDPE film........................................... 49
Scheme 3.2. The structure of Rhodamine B, Crystal violet, Methylene blue and Fuchsin.
................................................................................................................................................. 63
Scheme 3.1 Singlet oxygen trapping reaction................................................................. 90
Scheme 4.1 Reaction of singlet oxygen with 9,10 anthracene dipropionic acid .......... 108
Scheme 6.1 General structure of Pluronic surfactants. ............................................. 150
Chapter 1. Introduction

Superhydrophobic surfaces are non-wettable by water. When a water droplet is placed on a superhydrophobic surface, it exhibits a contact angle (CA) greater than 150° and a contact angle hysteresis (the difference between advancing and receding CAs) of typically less than 10°. Many biological surfaces in nature exhibit superhydrophobicity, such as lotus leaves, water strider legs, cicada wings, mosquito eyes, etc. Superhydrophobic surfaces have been investigated intensively over the last few decade, in order to understand the relationship between surface chemistry, morphology, and wettability. From an application view, superhydrophobic surfaces will become particularly useful when several functions are integrated together such as: electrical conductivity, stimuli-responded switching of wettability, transparency, photocatalytic properties and self-healing after damage. However, practical applications typically require simplified low-cost and scalable fabrication procedures and robust stability. In the first part of this thesis, I will discuss the preparation of several types of robust multifunctional superhydrophobic surfaces, including transparent, photocatalytic and bio-reactive superhydrophobic surfaces using economically viable methods. Fundamental chemical reaction studies were performed on the fabricated surfaces. In the second part of this thesis, I will discuss two projects where surfaces with controlled wettability were used to precisely dispense fluids for biological assays or electronic fabrication. One project focused on the sensitive detection of biomolecules. The other project studied the effect of surfactant chemistry on the distribution of hydrophobic microbeads (35 µm) in aqueous droplets. The number of microbeads dispensed was controlled by tuning the relative concentration of microbeads and surfactant.
1.1 Wetting theories

The wetting behavior of solid surfaces by a liquid is usually characterized by the contact angle (CA). The CA is an important fundamental concept in all solid-liquid/vapor interfacial phenomena.\textsuperscript{[51,52]} The measurement of CA usually assumes a water droplet is on a smooth, planar, rigid and homogeneous surface. The wettability of a solid surface by a liquid can be quantified by Young’s equation. For a liquid drop in coexistence with a vapor phase that is on a solid substrate, Young’s equation (Equation 1.1) describes the energy balance between the interfacial tensions at the solid-liquid-vapor contact line.

\[
\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta_y
\]  

(1.1)

In Young’s equation, \(\theta_y\) is the observed contact angle and \(\gamma_{SV}\), \(\gamma_{SL}\) and \(\gamma_{LV}\) refer to the interfacial surface tensions between vapor (V), liquid (L), and solid (S) (Figure 1.1).

![Figure 1.1. Surface tension diagram of Young’s equation and associated contact angle.](image-url)
Another important concept to describe the behavior of drops at the solid-liquid interface is contact angle hysteresis (CAH).\textsuperscript{[18]} The contact angle hysteresis is defined as the difference between advancing contact angle ($\theta_a$) and receding contact angle ($\theta_r$): $\Delta \theta = \theta_a - \theta_r$. Different from static contact angle, advancing and receding contact angles are also called dynamic contact angle. \textbf{Figure 1.2} shows the dynamic contact angles (advancing and receding) of a water droplet on a tilted surface. The water drop advances at the lower side and recedes at the upper side. In order to make the drop to slide, the tilt angle should larger than a critical value, which is the sliding (or slip) angle. The CAH depends upon the surface roughness as well as the surface chemistry; it stays constant during the sliding process as long as the surface is uniform.

Since Young’s equation describes an ideal system in its state of thermodynamic equilibrium,\textsuperscript{[53,54]} some other factors are not considered in this ideal surface, such as surface roughness, chemical heterogeneity, swelling, etc. Surface roughness is an important factor on the wettability of solid surfaces, which results in a deviation of the contact angle from the value established by Young’s equation.\textsuperscript{[24]} Generally, static contact angle increases as a result of surface roughness and structure topography. Two models were developed to describe the apparent contact angle on a rough surface, which known as the
Wenzel and Cassie-Baxter models.\textsuperscript{[55-59]} In the Wenzel model, the apparent contact angle is related to the surface roughness. It can be written as \textbf{Equation 1.2}:

\[
\cos \theta_w = r \cos \theta
\] (1.2)

where \(\theta_w\) is the apparent contact angle on the rough surface, \(\theta\) is the contact angle on an ideal smooth surface, \(r\) is the roughness factor, which defined as the ratio of the actual area to its projection, i.e. \(r = \frac{\text{area}_{\text{rough}}}{\text{area}_{\text{smooth}}}\). Since \(r\) is always greater than 1, the Wenzel’s equation suggests that if \(\theta < 90^\circ\) then \(\theta_w\) is smaller than \(\theta\). In this case, the roughness will enhance the wetting. On the other hand, when \(\theta > 90^\circ\) then \(\theta_w\) is greater than \(\theta\) and the hydrophobicity of the surface is enhanced by roughness. The Wenzel’s model assumes the liquid penetrates into the surface roughness (Figure 1.3b), and therefore it describes the homogeneous wetting state. The Cassie-Baxter’s model corresponds to the heterogeneous wetting state, in which the liquid drop only contacts with the top region of the surface with a layer of air underneath (Figure 1.3c). So the contact area can be seen as composed of two phase: solid and vapor, with a fraction of \(f_s\) and \(f_v\) respectively, where \(f_s + f_v = 1\). The Cassie-Baxter model can be expressed by \textbf{Equation 1.3}:

\[
\cos \theta_{CB} = f_s \cos \theta_s + f_v \cos \theta_v
\] (1.3)

where \(\theta_{CB}\) is the apparent contact angle in Cassie-Baxter model, \(\theta_s\) is the contact angle on the smooth solid surface, \(\theta_v\) is the contact angle in the vapor phase. Since \(\theta_s = \theta\) and \(\theta_v = 180^\circ\), the Cassie-Baxter equation can be simplified as \textbf{Equation 1.4}:

\[
\cos \theta_{CB} = f_s (\cos \theta + 1) - 1
\] (1.4)
Different from the Wenzel’s model, the apparent contact angle in Cassie-Baxter model will increase as increasing surface roughness even on an intrinsic hydrophilic ($\theta<90^\circ$) surface, because of the superhydrophobic vapor component of the surface.

![Typical wetting models of a droplet on solid substrates](image)

**Figure 1.3.** Typical wetting models of a droplet on solid substrates, (a) A liquid drop on a flat substrate (Young’s model). (b) Wetted contact between the liquid and the rough substrate (Wenzel’s model). (c) Non-wetted contact between the liquid and the rough substrate (Cassie’s model).[60]

### 1.2 Multifunctional superhydrophobic surfaces

#### 1.2.1 Transparent Superhydrophobic surface

Superhydrophobic surfaces provide the self-cleaning properties that can prevent dirt accumulation, fouling, and icing, which are beneficial for optical equipment and devices, such as lenses, photovoltaic panels, and windows. For these optical applications, high transparency is required. However, the superhydrophobic property is usually achieved by combining surface roughness and low surface energy. When considering the surface roughness, transparency is competitive with the hydrophobicity, because surface roughness may lead to light scattering when the roughness scale is greater than a quarter of the wavelength of visible light. Thus, the roughness has to be controlled to fabricate a superhydrophobic surface with high transparency. Generally, the scale of surface

---

5
roughness should be lower than one-quarter of the wavelength of visible light, so that the scattering of light is minimized. \[42,61\] Recently, transparent superhydrophobic surfaces/coatings have been prepared on different substrates (glass, polymers) by using several different methods, such as spray coating,\[62,63\] spin coating,\[64,65\] dip coating,\[66-67\] modified blade-coating,\[68\] lithography,\[69\] templating,\[43\] plasma polymerization,\[70\] layer-by-layer coating,\[71\] and chemical vapor deposition.\[72\] Most of these methods required a post-treatment with low surface energy material, such as fluorosilane, chlorosilane, perfluorocarbon and fluoropolymers. For the applications, mechanical robustness is particularly important because the nano-scale roughness (≤150 nm) could be easily destroyed, leading to the loss of superhydrophobicity. In my study, I developed a method to fabricate transparent superhydrophobic surfaces by dip coating and lamination on a transparent polymer substrate. The lamination process significantly improved the mechanical stability of the fabricated surface. To quantify the mechanical stability a flowing water test was performed. This work is discussed in Chapter 2.

1.2.2 Photocatalytic Superhydrophobic surface

The photocatalytic properties of TiO\(_2\) for splitting water was first discovered by Fujishima and Honda in 1972. Over the past decades, the use of TiO\(_2\) as a semiconducting heterogeneous photocatalyst for the photodegradation of organic pollutants has been extensively investigated. TiO\(_2\) is a semiconductor material which can generate positive holes and negative electrons upon the absorption of UV light corresponding to the band gap energy (about 3.2 eV) (Figure 1.4).\[73-76\] The positive hole will oxidize H\(_2\)O to
generate hydroxyl radicals, and the negative electron will reduce O$_2$ to form superoxides:$^{[73]}$

\[
\begin{align*}
\text{TiO}_2 + h\nu &\rightarrow e^- + h^+ \\
\text{OH}^- + h^+ &\rightarrow \cdot\text{OH} \\
\text{H}_2\text{O} + h^+ &\rightarrow \cdot\text{OH} + \text{H}^+ \\
\text{O}_2 + e^- &\rightarrow \cdot\text{O}_2^- \\
\cdot\text{O}_2^- + \text{H}^+ &\rightarrow \cdot\text{OOH}
\end{align*}
\]

The radicals formed in the above process are powerful oxidation agents, which can oxidize a wide range of organic molecules (e.g. pollutants) to mineralize products (e.g. carbon dioxide and water) and other small molecule products.

**Figure 1.4.** Schematic illustration of the formation of photogenerated charge carriers (holes and electrons) upon absorption of UV light.$^{[77]}$
Photocatalytic materials, such as TiO$_2$, can enhance the self-cleaning properties of superhydrophobic surfaces by oxidizing organic contaminants with the generated reactive oxygen species upon UV irradiation. Common semiconductor oxide materials, such as TiO$_2$, ZnO, and V$_2$O$_5$ which are widely studied for their photocatalytic properties, have been used to fabricate superhydrophobic surfaces.$^{[78-85]}$

In addition, TiO$_2$ surfaces have been shown to exhibit light-induced switching of wettability between hydrophobic and hydrophilic states. The UV light-induced amphiphilicity of TiO$_2$ surfaces for both water and oil liquids was first reported by Fujishima et al. in 1997.$^{[14]}$ They found that before UV irradiation, the TiO$_2$ film exhibited a water contact angle of about 72°, while after UV irradiation, water droplets spread completely on the film, resulting in a water contact angle of about 0° (Figure 1.5).$^{[14]}$ Also, a similar behavior was found for organic liquids, such as hexadecane and glycerol trioleate. The wettability of TiO$_2$ surfaces was reversible between superhydrophilic and hydrophobic under alternative UV irradiation and long-term storage in the dark.
**Figure 1.5.** Switchable wettability of TiO$_2$ surfaces. (a) A hydrophobic surface before UV irradiation. (b) A superhydrophilic surface after UV irradiation. (c) Exposure of a hydrophobic TiO$_2$-coated glass to water vapor. The formation of fog (small water droplets) hindered the view of the text on paper placed behind the glass. (d) Creation by UV irradiation of an antifogging surface. The high hydrophilicity of the surface prevents the formation of water droplets, making the text clearly visible.$^{[14]}$

It was suggested that the contact angle of TiO$_2$ surface is related to the density of surface hydroxyl groups. The reversible formation of hydroxyl groups on the TiO$_2$ surface under UV is illustrated in **Figure 1.6.**$^{[86]}$ Photoexcited Electrons are excited by UV light and captured by the molecular oxygen. As a result, the positive holes diffuse to TiO$_2$ surfaces, and are trapped at lattice oxygen atoms (**Figure 1.6b**). Then the binding energy between Ti and the oxygen is weakened, and the bond is broken by the water molecule, resulting in the formation of new hydroxyl groups (**Figure 1.6c**). The TiO$_2$ surface with hydroxyl groups produced by UV light irradiation has high surface energy, which is more hydrophilic than the original surface. During the dark storage, the thermodynamically less stable hydroxyl groups gradually detached from the surface in the form of H$_2$O$_2$ or H$_2$O and O$_2$. This indicates that the TiO$_2$ surface converts back to its initial condition, which is less hydrophilic.
Figure 1.6. Hydroxyl groups formation on TiO$_2$ during UV irradiation process. a. Before UV irradiation, the OH group is bound to oxygen vacancy; b. At the transition state, the photo-generated hole is trapped at the lattice oxygen; c. after UV irradiation new OH groups are formed.$^{[86]}$

In Chapter 3, I describe a simple and inexpensive lamination method (with and without templating) for fabricating superhydrophobic TiO$_2$-polymer nanocomposite surfaces that exhibit UV-induced reversible wettability and photocatalytic properties. Photodegradation of organic dyes using the prepared TiO$_2$-polymer nanocomposite surfaces was studied. A set of bacteria deactivation experiments were performed to show the potential application of TiO$_2$ film in water purification. Based on these studies, I also designed an experiment for an undergraduate analytical laboratory to introduce students to the basic photochemical reactions and the characterization of the photooxidation rates by standard UV-vis spectroscopy analytical methods.

TiO$_2$ is a hydrophilic material, so fabricating a superhydrophobic surface with TiO$_2$ is especially challenging and so usually requires a surface modification with low
surface energy material and/or a high surface roughness. It is even challenging to fabricate a superhydrophobic TiO$_2$ surface that exhibits stable superhydrophobicity, i.e. maintaining Cassie state, during photocatalytic reactions because the TiO$_2$ surface shows UV-induced increased wettability and the photoreactivity of TiO$_2$ may oxidize the hydrophobic surface modification. To address these challenges, a method was developed to fabricate a TiO$_2$ photocatalytic surface with stable superhydrophobicity by immobilizing TiO$_2$ nanoparticles on an array of printed PDMS posts. The hierarchical roughness is sufficient to stabilize water in the Cassie state even when the TiO$_2$ particles become more hydrophilic after UV exposure. This method is compatible with any type of particles. We also fabricated superhydrophobic surfaces with silicon-phthalocyanine photosensitizer particles and studied single oxygen generation and trapping reactions on such surfaces. This work is discussed in Chapter 3.

1.2.3 Reactions in droplets: microreactors on multifunctional superhydrophobic surfaces

Micro-reactors offers many fundamental and practical advantages to today’s chemical and pharmaceutical industries, including miniaturization, ease of analysis, and controllability.[87-93] Miniaturization can expand existing bioassays, separation technologies, and chemical synthesis techniques. In micro-reactors, smaller amounts of chemicals are required compared to a batch reactor, and a large number of parallel experiments can be done in less time.[94-98]

Micro-reactors can be realized in two types: droplet-based microfluidics[99,100] and individual micro-droplets.[101,102] In microfluidics, a droplet reactor can be formed by
combining different reagents flows into a single stream.\textsuperscript{[103-106]} The control of mixing is very important for these reactions. If mixing is not induced, all reactions would be diffusion limited.\textsuperscript{[107]} Controlled rapid mixing in microfluidics can be achieved through several ways such as winding channels\textsuperscript{[105,106]} and channel structure modification.\textsuperscript{[108,109]} However, some applications require a time lag between droplet generation and sample reaction (e.g., kinetics, synthesis). This time lag is controlled by adjusting channel length, flow rate, or external force, but require a complicated design and fabrication process. To facilitate the time requirement for reactions, the droplets in microfluidics can be docked in traps,\textsuperscript{[110-112]} or trapped on vertical posts,\textsuperscript{[113]} in which continuous flow is required for the droplets to maintain docked. Weitz \textit{et al} reported a ‘Dropspots’ device,\textsuperscript{[114]} in which droplets were confined in an array of round chambers connected by narrow constrictions, the time that droplets are trapped was controlled by the flow. Although these methods can provide the time lag for a reaction, complex channel geometries are required. Mixing during trapping remains a challenge.

Individual droplets formed on a superhydrophobic surface can also act as micro-reactors.\textsuperscript{[101,115]} Mixing within such individual droplets can be induced using an external force, such as a magnetic field\textsuperscript{[16]} or heating.\textsuperscript{[116,117]} On a heated substrate, the temperature gradient will accelerate evaporation and induce convective mixing within a droplet. However, the temperature increase will also change the reaction kinetics and may adversely affect biological molecules. Evaporation of droplets on non-heated superhydrophobic surfaces was also studied for biosensor applications where molecule detection was required.\textsuperscript{[118,119]} However, convection within the evaporating droplet was not controlled and quantified.
In Chapter 4, I describe a method to induce and control convective mixing in sessile droplets on multifunctional superhydrophobic surfaces. Two reactions were studied as a function of convection rate.

1.3 Precise dispensing system: Nanoliter virtual well microplate (nVMP) and nano-droplet array plate (nDAP)

Precise dispensing of a large number of nanoliter droplets containing bioreagents\textsuperscript{[120-124]} is one of the most crucial steps for achieving reliable assay results and is highly desired for high throughput/content screening of new drugs or biomarkers.\textsuperscript{[125]} The need for precise dispensing of nanoliter (nL) quantities is especially acute when the source sample volume is limited, such as naturally occurring venom from snakes, spiders or other natural products.\textsuperscript{[126]} Conventional contact dispensing techniques do not offer sufficient precision for dispensing droplets less than 50 nL\textsuperscript{[127,128]} and microwell plates are too large to handle such small volumes. Dispensing volumes below 50 nL is challenging because the dispensing process is dominated by interfacial adhesion,\textsuperscript{[123,129]} and factors such as surface tension, capillary forces and local microstructures that affect the transferred volume.\textsuperscript{[130-132]} As a result, error increases significantly as the dispensed volume decreases from microliters to nanoliters. Alternatively, non-contact dispensing systems create a jet of nanoliter droplets and so relieve the tolerances imposed on positioning and substrate planarity, but increase the cost and complexity of the delivery system used,\textsuperscript{[133-140]} as well as subjecting the solution to high temperatures and/or shear forces that can damage large molecules and cells. Dispensing errors associated with jetting systems remain high, approximately ±10% with 20 nL droplets.\textsuperscript{[141]}
A different approach to dispensing is to control the size and wettability of the surface itself. Virtual microwells for high-throughput screening applications have been described where the liquid was transferred to hydrophilic arrays patterned within a hydrophobic substrate.\textsuperscript{[142]} The liquid sample is constrained by the boundaries between the hydrophobic and the hydrophilic surface. To dispense fluids, either a special cover with matching arrays of features or a micro-dispenser as described above is required. Thus although this surface aids in the formation of small droplets, dispensing still requires specialized materials and equipment to achieve high precision. To facilitate the dispensing of aqueous fluids by using differences in wetting, some researchers have dispersed hydrophilic regions on a superhydrophobic substrate.\textsuperscript{[143,144]} \textit{Salvinia molesta}, a plant that floats on water, uses a combination of hydrophilic and superhydrophobic features to generate a high free energy barrier.\textsuperscript{[145]} Although these types of hydrophilic/superhydrophobic surfaces facilitate droplet positioning, they do not enhance control of the dispensed volume because the energy barrier between the two regions is too small; dispensing accuracy still relies on accurate dispensing tools.

For the surface to influence the dispensed volume, the activation barrier between hydrophilic and hydrophobic regions must be sufficiently large that the solid-liquid-vapor triple contact line (TCL) is pinned. To achieve such a high energy barrier, a sharp edge at the boundary of the hydrophilic region is required. Superhydrophobic surfaces have been prepared with such high energy barriers including nanonail\textsuperscript{[146]} and microhoodoo\textsuperscript{[147,148]} structures.

Inspired by these studies, the Lyons’ group previously developed a superhydrophobic microumbrella surface made from hydrophilic polymer materials with
re-entrant features.\textsuperscript{[149]} The high energy barrier pinned the TCL, causing a concave meniscus to form when the droplet bridged between adjacent micro-umbrella features; on a hydrophobic surface of the same geometry, the water meniscus is convex. Thus, a combination of a hydrophilic surface with an abrupt boundary (i.e., sharp edge) results in a structure with a pinned TCL and a sufficiently high energy barrier to prevent wetting beyond the edge of the surface. Although these high energy barrier surfaces have been demonstrated on micrometer length scales, they are expensive to fabricate and are easily damaged due to their fragility. Thus, there is a need for a surface that is inexpensive to fabricate, mechanically robust, and of the appropriate size that can be used to precisely dispense arrays of nanoliter droplets.

In Chapter 5, a novel nanoliter droplet virtual well microplate (nVWP) for precisely dispensing nanodroplets on top of isolated glass pedestals is described. High precision (better than ±1.6\%) was achieved through the combination of local surface chemistry and the geometry of the pedestals, i.e., the sharp edge created at the glass–air interface. Such a device relieves the mechanical alignment tolerances required for dispensing compared to conventional dispensing techniques and provides a significantly improved method to accurately dispense and manipulate nanoliter droplets from source sample volumes as small as 3 μL. As an application of nVWP, I demonstrate how the high-precision nVWP dispensing platform can be used for a variety of assays, including sensitive detection of proteins and peptides by both fluorescence microscopy as well as MALDI-TOF. In addition, the glass pedestal surface was functionalized to enable the selective adsorption of specific peptides/proteins from biomolecule mixtures. KcsA ion channel proteins were bound to
nickel chelate resin coated glass pedestals, and the surface was shown to selectively adsorb Tx7335 peptide from snake venom.

Using a similar fabrication process, the Lyons’ group developed a second precise dispensing system: nano-Droplet Array Plate (nDAP), in which no glass pedestal is required. This system has several advantages. However, the circular pedestal is especially helpful as it enables the direct measurement of droplet volume by measuring droplet height. By using this system, I was able to study the dispensing of hydrophobic microbeads dispersed in a water drop. The number of dispensed microbead is related to how the microspheres dispersed in aqueous solution at the aid of surfactants. The effect of surfactants on dispersing of hydrophobic microbeads is discussed in Chapter 6.

1.4 **Surfactants for dispersed system**

Surfactants are a group of chemical compounds which are surface active; they can reduce the surface tension and interfacial tension of solutions. The word “surfactant” originally comes from “surface active agents”. One unique behavior of surfactants is that they can self-assemble at interfaces (air-water; oil-water; and solid-water) and form tightly packed structures, such as monolayers and aggregates; or form micelles in solution. Surfactants are amphiphilic molecules that interact with both polar and non-polar environments. The general structure of a surfactant molecule is illustrated in Figure 1.7. The molecule has one hydrophilic head group and one hydrophobic tail. The surfactants can be classified by the values of hydrophilic-lipophilic balance (HLB), which describes the relative ratio of hydrophilic and hydrophobic groups in surfactant molecules. HLB was originally applied to nonionic surfactants, as described by Griffin’s method.
\[ HLB = 20 \times \frac{M_h}{M} \], where \( M_h \) is the molecular weight of the hydrophilic part of the molecule, and \( M \) is the total molecular weight. In Griffin’s method, HLB has a scale of 0 to 20. Later, HLB was extended to ionic surfactants and can be calculated by Davies’ method:\(^{[152]}\)

\[ HLB = 7 + \sum_{i=1}^{m} H_i - n \times 0.475 \], where \( m \) is the number of hydrophilic groups in the molecule, \( H_i \) is the value of the group number for each hydrophilic group, and \( n \) is the number of lipophilic groups in the molecule. The scale of HLB is extended up to 60. The advantage of Davies’ method is that it takes account the effects of different hydrophilic groups in both ionic and nonionic surfactants.

**Figure 1.7.** Schematic structure of a surfactant.

Because of the amphiphilic property, surfactants may be applied as wetting agents, detergents, emulsifiers, foaming agents, and dispersants. Usually, surfactants are classified based on their polar head group and divided into nonionic, anionic, cationic, and zwitterionic. A nonionic surfactant has no charge on its polar head group. If the head group has a negative charge, the surfactant is called anionic; on the other hand, if the charge is positive, it is called cationic. When a surfactant has a polar head containing two oppositely charged groups, it is called zwitterionic. (**Figure 1.8**)
1.4.1 Polymeric nonionic surfactants

Different from ionic surfactants whose properties are controlled by electrostatic interactions, nonionic surfactants are regulated by hydrophilic interactions. So the nonionic surfactant system is not sensitive to salts. The most common type of nonionic surfactant is that with an oligo(oxyethylene) group as the polar head, and an alkyl chain as the lipophilic part, such as oxypropylene (PO). Polymeric surfactants, such as Pluronic (BASF trade name of EO-PO-EO triblock copolymers), is a very important type of nonionic surfactant. The growing interest in polymeric surfactants is generally because of their characteristic features: 1. They are effective at low total concentrations. 2. They show little sensitivity to salts. 3. The length of the hydrophilic chain is tunable over a large range, and the surfactant can be retained at hydrophobic-hydrophilic interfaces. For the low molecular weight surfactants, if the hydrophilic chains become very long, the surfactants tend to desorb from the interface and dissolve in the aqueous phase. Thus, polymeric surfactants are very efficient stabilizers for dispersed systems.

Figure 1.8. Surfactant classification according to the composition of the head group.
1.4.2 Adsorption of polymeric nonionic surfactant at solid hydrophobic surfaces

Adsorption of surfactants at solid surfaces is of great importance in many technical processes, for example, in the stabilization of suspensions, in detergency, and in lubrication. The surfactant adsorption from aqueous solutions is driven by two factors: (i) the energy gained by changing a surface–water contact into a surface–surfactant contact and (ii) the hydrophobic effect, that is, the tendency of the surfactant hydrocarbon moiety to avoid the aqueous environment.

Adsorption of a polymer at a surface depends upon the partitioning of the polymer molecule between the surface phase and the solution phase. Strong adsorption can be achieved by either a strong interaction between the polymer segments and the surface or alternatively a poor interaction between the polymer and water.\[^{156}\]

When a polymer molecule adsorbs at a surface, it loses some of its conformational entropy and this opposes adsorption. Hence, there needs to be some net favorable enthalpic interaction between polymer segments and the surface for adsorption to occur. As stated above, another reason could be poor solubility, that is, the adverse interaction between polymer and water in the solution forcing the polymer to the surface.

The adsorption of surfactants on a solid surface will reach a limiting value, $\Gamma_{\text{max}}$, when the solution concentration is above the critical micelle concentration (CMC). The surfactant activity in the solution is constant when the concentration is higher than CMC. As a result, the adsorbed amount should not increase. For polymeric surfactants, the $\Gamma_{\text{max}}$ could also be reached when the surface is saturated by large polymer molecules.
The adsorption differs depending on whether the surface is hydrophobic or hydrophilic. On hydrophobic surfaces, the surfactants are adsorbed with their hydrocarbon chains laying down. High molecular weight ($M_w$) species are more readily adsorbed than low molecular weight polymers. This is understandable as we know that high $M_w$ species are less soluble in solution than lower $M_w$ analogs because the entropy gained is smaller as increasing the chain length. The radius of gyration ($R_G$) for polymers forming random coils in the solution phase is proportional to the molecular weight. When a polymer surfactant is adsorbed on a hydrophobic microbead surface, the hydrophobic blocks interact with the surface, and the hydrophilic blocks form random coils in the adjacent aqueous phase. So the coverage of polymer surfactant on microbeads depends on two factors: a. interactions between hydrophobic blocks and microbead surface, which determines the amount of surfactant adsorbed; and b. the molecular weight of hydrophilic chains, which determines the area the surfactant molecules cover.

1.4.3 Stabilization of suspensions with surfactants

Particle dispersions can be stabilized by adsorption of surfactants. As described in Chapter 6, I used Pluronic nonionic surfactants to treat hydrophobic polyethylene (PE) microbeads and studied the effect of surfactants hydrophilicity/hydrophobicity on the dispersion of microbeads in aqueous solution. The stability of hydrophobic microbeads in water is related to the surfactant effect, which describes how the microbead surface is stabilized by surfactants. The microbeads will be more hydrophilic and stable in the aqueous phase when more surface area is covered by the hydrophilic chains of Pluronic surfactants. To investigate the surfactant effect on microbead dispersion, I selected
Pluronic surfactants with different hydrophilic and hydrophobic chain lengths and studied the stability of microbeads in aqueous dispersion. This stability was quantified by the number of microbeads dispensed onto nDAP posts.
Chapter 2. Fabrication and characterization of transparent superhydrophobic surface

2.1 Introduction

Lotus leaves, in nature, exhibit extraordinary water repellency on their upper side, which is attributed to the micro/nano scale morphology of the surface. By mimicking this hierarchical surface structure, numerous artificial superhydrophobic surfaces were developed.\textsuperscript{[1-7]} They are of interest because of their potential industrial applications, such as self-cleaning, anti-fogging, anti-icing, etc. Particularly, transparent superhydrophobic surfaces are of interest because of potential applications for solar-cell panels, optical lens, and automobile windshields.

The major problems limiting the real applications of superhydrophobic surfaces are the complexity of fabrication and low mechanical durability. Recently work from our group addressed these two issues,\textsuperscript{[8]} by using a simple and inexpensive lamination templating method to fabricate a superhydrophobic polymeric surface with excellent abrasion resistance. Herein, I develop a method of fabricating transparent superhydrophobic surfaces with acceptable mechanical durability.

2.2 Experimental

2.2.1 Fabrication of transparent superhydrophobic surface

A transparent polymer film is an ideal substrate to fabricate transparent superhydrophobic surfaces because polymers are flexible and easy to process. Polymers
are synthetic materials, the structures and properties of polymers can be tailored, so there is a wide range of polymer candidates available.

To select a proper polymer material for preparing the transparent superhydrophobic surface, we need to consider the following criteria:

1. Highly transparent in the visible light range;
2. Hydrophobic;
3. Proper glass transition temperature.

A list of common transparent polymers is shown in Table 2.1\textsuperscript{[9-11]}; they all have good transparency in the visible light range. We chose cyclic olefin polymer (COP) as the substrate because they are amorphous and would maintain transparency after thermal processing. Another important factor for transparency is refractive index (RI) which is related to reflection. Since we want to use hydrophobic silica nanoparticles to coat and laminate with the polymer substrate, similar refractive indices of polymer and silica will minimize unwanted reflections at the interface. The relationship between reflection and media indices with normal incidence light is described as by Equation 2.1:

\[ R = \left( \frac{n_1 - n_2}{n_1 + n_2} \right)^2 \]  

(2.1)

where \( n_1 \) and \( n_2 \) are the refractive indices of two adjacent materials, the larger difference in \( n \) values results in higher reflections. The refractive index of fumed silica is about 1.47.\textsuperscript{[12]} TPX\textsuperscript{®} polymethylpentene would be a good candidate for the transparency purpose, however the high melting point and high crystallinity make it more difficult to process. The cost of polymers is another reason that we prefer to choose polycyclic olefins as the polymer substrate.
Two types of hydrophobic silica nanoparticles received from Evonik (AEROSIL R202 and AEROSIL R812S) were used in preparing the transparent superhydrophobic surfaces. The surface area and particle size of the silica nanoparticles were listed in Table 2.2.\cite{9}

Table 2.1. Properties of candidate polymers.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>RI (589nm)</th>
<th>Glass Transition Temperature</th>
<th>Light Transmission (3mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA</td>
<td></td>
<td>1.491</td>
<td>80-100°C</td>
<td>92%</td>
</tr>
<tr>
<td>Polystyrene</td>
<td></td>
<td>1.502</td>
<td>95°C</td>
<td>92%</td>
</tr>
<tr>
<td>PVC</td>
<td></td>
<td>1.57</td>
<td>80-90°C</td>
<td>87%</td>
</tr>
<tr>
<td>ZeonorFilm (poly cyclic olefins)</td>
<td></td>
<td>1.53</td>
<td>163°C</td>
<td>92%</td>
</tr>
<tr>
<td>TPX Polymethylpentene</td>
<td></td>
<td>1.46</td>
<td>melting point 240°C</td>
<td>93%-94%</td>
</tr>
</tbody>
</table>

Table 2.2. Physical properties of AEROSIL silica nanoparticles

<table>
<thead>
<tr>
<th></th>
<th>BET Surface Area (m$^2$/g)</th>
<th>Primary Particle Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEROSIL R202</td>
<td>100±20</td>
<td>14</td>
</tr>
<tr>
<td>AEROSIL R812S</td>
<td>220±25</td>
<td>7</td>
</tr>
</tbody>
</table>
The properties of the COP ZF16 film were characterized by Differential Scanning Calorimetry (DSC), Thermal Gravimetric Analysis (TGA) and UV-vis spectroscopy. The glass transition temperature of ZF16 film was obtained from the DSC diagram (Figure 2.2), which is 164°C. The glass transition temperature ($T_g$) is an important characteristic of every polymer, because the mechanical behavior of the polymer changes markedly. The processing of polymers is usually operated at temperatures above $T_g$, and the use temperature of a polymer product is lower than $T_g$. For the lamination process, the temperature should be higher than $T_g$, at which the polymer can flow and can be laminated to other materials.

![DSC plot of ZF16 polymer film](image)

**Figure 2.1.** DSC plot of ZF16 polymer film.
The thermal stability of the ZF16 polymer film was measured by TGA. From the TGA plot (Figure 2.2), the ZF16 is shown to be very stable and has no degradation until the onset of degradation at 444.5°C. The UV-Vis spectrum of ZF16 film is shown in Figure 2.3. The light transmittance is above 91% in the range from 450 nm to 800 nm.

Figure 2.2. TGA plot of ZF16 polymer film.
Figure 2.3. UV-Vis spectrum of ZF16 polymer film.

To obtain a transparent superhydrophobic surface, the COP film was first dip-coated with hydrophobic silica nano-particles (~20nm) solution for multiple times and dried at room temperature. The dip coating instrument is shown in Figure 2.4. A COP film measured 25mm by 30mm is attached to a glass slide which fixed on the moving head of the instrument. The COP film is first dipped into the isopropanol solution of silica nanoparticles and then stays for 10 seconds before being withdrawn from the solution. The withdrawing speed is controlled to be 8 cm/min. The weight concentration of silica nanoparticle solution is 2%. One side of the COP film was covered with a protection film, so that only one side was coated with silica nanoparticles. The protection film was removed before lamination.
After drying at room temperature, polymer film was laminated between two glass plates at moderate pressure and the temperature above its glass-transition temperature ($T_g$). **Figure 2.4** shows the lamination process. The reason of using smooth glass slides at both sides of polymer film during the lamination is to minimize the surface roughness, which can cause light scattering.

![Lamination Process Diagram](image)

**Figure 2.4.** Lamination of dip-coated polymer film.

The lamination has to be done at the temperature above the glass transition temperature ($T_g$) of polymer, which is about 161°C. And the pressure varies from 17 psi to 215 psi, which will affect the transparency and mechanical durability of the surface.

### 2.2.2 Characterizations

The transmittance spectra of the prepared superhydrophobic film at visible light range were collected by using Perkin Elmer (Lambda 650) spectrophotometer. The surface morphology was characterized by AMRAY 1910 Field Emission Scanning Electron Microscope (FESEM). The thermal properties of the COP film were measured by
differential scanning calorimetry (DSC) using a TA Instruments model Q100 at a heating rate of 10 °C min\(^{-1}\). Thermogravimetric Analysis (TGA) was performed to study the thermal stability of polymer film. Approximately 10 mg of sample was heated from 40 °C to 700 °C at a heating rate of 10 °C/min under a nitrogen atmosphere. The static contact angle (CA) and slip angle were measured with a goniometer (250-F1, rame-hart Instrument Co). Droplets of DI water, with a volume of 5 μL were placed gently onto the surface at room temperature and pressure. The static CA were measured five times at different locations. The sliding angle (SA) was measured by placing a water droplet of 10 μL on an initially horizontally substrate and then tilting the substrate until the water droplet rolled off. The chemical composition of the surfaces was studied by X-ray photoelectron spectroscopy (XPS) using an Omicron Nanotechnology system (EA-125) with monochromatic radiation from an Al target. The XPS examination was carried out immediately after surface fabrication as well as after the water-tunnel flushing test.

2.2.3 Surface durability

The mechanical durability of transparent superhydrophobic surfaces was evaluated by a water-tunnel flushing test described in Figure 2.5. The sample was attached to the end of a rubber stopper and inserted in a water-tunnel, such that the film was protruded 5 mm into the tube. The water-tunnel had a inner dimension of 2.5 cm × 2.5 cm × 60 cm, Water in the tunnel runs through the sample surface at a flow rate of 2.9 m/s. The water contact angle of the prepared surface was measured before and after the water tunnel test.
2.3 Results and discussions

2.3.1 Effect of dip-coating cycles

The polymer film was dip-coated into the silica particle dispersion multiple times using 2% R202 silica particles. By increasing the dip-coating cycles, more silica nanoparticles were deposited on the polymer surface. This was confirmed by SEM images in Figure 2.6. As we can see from the SEM images, the surface coverage of silica nanoparticles is few when dip-coated only once (Figure 2.6a). Increasing the number of dip-coating cycles to three (Figure 2.6c), results in most of the surface area being covered by silica nanoparticles. Increasing the dip-coating cycle to five (Figure 2.6d), results in thicker and more uniform coverage of silica nanoparticles on the. The water contact angles and stability of the samples prepared using different number of dip-coating cycles are compared in Table 2.3. The stability of the superhydrophobic surface is defined as the time the water contact angle exceeds 150° in the water tunnel test. The results indicate that one dip-coating cycle was not enough to achieve superhydrophobicity, increasing the number of dip-coating cycles increases the water contact angle gradually from 155.2° (2
cycles) to 164.9° (5 cycles) and also the stability of superhydrophobic surfaces from 2 hours (2 cycles) to 8 hours (5 cycles).

Higher coating density can give better hydrophobicity, however the transparency decreases as shown in Figure 2.7. Surface fabricated from 3 dip-coating cycles has high coating density and also good transparency of 88% transmittance at 500nm. Surface fabricated with 5 dip-coating cycles has a lower transparency of 82% transmittance at 500nm than that fabricated with three dip-coating cycles, but it has a greater water contact angle of 164.9°, a smaller sliding angle of 3.8° and a better stability in water tunnel test (8 hours vs. 6 hours). This is because of the higher coating density on polymer surface. The transmittance loss in the 300-500 nm range of 5 dip-coated sample is more significantly, because the wavelength is about the same size of particle agglomerates on the surface.
Figure 2.6. SEM images of dip-coated surfaces: a. dip-coated one time; b. dip-coated two times; c. dip-coated three times; d. dip-coated five times. All samples were dip-coated in solution of 2%wt R202 silica nanoparticles and laminated at 175°C and 167 psi.

Table 2.3. Water contact angles and stability of prepared superhydrophobic surfaces with different dip-coating cycles.

<table>
<thead>
<tr>
<th>Dip-coating cycles</th>
<th>Contact Angle (°)</th>
<th>Sliding Angle (°)</th>
<th>Stability in water tunnel test (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>142.6</td>
<td>36.1</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>155.2</td>
<td>8.3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>161.3</td>
<td>4.2</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>164.9</td>
<td>3.8</td>
<td>8</td>
</tr>
</tbody>
</table>
**Figure 2.7.** Light transmittance spectra of fabricated superhydrophobic films and ZF16 without treating.

### 2.3.2 Effect of lamination pressure

Lamination pressure is another important factor for the fabrication of transparent superhydrophobic surfaces. To discuss the effect of lamination pressure, samples were made at different pressures while keeping other processing factors constant: temperature was 185°C, polymer film was dip-coated 5 times, the concentration of silica nanoparticles was 2 wt %. At each pressure, three samples were fabricated and tested. **Figure 2.8** showed the transmittance of films plotted as a function of lamination pressure.
There are two factors that affect the light transmittance through the film: surface roughness and refractive index difference between the polymer and the nano-particles. As illustrated in Figure 2.9, when the pressure is low the particles are primarily at the surface of polymer film (Figure 2.9a), increasing the pressure to moderate pressure (Figure 2.9b) and high pressure (Figure 2.9c) result in more particles being pressed into the polymer film. At low pressure, the surface roughness is relatively high so the transmittance at 500 nm is low. When increasing the pressure, the silica particles are pressed deeper into the polymer substrate, so the surface roughness becomes lower, resulting in increased light transmittance. However, the refractive index of polymer and silica nano-particles are different (1.53 and 1.46). By pressing more particles into the polymer and create larger particle agglomerates, reflections may increase at the particle/polymer interfaces within the composite which can lower the overall light transmittance.
Figure 2.9. Illustration of effect of pressure on lamination process.

The contact angle and stability of the superhydrophobic surfaces fabricated at different lamination pressures were measured, the results are summarized in Table 2.4. All the samples showed good superhydrophobicity with contact angles larger than 160°. The samples laminated at higher pressure exhibited longer stability in the water tunnel test. This is because the higher pressure makes the particles penetrate deeper into the polymer substrate and the adhesion between particles and polymer increases as the contact area becomes larger.

Table 2.4. Water contact angles and stability of prepared superhydrophobic surfaces at different lamination pressure.

<table>
<thead>
<tr>
<th>Lamination pressure (psi)</th>
<th>Transmittance (% at 500nm)</th>
<th>Contact Angle (°)</th>
<th>Stability in water flushing test (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>83.8</td>
<td>166.8±0.5</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>84.0</td>
<td>165.2±0.6</td>
<td>4</td>
</tr>
<tr>
<td>42</td>
<td>87.0</td>
<td>164.8±0.5</td>
<td>6</td>
</tr>
<tr>
<td>83</td>
<td>86.5</td>
<td>164.5±0.6</td>
<td>7</td>
</tr>
<tr>
<td>167</td>
<td>86.1</td>
<td>164.8±0.8</td>
<td>8</td>
</tr>
<tr>
<td>208</td>
<td>84.6</td>
<td>163.1±1.0</td>
<td>7</td>
</tr>
</tbody>
</table>
2.3.3 Effect of lamination temperature

To discuss the effect of lamination temperature, samples were fabricated maintaining other variables constant: 42 psi, 5 dip-cycle, 2 wt% R202 silica nanoparticles and 3 dip coating cycles.

As seen in Figure 2.10, at beginning when increasing temperature from the 175°C, the transmittance increases. This is due to the decreasing of surface roughness as discussed above. But when increasing the temperature to higher than 200°C, the transparency of the superhydrophobic surface decreases dramatically. This rapid change in light transmittance is because the better mobility of polymer enabled more nano-particles to become embedded into the polymer. The polymer has a lower viscosity at high temperature, the resulting increased flow causes some air to be trapped into the polymer during lamination, making
the surface became uniform. The increased light scattering and reflection results an increased loss of transmittance light. As shown in Table 2.5, the water contact angle decrease as increasing the lamination temperature, which is due to the decrease of surface roughness. However, the silica nano-particles were embedded deeper into the polymer film at higher temperature, they were more difficult to be removed by flowing water. As a result, the stability of surface in water tunnel test became longer when the lamination temperature increases from 175 °C to 185 °C. Keep increasing the lamination temperature, the stability did not increase (200 °C) and even became lower (300 °C), which is because the initial contact angle decreased. In summary, the sample prepared at 185 °C gives the best overall performance.

Table 2.5. Water contact angles and stability of prepared superhydrophobic surfaces at different lamination temperatures.

<table>
<thead>
<tr>
<th>Lamination temperature (°C)</th>
<th>Transmittance (% at 500 nm)</th>
<th>Contact Angle (°)</th>
<th>Stability in water tunnel test (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>175</td>
<td>83.5</td>
<td>165.6±0.7</td>
<td>5</td>
</tr>
<tr>
<td>185</td>
<td>87.0</td>
<td>164.8±0.5</td>
<td>6</td>
</tr>
<tr>
<td>200</td>
<td>84.7</td>
<td>162.3±0.6</td>
<td>6</td>
</tr>
<tr>
<td>215</td>
<td>75.1</td>
<td>153.5±0.8</td>
<td>3</td>
</tr>
<tr>
<td>300</td>
<td>59.9</td>
<td>148.8±0.9</td>
<td>N/A</td>
</tr>
</tbody>
</table>

2.3.4 Effect of nano-particle type on mechanical stability in water tunnel test

In this section, the transparent superhydrophobic surfaces were fabricated at 185°C (5 dip-coating cycles, 2 wt% silica nano-particles), which had the overall performance as
discussed previously. Two types of silica nanoparticles were used (R202 and R812S). The lamination pressure was controlled at 42 psi or 167 psi. These two pressures were chose because surface fabricated at 42 psi had best transparency, surface fabricated at 167 psi had highest water contact angle and best stability (as shown in Table 2.4). Four types of sample were fabricated at different conditions (Table 2.6).

**Table 2.6.** Transparent superhydrophobic surface prepared at different conditions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nanoparticle type</th>
<th>Lamination pressure (psi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>R202</td>
<td>42</td>
</tr>
<tr>
<td>B</td>
<td>R202</td>
<td>167</td>
</tr>
<tr>
<td>C</td>
<td>R812S</td>
<td>42</td>
</tr>
<tr>
<td>D</td>
<td>R812S</td>
<td>167</td>
</tr>
</tbody>
</table>

The flow rate in the water tunnel was 2.9 m/s. Five samples from each type were tested. During the water tunnel test, samples were removed from the tunnel for contact angle and UV-vis spectroscopy measurements at 60-minute intervals. The contact angle was measured 5 times at different locations on the surface.

The results of contact angle and sliding angle measurements for sample A are plotted in Figure 2.11.
Figure 2.11. Stability of superhydrophobic surface under water flushing test.

The water contact angle of the surface decreases with time in the water tunnel test, whereas the sliding angle increases. This is because of the removal of hydrophobic silica nanoparticles from the surface by the flowing water. The roughness of the surface also decreased as fewer nanoparticles remained on the surface. The removal of silica particles was confirmed by a surface chemical composition analysis by XPS. The relative atomic concentration of the individual elements were calculated from Equation 2.2:

$$C_i = \frac{A_i / S_i}{\sum_j^n A_j / S_j}$$  \hspace{1cm} (2.2)

where \(A_i\) is photoelectron peak area of the element \(i\); \(S_i\) is sensitivity factor for element \(i\) and \(m\) is the number of elements measured.\[^{13,14}\]
The XPS energy spectra of sample A before and after 6-hour water tunnel test are shown in Figure 2.12. The ratios (Si/C and O/C) of elements from silica and polymer are calculated based on relative atomic concentration, and listed in Table 2.7. Both the Si/C and O/C ratios decreased after 6-hour water tunnel test, which indicates silica particles were removed from the polymer surface.

Figure 2.12. XPS spectra of superhydrophobic surface A before and after water flushing test. a. full scale spectra, b-d. zoomed in spectra to show detail peaks.
Table 2.7 Element ratios on transparent superhydrophobic surfaces.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Si/C</th>
<th>O/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before water tunnel test</td>
<td>2.74</td>
<td>2.60</td>
</tr>
<tr>
<td>After 6-hour water tunnel test</td>
<td>0.72</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Another consequence of loss of silica nano-particles from the surface is an increase in transparency of both samples A and B as shown in Figure 2.13. This is because, as the particles are removed, the area of interface between particle and polymer becomes smaller, so the overall reflection at the interface is also reduced.

The stability of superhydrophobic surfaces fabricated at different conditions were compared in Figure 2.14. The transparent superhydrophobic surface fabricated with R202 at 167 psi lamination pressure maintains the superhydrophobicity for up to 8 hours under continuously water flow. When R812S particles were used instead of R202, the superhydrophobic properties decreased more rapidly. The CA decrease below 145° after 3 hours, whereas the surface made with R202 particles maintained a CA > 145° for 8 hours. The poor stability of R812S surface may because of the low adhesion between particles and polymer.
Figure 2.13. The change of light transmittance during water tunnel test.

Figure 2.14. Stability of superhydrophobic surfaces fabricated at different conditions.
2.4 Conclusions

Transparent superhydrophobic surface was fabricated by a lamination method. The hydrophobic silica nanoparticles were dip-coated on a polymer substrate, which provided high surface roughness and low surface energy. The adhesion between silica nanoparticle and polymer substrate was significantly increased by the lamination process. The fabricated surfaces showed excellent superhydrophobicity and good transparency. One advantage of polymer substrate is the flexibility, which provide the potential application in the self-cleaning surface of irregular shapes. The stability of the surface was evaluated by a water tunnel test. The surface can maintain superhydrophobic properties for up to 8 hours under a water tunnel of 2.9 m/s. The effects of dip-coating cycles, lamination pressure and lamination temperature were studied. Two types of silica nanoparticles were used for the fabrication. The larger particles with lower surface area (AEROSIL R202) showed better adhesion with the polymer substrate and provided better stability of superhydrophobic properties in water tunnel test compared to those made with R812S.
Chapter 3. Fabrication and characterization of polymer nanocomposites with superhydrophobic and catalytic properties

3.1 Introduction

Titanium dioxide is a well-known semiconductor that exhibits great photo-reactivity. Since the discovery of photocatalytic splitting of water on TiO$_2$ electrodes,$^{[1]}$ studies in understanding the fundamental mechanism and in enhancing the photocatalytic efficiency of TiO$_2$ has extensively performed by chemists, physicists, and chemical engineers. In early years, the studies are often related to energy renewal and energy storage.$^{[2-6]}$ Since the end of last century, the environmental and biological applications of TiO$_2$ became an active area in heterogeneous photocatalysis. This is inspired by the potential applications of TiO$_2$-based photocatalytic systems for the total destruction of organic compounds and bacteria in polluted air and water.$^{[7-10]}$

TiO$_2$ photocatalytic film can be prepared by many different methods, such as sputter coating,$^{[11]}$ spray pyrolysis,$^{[12]}$ sol-gel dip-coating,$^{[13]}$ anodic oxidation,$^{[14,15]}$ chemical vapor deposition (CVD),$^{[16-18]}$ plasma spray,$^{[19,20]}$ etc. The preparation of TiO$_2$ using methods are good for fundamental research, but for applications these methods have disadvantages of complexity, costly, and difficult to scale up.

In this chapter, I presented a simple and inexpensive lamination method for fabricating a TiO$_2$-polymer nanocomposite surface with superhydrophobic property. Not like the conventional methods to prepare composite which both compositions are uniformly dispersed in the matrix, the lamination process have TiO$_2$ particles primarily locate at the surface of the composite and effective for photocatalysis. The TiO$_2$-polymer
nanocomposite surfaces were prepared thru two types of lamination methods: 1. Lamination with a mesh template, the prepared surface has a high roughness which shows superhydrophobicity; 2. Lamination without templating, which is a simpler and more easily scale-up method. The surfaces prepared by these two methods are both exhibit photocatalytic properties. The photodegradation of organic dyes were investigated on the TiO$_2$-polymer nanocomposite surfaces. Furthermore, two applications of the TiO$_2$-polymer nanocomposite film prepared by simple lamination are discussed: one application is degradation of dyes used in undergraduate instructional laboratories; another application is photocatalytic sterilization, the film shows effective in killing bacteria and can be used for water purification. For an educational purpose, we also designed an experiment for undergraduate inorganic/analytical chemistry laboratory, in which the students are encouraged to study general photo-chemical reactions and utilize UV-vis spectroscopy to quantitatively characterize the reactions.

Since TiO$_2$ and most semiconductors are hydrophilic, fabrication of a photocatalytic surface with stable superhydorphobicity is challenging. I presented a novel method to prepare a photocatalytic superhydrophobic surface which can maintain stable Cassie state (a layer of air under the aqueous solution) during the photo-reactions. This surface is fabricated by incorporating TiO$_2$ nanoparticles into a superhydrophobic surface with polydimethylsiloxane (PDMS) posts in well-defined arrays. By printing the surface on a porous support, oxygen could be flowed through the plastron resulting in significantly higher photooxidation rates relative to a static ambient. Photooxidation of Rhodamine B (RhB) and Bovine Serum Albumin (BSA) protein was studied on these TiO$_2$-containing surfaces. TiO$_2$ nanoparticles could be isolated in the plastron, preventing contact with the
solution. This approach may prove useful for water purification and medical devices where isolation of particles from the solution is necessary and so Cassie stability is required.

This method is technically compatible with any arbitrary photo-catalyst particles. A type of photosensitizer, silicon-phthalocyanine (Pc), is immobilized on the PDMS posts and exhibits stable superhydrophobicity. Singlet oxygen ($^{1}\text{O}_2$) can be generated on this surface when irradiated with red laser (670 nm) and be trapped by a water-soluble anthracene compound. The generation and trapping reactions were studied.

3.2 Superhydrophobic TiO$_2$-polymer nanocomposite surface with UV-induced reversible wettability and photocatalytic property

3.2.1 Experimental

3.2.1.1 Materials

A commercially available thermoplastic sheet of HDPE from McMaster-Carr was used as the polymer substrate. A precision woven nylon mesh (371 × 371, from McMaster-Carr) was used as a template to create microstructures on the polymer surface. The wire diameter and the pore size of the nylon mesh are 33 and 36 μm, respectively. Two kinds of nanoparticles were used to create nanostructures on the polymer surface. One was TiO$_2$ nanoparticles (634662, from Sigma-Aldrich) with a size ranging from 20 to 100 nm.

3.2.1.2 Fabrication of TiO$_2$-PE nanocomposite films

The procedure for fabricating surfaces involves two processing steps as shown schematically in Figure 3.1. In the first step, a piece of HDPE sheet, a mesh template and a layer of nanoparticles are laminated together under heat and pressure with the targeted
polymer surface facing the mesh template and the nanoparticles. The layer of nanoparticles was coated using a Doctor Blade method. The stack-up was heated to 138 °C under a pressure of 4000 psi for 30 min and then cooled to room temperature. In the second step, the mesh template and excess nanoparticles are separated from the polymer film. The fabricated superhydrophobic surface is formed and exposed during the peeling process. The surface was cleaned using ultrasonic bath before use.

![Figure 3.1. Schematic of the formation of the microstructures during the processing.](image)

**3.2.1.3 UV illumination experiments**

The UV light was generated by a UV spot lamp (Bluewave 200, from Dymax) using a 5 mm diameter liquid light guide. The power density was set at 33 mW·cm\(^{-2}\). The wavelength of the UV light ranged from 320 to 450 nm. The UV illumination was conducted with and without water on the superhydrophobic surface at room temperature (∼25 °C). The change of the CA under the UV illumination was monitored at specific time
intervals. The surface illuminated under water was dried by compressed air before the CA measurements. For thermal recovery of superhydrophobic properties, the surface was heated in a dark oven at 105 °C for 1.5 h after UV illumination.

### 3.2.1.4 Characterization

Surface structures were studied by field emission scanning electron microscopy (FESEM, Amary) and optical microscopy (Nikon-SMZ 1500 and Laborlux 12ME). The static CAs and slip angle were measured with a goniometer (250-F1, rame-hart Instrument Co). The chemical composition of the surfaces was studied by X-ray photoelectron spectroscopy (XPS) using an Omicron Nanotechnology system (EA-125) with monochromatic radiation from an Aluminum target. The XPS examination was carried out immediately after the surface fabrication as well as after UV irradiation and heat treatment. The distribution of TiO2 particles on the fabricated surfaces were detected by energy-dispersive X-ray spectroscopy (EDX) at a scanning voltage of 10 KV.

### 3.2.1.5 Photodegradation experiment

To study the photocatalytivity of superhydrophobic TiO2-HDPE film, a dynamic photodegradation experiment was designed. As illustrated in [Scheme 3.1](#) a 200µL droplet of Rhodamine B (RhB) is attached to a PMMA bar, which can move back and forth to coat the RhB solution to the hydrophilic TiO2 spots. The coated area is under UV irradiation, RhB is degraded by TiO2 photocatalysis. The reaction system is covered by a plastic bag to prevent evaporation. After reaction, the droplet is collected and the remaining RhB concentration is determined by UV-Vis spectroscopy.
3.2.2 Results and discussion

3.2.2.1 Fabrication of TiO$_2$-polymer nanocomposite surface

To achieve superhydrophobicity, a micro and/or nanoscale rough surface structure is necessary because it can dramatically reduce the liquid–solid contact area, and thus the adhesion forces between water and the solid surface. Superhydrophobic properties can be further improved by creating hierarchical levels of roughness such that the primary, relatively large scale, roughness keeps the droplet elevated above the surface for stability and reduces the overall liquid–solid contact area while the fine scale roughness (i.e., secondary roughness) minimizes solid–liquid contact in the area of the primary roughness. The combined primary and secondary roughness length scales forms a hierarchical roughness that is essential for fabricating a robust superhydrophobic surface.

To create a surface with multiple roughness scales, we used a lamination templating method. A precision polymer nylon woven mesh with a wire diameter of 33 μm and a square pore size of 36 μm was used to create primary roughness microstructures (Figure
3.1 a,b), and TiO$_2$ nanoparticles (Figure 3.1 c,d) were used to create the fine scale roughness nanostructures. As shown in images c and d in Figure 3.1 the TiO$_2$ nanoparticles are composed of single TiO$_2$ particles with a size ranging from 15 to 100 nm, which forms the primary roughness. The agglomerates of individual particles have a size of $\sim$500 nm, which forms the secondary roughness.

A lamination temperature of 138 °C was used to ensure that the HDPE was above the crystalline melt point of 132.6 °C. During lamination, the molten polymer flows into the open pores (36× 36 μm) of the mesh and adheres to the TiO$_2$ agglomerates as shown schematically in Figure 3.1 a-c. Flow is limited by the viscosity of the polymer (where the viscosity depends upon the temperature and molecular weight of the polymer) and applied pressure. After lamination, the stack was cooled to room temperature (25 °C) and the mesh was separated from the polymer film by peeling. Because the nanoparticles prevent the molten polymer from flowing around the mesh wires, the mesh could be easily peeled off the HDPE surface. Excess TiO$_2$ nanoparticles were removed during the peeling step, however ultrasonicating the surface in distilled water ensured that all excess particles were removed (Figure 3.1 d, e).
Figure 3.2. SEM images of the fabricated surfaces: (a-c) top view and (d, e) cross view tilted at 80° and 87° respectively. (f) EDX image, showing the TiO₂ distribution on the surface.

The structure of the fabricated surface was studied by SEM to gain insight into surface roughness hierarchy. Typical SEM images recorded at low and high magnifications and from different viewing angles are shown in Figure 3.2 a-e. In images a and b in Figure 3.2, it can be seen that the fabricated surface is composed of microscale square posts (69 μm pitch) surrounded by curved grooves formed from the embossed wire mesh template. The side length of the square posts is about 36 μm, and the height of the posts varies with the curvature of the woven mesh (Figure 3.2 d). Nanoscale features on the top surface of the posts can be clearly discerned under higher magnification (Figure 3.2 e), and these nanostructures are composed of both TiO₂ nanoparticles and HDPE. The polymer forms a web-like structure with a filament diameter ranging from 80 to 500 nm, whereas the aggregates of TiO₂ nanoparticles are fully or partially embedded into the polymer surface.

Figure 3.2 e is taken from 87° angle under high magnification and shows that the filaments appear aligned with each other and perpendicular to the substrate surface. To investigate the effect of concentration and distribution of the TiO₂ nanoparticles, we used energy-dispersive X-ray spectroscopy (EDX) for mapping the location of TiO₂ nanoparticles on the surface. The surface was coated with carbon to improve the conductivity for imaging. It can be seen that the TiO₂ nanoparticles are primarily located on the tops of the posts; only a very few TiO₂ particles can be detected on the grooves surrounding the posts. This distribution is consistent with the process described in the preceding paragraphs. Although the surface was precoated with carbon for imaging, the
detected weight ratio of elemental Ti to C is about 37:53, indicating a high concentration of TiO$_2$ nanoparticles on the posts.

### 3.2.2.2 Superhydrophobic properties

The static water CA measured on the TiO$_2$–HDPE polymer nanocomposite surface was 158° using a water droplet of 5 μL as shown in Figure 3.3. The sliding angle of 10 μL water droplets was measured to be $\sim$8° by using a tilting base method (base angle was increased from 0° at a rate of 5°/s). A much lower SA, <4°, was measured when placing water droplets on a pre-tilted surface.

![Static contact angle measurement on TiO$_2$-polymer surface.](image)

**Figure 3.3.** Static contact angle measurement on TiO$_2$-polymer surface.

### 3.2.2.3 UV-induced reversible wettability

The TiO$_2$ nanoparticles used to fabricate these nanocomposites are exposed on the surface of the nanocomposite and have untreated surfaces (i.e., no silanes or surfactants). As a result, the particles are able to interact directly with UV light. UV light has been shown$^{[21]}$ to significantly increase the wettability of TiO$_2$ surfaces as manifested by a decrease in contact angle. The effect of UV light on these hybrid TiO$_2$–HDPE surfaces,
however, has not been explored previously and so the contact angle of a droplet on the surface was measured as a function of UV exposure. Exposure experiments were carried out using a UV spot lamp (33 mW·cm$^{-2}$) with a broad output from 320 to 450 nm. The exposure was conducted at room temperature in two environments: dry and with a layer of water resting on the fabricated surface. The change in CA with UV illumination time was monitored and the results are shown in Figure 3.4a. It can be seen that the CA decreases with the increase of UV illumination time for both surfaces. When submerged in water during illumination, the CA decreased rapidly, falling to 120° in less than 30 min. For the dry surface, the CA decreased slowly at first, dropping only a few degrees during the first 30 min of illumination. Approximately 90 min was required to reach the ultimate contact angle of 120°, more than 3.5 times longer than when the sample was submerged. Under water, no similar induction period was observed. After illuminating for 30 min under water, the TiO$_2$-HDPE surface was heated in a dark oven at 105° for 1.5 h to dry the surface. Superhydrophobicity was restored by this heating process as shown in Figure 3.4b. This process could be continuously repeated demonstrating good reversible wettability; four cycles are shown in Figure 3.4b. It can be seen from Figure 3.4a that the lowest contact angle for a water droplet on the fabricated surface was measured to be $\sim$120°; additional UV illumination would not further decrease the CA below 120°. In contrast, surfaces composed of uniformly distributed TiO$_2$ particles exhibit superhydrophilic properties upon UV exposure$^{[22-26]}$ with a CA <10°, whereas HDPE exhibits a CA of 105°, which is independent of UV exposure. On the hybrid surfaces reported here, there are multiple roughness scales and the hydrophilic TiO$_2$ particles are not distributed uniformly, but localized on the posts. As a result, a droplet could transition from a Cassie state to a Wenzel
state and fully wet the TiO$_2$-coated posts as shown in Figure 3.4d. Such a change on the
top of the posts triggers the wetting of the HDPE grooves as well. The TCL of a water
droplet would be pinned at the edge of the posts. As a result, the contact angle decreased
to 120° upon the UV induced wetting of TiO$_2$. This CA is similar to the value reported in
our previous study,$^{51}$ where we found that the contact angle of a template-embossed pure
polyethylene surface (no particles) was 125° in agreement with predictions from the
Wenzel equation.
Figure 3.4. The change of CA as a function of illumination time. (a) Changes of CA with the UV illumination time with and without water on the surface. (b, c) Reversible wettability changes during cyclic alternation of UV Illumination for 30 min with water, and heating at 105 °C for 1.5 h. (d) Reversible wetting–nonwetting transmission on the fabricated TiO₂–polymer nanocomposite surface with hierarchical structures.
The chemical changes on the TiO$_2$ nanocomposite surface were examined by XPS. Ti–O–H groups would significantly enhance the hydrophilicity of the TiO$_2$ nanoparticle surface and so account for the elimination of the free-energy barrier separating the Wenzel and Cassie state.

3.2.2.4 Photodegradation of RhB

The photodegradation of RhB was quantified by UV-vis spectroscopy. The spectra are shown in Figure 3.5. A control experiment was also performed, in which no UV light was applied. The spectra of RhB solution before and after 2 hours on TiO$_2$-HDPE film were shown in Figure 3.6. A slight increase of absorbance at $\lambda_{\text{max}}$ is observed, which is due to the evaporation during the experiment. The concentration changes are plotted in Figure 3.7. There’s a good linear relationship between lnA and time, which indicate this photocatalytic reaction on superhydrophobic surface follows a first-order reaction mechanism. The rate constant $k$ is calculated to be 0.0134 min$^{-1}$.
Figure 3.5. UV-Vis spectra of RhB solution at different UV exposure time on TiO$_2$ photocatalytic film.

Figure 3.6. UV-Vis spectra of RhB solution on TiO$_2$ photocatalytic film without UV exposure.
**Figure 3.7.** Plot of RhB concentration change during photodegradation: a. linear scale; b. natural logarithm scale.

### 3.2.3 Conclusions

In summary, a photocatalytic superhydrophobic TiO$_2$-HDPE nanocomposite surface, where TiO$_2$ nanoparticles were segregated into a regular square array pattern, was fabricated by a simple template lamination method. The static CA reaches 158° and the slip-off angle is as low as 8°. The TiO$_2$–HDPE nanocomposite surface shows a UV-thermal induced reversible wettability which can be repeated over numerous cycles. As shown by XPS analysis, the reversible wetting properties are due to hydrolysis of the TiO$_2$ nanoparticle surface upon irradiation with UV light. The photocatalytic property was illustrated by degradation of RhB.

### 3.3 Photocatalytic TiO$_2$-nanocomposite films for organic dyes degradation

In this section, a TiO$_2$-polymer nanocomposite film was fabricated by a simple lamination process. Photodegradation of a dye solution (RhB) on the surfaces was studied.
The fabricated film was demonstrated useful in two applications: treatment of organic waste from undergraduate laboratory and killing bacteria.

3.3.1 Experimental

3.3.1.1 Fabrication of TiO$_2$-polyethylene nanocomposite films

The TiO$_2$-polyethylene nanocomposite film was fabricated by a lamination method. P25 TiO$_2$ nanoparticles from Evonik (diameter $\approx$ 25nm) were selected because they are widely used and relatively low-cost. Ultra-high molecular weight Polyethylene (UHMW PE500 manufactured by Saint-Gobain) film with a thickness of 500 $\mu$m (0.02") was used as the polymer substrate. A thin layer of TiO$_2$ particles was spread on a mold and covered by a piece of PE film (2 inches by 3 inches). The mold was put in between two metal plates in the lamination press, and then temperature and pressure were applied to the system. Temperature was set to a value higher than the UHMW PE melting point (133$^\circ$C) in order to lower PE film viscosity such that it could flow. Under high temperature and pressure, the PE polymer melted and flowed into the TiO$_2$ particle layer causing TiO$_2$ particles to become encapsulated within the polymer matrix as well as partially embedded on the polymer surface leaving a portion of the particles exposed on the surface. After cooling to room temperature, excess, non-adhered, TiO$_2$ particles were removed by blowing the surface with compressed air. The TiO$_2$-PE nanocomposite films were washed with distilled water and dried at 65$^\circ$C for 2 hours before use. For photocatalytic experiments, each film was put in water for 24 hours to improve the photocatalytic efficiency.$^{[27]}$ The water treatment can modulate the surface chemistry and electronic structure of TiO$_2$ and promote the ability of TiO$_2$ to catalyze dyes excited by visible-light irradiation.$^{[27]}$
3.3.1.2 Degradation of organic dyes used in undergraduate instructional laboratories

Undergraduate biology and biochemistry laboratory courses generate significant levels of organic effluent, including dyes that are non-hazardous wastes yet difficult to degrade in sanitary sewer systems. Our objective is to decompose of these wastes by using sunlight and a novel TiO$_2$ nanocomposite catalytic material, developed in our laboratory, before they are disposed. We are proposing to use the results from our materials science research to make our campus greener.

In campus laboratories, all hazardous chemical wastes must be disposed of by a specialist contractor in order to meet safety, health, and legislative requirements. The non-hazardous chemicals that may be flushed directly into the sink are limited to: water-soluble, nonflammable, noncorrosive, nonreactive, and nontoxic materials, as defined by the US Environmental Protection Agency. The teaching laboratories on our campus generate roughly one hundred gallons of this non-hazardous waste each year. Being cautious, our campus chooses to send this waste to a hazardous waste contractor at a cost of ~$2000/year. A similar situation exists at other colleges, which means there are thousands of gallons of non-hazardous waste generated every year in the City. Although defined as non-hazardous waste, these dye solutions may still lead to unwanted contamination if disposed in the sanitary sewer system, or generate added expense if disposed by a contractor. Experience gained by the treatment of non-hazardous waste will be used to research techniques for treating hazardous waste in the future.

3.3.1.3 Bacteria inactivation experiment
Access to clean drinking water is a perennial challenge in third-world countries. Problems resulting from poor education, health, infrastructure and weather extremes all play a role and contribute to the difficulty in maintaining good water quality. Our project is focused on studying the effectiveness of a solar-activated photocatalytic film to kill microbes responsible for water-borne diseases. Our approach leverages an established method called Solar Water Disinfection (SODIS). SODIS uses sunlight and heat to deactivate pathogens present in water by using filled plastic bottles that are exposed to sunlight via placing them on rooftops for a span of 6 hours to 2 days. Compliance is difficult to insure because of the long exposure time required to deliver the effectiveness of the SODIS system. By inserting a low-cost polymer film containing photo-catalytically active titanium dioxide (TiO₂) particles into the bottle, the time required to deactivate the pathogens and disinfect the water is decreased. The faster purification times are expected to increase user compliance and provide an inexpensive and reusable source of safe drinking water to remote areas in underdeveloped countries.

The goal of the research is to quantify the rate of E. coli de-activation in the presence of a TiO₂ nanoparticle-embedded polymer film surface. Control surfaces will be used so that the effectiveness of the photocatalytic film can be compared against a container with no TiO₂ film (similar to the conventional SODIS process). To verify that the film itself is not harmful to bacteria, a second control will be used where the TiO₂ film is kept in the dark. All experiments will be conducted at 20°C ± 1°C to prevent thermal deactivation of bacteria. An artificial light source will be used in the laboratory tests.

Three sets of experiments were performed:
1. With TiO$_2$ film and UV Light: Place sealed bag under Dymax UV light source with exposure of 10 mW/cm$^2$.

2. With TiO$_2$, but no UV light: bag wrapped in aluminum foil. Designed to show that the film, itself, is inert when not exposed to UV light.

3. UV Light Only: Bag without film exposed to UV light. Designed to quantify the effectiveness of UV light on bacteria with no TiO$_2$ present.

For each experiment, 10 µL aliquot of solution was collected and place into wells of a 96-well plate at starting of the experiment. Add 90 µL of TSB broth to each well. Repeat the aliquot extraction after 30 min, 60 min, and 120 min. All samples were maintained at 20°C ± 1°C during the two-hour exposure experiment. Once the samples are collected at the allotted time the 96-well plate is covered and kept in refrigerator. After all samples are collected, the 96-well plate was placed on a shaker and kept in incubator for 3 hours at 37°C. After incubation, the optical density of samples was measured by using a microplate reader. The percentage of bacteria deactivation was calculated as compare to the initial bacteria solution.

### 3.3.2 Results and discussions

#### 3.3.2.1 Degradation of organic dyes used in undergraduate instructional laboratories

The degradation of organic dyes from undergraduate instructional laboratories were studied by using the fabricated TiO$_2$ film and sunlight irradiation. The dyes include methylene blue, crystal violet, basic fucsin and a mixture of the three dyes (the structures are shown in Scheme 3.2).
To understand the photodegradation kinetics, controlled studies of dye molecules on the nanocomposite films were performed using a UV lamp (BlueWave 200 with primary wavelength of 365nm). Results showed TiO$_2$-PE films could degrade both individual dyes and dye mixtures. In these tests, a pure dye solution (20mL of a 10 mg/L solution) was added into the fabricated plastic bag and irradiated under UV light (1 mW/cm$^2$, similar intensity of the UV portion in sunlight) for 2.5 hours. The results are summarized in Table 3.1. More than 90% of dye molecules decomposed after 2.5h. The degradation reactions followed a first order reaction mechanism.

**Scheme 3.2.** The structure of Rhodamine B, Crystal violet, Methylene blue and Fuchsin.
Table 3.1. Dye solution concentration before and after 2.5h UV irradiation.

<table>
<thead>
<tr>
<th>Dye</th>
<th>Initial conc. (mg/L)</th>
<th>Final conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal Violet</td>
<td>10.00</td>
<td>0.72</td>
</tr>
<tr>
<td>Methylene Blue</td>
<td>10.00</td>
<td>0.81</td>
</tr>
<tr>
<td>Basic Fuchsine</td>
<td>10.00</td>
<td>0.43</td>
</tr>
</tbody>
</table>

To utilize natural sunlight as the energy source, outdoor experiments were conducted. The same solutions were used as for the UV lamp experiments described above. On a sunny day in March (temperature: 45-50°F, sunlight power density: 1-3mW/cm²), the samples were exposed under sunlight for 3 hours. The fading of the solution color in bags during the experiment were shown in Figure 3.8. The bright color disappeared almost completely after the 3 hour exposure, which indicated most of the dye molecule rings were decomposed.

Figure 3.8. Outdoor exposure experiment. (Upper left: three dyes mixture; upper right: Methylene blue; bottom left: crystal violet; bottom right: basic fuchsin).
For basic fuchsin (a), crystal violet (b), methylene blue (c), and a mixture of the three dyes (d), the decay of their absorbance peaks is shown in Figure 3.9. Before sunlight exposure, there was an intensity decrease for the dye solutions because dye molecules were adsorbed onto the TiO$_2$-PE surface. A control experiment was conducted by keeping four bags containing the individual dyes (Rhodamine B, Methylene Blue, Crystal violet and Basic fuchsin) in the dark for three hours. The concentrations decreased by 26.7% for RhB, 32.6% for Methylene blue, 55.1% for Crystal violet and 21.1% for basic fuchsin was observed due to the adsorption of dye molecules onto the rough TiO$_2$-PE surface. More than 80% of dye molecules were decomposed after one-hour exposure to sunlight. After 3 hours, only 0.41% of basic fuchsine, 0.23% of crystal violet and 0.43% of methylene blue were detected.
Figure 3.9. UV-vis spectra of outdoor experiments. (a: basic fuchsine, b: crystal violet, c: methylene blue, d: three dyes mixture)

The experimental results using both a UV lamp and sunlight demonstrated our photocatalytic bags worked well to degrade both single dye solutions as well as dye mixtures. The reaction rates from the UV lamp experiments are similar to those from sunlight exposure as expected due to the similar power densities. We also performed the experiment using our photocatalytic bags to decompose the waste dye mixture from teaching labs. We determined the approximate composition of the waste by UV spectroscopy. This acidic solution (pH=2.7) contains methylene blue (2.75 mg/L), crystal violet (2.50 mg/L), and basic fuchsine (8.00 mg/L). The pH of this solution was adjusted to pH 7.0 before filling the plastic bags. After a three-hour exposure to sunlight, more than 99.3% of the dye molecules were decomposed and the solution became colorless, as shown in Figure 4. In the early reaction stage, demethylation of the dye molecules occurs to form derivatives such as N-oxides and thionin. After multiple steps, small molecules like CO₂ and H₂O will form. Based on literature studies [29-31], the total mineralization time is estimated to be 6-7 hours, which enables the outdoor experiments to be complete within one day.

3.3.2.2 Reusability test of plastic TiO₂ bags

To test the reusability of our TiO₂-PE plastic bag, 10 cycles of UV radiation of the dyes using the plastic bags were conducted. The average decomposition was 92.1%. There was essentially no change of the decomposition rate, compared to the first cycle (93.0%). The results are shown in Figure 3.10. The good reusability of TiO₂-PE plastic bag is due
to the large concentration of TiO₂ particles on the PE surface. The particles can shadow the underlying PE in which the TiO₂ particles are embedded. The shadowing would reduce the direct oxidation of the PE by the reactive oxygen species (ROS) and so extend the lifetime of the composite films.

![Figure 3.10. Reusability of TiO₂-PE nanocomposite film.](image)

**Figure 3.10.** Reusability of TiO₂-PE nanocomposite film.

### 3.3.2.3 Bacteria inactivation experiment
Figure 3.11. Bacteria survival ratio. Blue square: average percentage living E. coli left after TiO₂ film is exposed to UV light source; Red dot: Bag with TiO₂ film is kept in dark, without exposing to light; Green triangle: Clear bag- SODIS imitation bag (no TiO₂ film).

The TiO₂ film proved effective in reducing the concentration of bacteria (E. coli) when exposed to UV light as compared to the controls. After 2 hours of UV exposure, the percent of viable bacteria decreased by $31 \pm 8\%$ in the bag containing the TiO₂ film, whereas the control bag without the TiO₂ film (SODIS simulation) showed no change in bacteria present. Keeping the TiO₂ film in the dark caused the bacteria to multiply, with the concentration increasing by $23 \pm 4\%$ after two hours in the dark.

3.3.3 Conclusion

A lamination method was developed to fabricate a new type TiO₂-polymer nanocomposite film for photocatalytic applications. By using the TiO₂ with sunlight
irradiation, we demonstrated the effective photodegradation of organic dyes as well as waste mixtures from undergraduate laboratory.

The results from bacteria deactivation experiments indicate that the photo-catalytic TiO$_2$ film, prepared by simple lamination, does increase the effectiveness of the SODIS method even at a temperature of 21°C, far below the temperature at which SODIS is effective.

3.4 Photocatalytic superhydrophobic surfaces

3.4.1 TiO$_2$-PDMS self-cleaning superhydrophobic surface

3.4.1.1 Experimental

3.4.1.1.1 Fabrication of TiO$_2$-PDMS surperhydrophobic surface

The PDMS posts were printed as a 17 × 17 square array with a pitch of 0.5 mm (8 mm × 8 mm array, 1 mm tall) on a 10 × 10 mm membrane surface (0.5 µm diameter, Millipore®). A layer of TiO$_2$ nanoparticles (~ 21 nm), or SiO$_2$ nanoparticles (~ 200 nm) was spread onto the posts immediately after printing. The viscous and thixotropic properties of the PDMS maintained their shape before cure; the particles became partially embedded into the uncured surface ensuring good adhesion between the particles and the PDMS posts. The surface was cured at 65 °C for 2 hours. Excess particles were removed by exposing the surface to high flows of compressed air and rinsed with deionized water.

In addition to the surface of PDMS posts covered by TiO$_2$ nanoparticles, the surface was modified by coating SiO2 nanoparticles (Cabot TS530) at the tips of posts. This modified surface was prepared by dipping the fully TiO$_2$ coated PDMS posts surface into a thin layer of uncured silicone (Corning 3140) and then dusted with SiO$_2$ nanoparticles,
following by curing in oven. In such surface, the tips of PDMS posts (~200μm) are covered by SiO₂ and the lower part of the posts (~800μm) are covered by TiO₂. This structure ensures that the TiO₂ nanoparticles isolated from contacting with aqueous solution, and enable the study of the generation of reactive oxygen species or intermediate at gas phase.

3.4.1.1.2 Photocatalytic reactions

For the plastron gas experiments, a three-phase photoreactor was constructed. The bottom of a PMMA disposable cuvette was removed to enable connection to the superhydrophobic photocatalytic surface. TiO₂-PDMS posts were printed onto a Millipore membrane (10 mm × 10 mm) with a pore size of 0.5 μm, coated with TiO₂ nanoparticles and cured. The printed membrane was then placed on a 1 cm² Delrin plastic (3 mm thick) support plate which defined the top of the plenum. Five holes (1 mm diameter each) drilled through the plate enable gas flow from the plenum to the plastron. The support plate was inserted half-way into a 1 cm² custom-molded silicone rubber chamber (3 mm thick), leaving a 1.5 mm deep plenum for gas purging. A 25G 1½’ needle was bent and inserted into the bottom of the plenum with the silicone forming a gas-tight seal. The other end of the needle was connected to a regulated gas supply; the gas flow rate was controlled with a rotometer. To prevent the wetting of side wall posts by solution, the bottom inner side (0.5 mm × 0.8 mm for each side) of cuvette was first coated with silicone (Corning 3140) and then dusted with SiO₂ nanoparticles to achieve superhydrophobicity. After curing 2 hours at 60 °C for 2 hours, excess particles were removed by deionized water and compressed air. The cuvette was placed in an Ocean Optics cuvette holder fitted with optical fibers connected to a light source (Mikropack HL2000) and spectrometer (Ocean Optics USB4000) such that the solution absorption spectrum could be measured in-situ
during irradiation from the top opening of the cuvette. In some cases, UV-visible spectra were collected using a Perkin Elmer (Lambda 650) spectrophotometer.

3.4.1.1.3 Self-cleaning demonstration

SiO$_2$ nanoparticles (TS-530) were coated on the PDMS posts and used as the control surface. Both TiO$_2$-PDMS and SiO$_2$-PDMS surface was put into the BSA Alexa Fluor 488 solution for 60 min. No obvious difference was observed before and after the protein deposition on the posts. Surfaces were then rinsed with deionized water and dried with compressed air. The surfaces were then exposed to UV light (150mW/cm$^2$) for 2 hours. The confocal imaging was taken before and after UV exposure. The confocal parameters used were: excitation wavelength, 488nm; laser power, 20%; and PMT gain, 560 V. The fluorescence range was set to be 498-540nm. The absolute scan height of the post was 300 μm with the scan number of 250 (1.2 microns between scans).

3.4.1.2 Results and discussions

3.4.1.2.1 Superhydrophobic properties

The course-scale primary roughness of the surface is formed by printing an array of PDMS posts using a robotic printer (1mm tall × 0.5mm diameter × 0.5mm pitch). After printing, particles were adhered to the uncured PDMS surface forming a secondary roughness; a tertiary roughness is formed from agglomerates of nanoparticles (Figure 3.12 a-c). The microstructure of the TiO$_2$-PDMS surface is shown in the SEM images (Figure 3.12 a-c). It is this hierarchical roughness, with relatively course agglomerates of nanoparticles forming a re-entrant surface geometry, that maintains liquid in a Cassie state (Figure 3.12 d,e).
Figure 3.12. SEM images of a PDMS post printed on 0.5 mm pitch, partially embedded with TiO$_2$ nanoparticles and optical images of a 20 µL water drop on the surface. (a) Whole post, (b) High magnification of a tip, (c) Higher magnification showing TiO$_2$ nanoparticle agglomerates, (d) Optical image of a 20 µL water drop on a printed surface, (e) Enlarged image of the solid-liquid-gas interface illustrating how the liquid surface deforms about the nanoparticles.

A 20 µL water droplet residing on the TiO$_2$-PDMS surface is shown in Figure 3.13. The Water contact angle of the TiO$_2$-PDMS surface is 162° ± 5°. The surface of TiO$_2$ particles becomes hydrophilic when exposed to UV light in the presence of water due to the formation of Ti-OH groups.\[32\] For our printed hierarchical TiO$_2$-PDMS surfaces, water is supported in the Cassie state after more than 3 hours of UV irradiation (4 mW/cm$^2$) as shown in Figure 3.14. Over this 3 hours of exposure, the position of the water-air interface descends only a small amount (< 90 µm) into the plastron. At the later stages of this process, some PDMS post tips transitioned from the liquid-air interface into the liquid phase due to
the increase in the hydrophilicity of the TiO$_2$ surface. However, the superhydrophobic properties remained stable with a fully intact plastron for two reasons: the TiO$_2$ particles away from the solid-liquid-air triple contact line (TCL) remain relatively hydrophobic and the length of the TCL increases as the interface descends due to the conical shape of the posts, preventing further encroachment.

**Figure 3.13.** 20 µL water droplet residing on TiO$_2$-PDMS surface.
Figure 3.14. Optical images of the liquid-air interface position relative to the PDMS post surface after UV irradiation on a TiO$_2$-PDMS surface. UV irradiation time for (a) 0, (b) 60, (c) 120 and (d) 180 minutes (water layer thickness: 3 mm). The water layer appears white, the plastron appears dark grey and the PDMS posts appear light grey.

3.4.1.2.2 Photo-catalytic reactions in a triphasic reactor

A three-phase catalytic chemical reactor device was constructed as shown schematically in Figure 3.15. This design enables the simultaneous irradiation of the catalyst and the monitoring of the solution concentration by UV-vis spectroscopy. In addition, a gas can be introduced into the plenum supporting the printed superhydrophobic surface such that it flows through the membrane and into the plastron. As shown in Figure 3.16, bubbles formed at the plastron-liquid interface would release and rise through the 2 ml of solution, increasing both the surface area and time over which the gas can dissolve into solution. After bubble release, a planar plastron reforms and the bubble sequence repeats. The solution remains in the Cassie state throughout the experiment.
**Figure 3.15.** Schematic drawing of the cuvette reactor with a gas bubble formed on the surface.

**Figure 3.16.** Optical photographs of a superhydrophobic surface composed of TiO$_2$ particles in cuvette photocatalytic reactor, showing: (a) Gas bubble formed over the surface, (b) and (c) Gas bubble releasing from the surface, (d) After bubble released, plastron layer reformed with a planar and reflective air-water interface.

Decoloration of RhB solution, monitored as a function of irradiation time is shown in **Figure 3.17**. The reaction was conducted under flowing oxygen (20 cc/min). The concentration of RhB (10 mg/L) decreased by 80.0 % after 3.5 h of irradiation with a rate constant of 0.0079 min$^{-1}$ (**Figure 3.18**). First order kinetics was observed; a plot of ln[RhB] vs time was linear ($R^2$ value ≥ 0.97) which is consistent with a Langmuir–Hinshelwood mechanism. $[^{33,34}]$
Figure 3.17. UV-vis spectrum of Rhodamine B at different irradiation times on a TiO$_2$-PDMS surface. Inset pictures are the Rhodamine B solution before (left) and after (right) UV photodegradation.

Figure 3.18. Change in RhB concentration as a function of irradiation time. a, Percent change of RhB concentration decreases as a function of UV irradiation time. b, ln concentration of RhB vs time (Rate constant is 0.0079 min$^{-1}$; $R^2$ is 0.97).
Flowing O₂ led to the most rapid rates of RhB decolorization. When the oxygen concentration was decreased by using either static ambient air or flowing N₂ gas, the rate was reduced significantly to 0.0054 or 0.0008 min⁻¹ respectively (Figure 3.19). The rate in flowing N₂ was 89% slower than in flowing O₂; however the rate was not reduced to zero due to trace O₂ and/or hydroxyl radicals generated by the photocatalytic decomposition of water.⁴⁵ Without the PDMS superhydrophobic surface with TiO₂ particles present, the RhB concentration change was less than 0.3%. The high rate of RhB decolorization in flowing O₂ is comparable with prior work on photodegradation of RhB dye using dispersions of TiO₂ nanoparticles in solution.⁴⁶,⁴⁷ The rate is ~7 times faster than the previously reported degradation of methylene blue (MB) on a superhydrophobic TiO₂-PTFE nanocomposite film.⁴⁸ The faster rate observed on the TiO₂-PDMS printed surface reflects the higher concentration of O₂ in the plastron as well as the greater TiO₂ particle surface area accessible to the aqueous solution. As shown in Figure 3.19, the error bars in the oxygen bubbling experiments are relatively large. This is because during the reaction, oxygen bubbles may exit from different locations across the PDMS posts for each separate experiment, affecting the liquid-solid contact area.
Figure 3.19. Degradation of RhB under different plastron gas conditions. At least three experiments were done for each condition.

To determine if any reactive oxygen species generated in the plastron can be transported into the solution and oxidize the RhB, a surface was constructed that physically separated the TiO$_2$ particles from the aqueous dye solution. A SiO$_2$ capped surface was prepared to isolate the TiO$_2$ nanoparticles from the aqueous solution. On such surface, the tips of PDMS posts (~200μm) were coated with hydrophobic SiO$_2$ nanoparticles, and lower part of PDMS posts were coated with TiO$_2$ nanoparticles. The photooxidation experiments were conducted under static ambient air conditions (no gas flow through the plastron) and the results are compared with other surfaces in Figure 3.20. On the fully coated TiO$_2$ surface, 54% of the RhB was photooxidized after 3.5 hours. However, when TiO$_2$ particles were isolated from RhB solution, the decrease of RhB concentration was only 12% after same UV irradiation time. This decrease is similar to the decrease observed
on a control surface (10%, blue curve in Figure 3.20) composed of only SiO2 nanoparticle coated PDMS posts. Since we know that RhB is stable when exposed to UV irradiation (~ 3% decrease after 3.5 hours, black curve in Figure Figure 3.20), the majority of the 10% decrease of RhB on SiO2 nanoparticle surfaces was due to adsorption of the dye on the silica surface. As the triple contact line descends into the surface over time, the concentration of dye in the solution decreases throughout the experiment. The coating of the SiO2 surface by RhB was confirmed by optical microscope inspection of the surface after the experiment. These results indicate that RhB in solution is photooxidized primarily when in direct contact with TiO2 particles. However, due the movement of the triple contact line during the experiment, a contribution from ROS transported across the plastron cannot be ruled out.

![Figure 3.20](image)

Figure 3.20. Degradation of RhB on different surfaces at static ambient air conditions. At least three experiments were done for each condition.
3.4.1.2.3 Self-cleaning properties

The surface can photooxidize organics both when in contact with a solution containing the molecules, as well as when the solution is removed leaving the molecules adsorbed onto a dry surface. RhB is continuously adsorbed from solution and photooxidized on the surface of the TiO$_2$ nanoparticles as shown in Figures 3.17. The aqueous solution is not required for photooxidation, however. When RhB is adsorbed onto the TiO$_2$-PDMS surface and then dried, UV irradiation of the dry surface also leads to decoloration as shown in Figure 3.21.

![Figure 3.21. Optical microscope images of RhB coated TiO$_2$-PDMS posts before and after UV irradiation (200 mW/cm$^2$ for 2 hours). (a) Before UV irradiation, (b) After UV irradiation.](image)

Proteins are ubiquitous surface contaminants that can lead to significant changes in surface wettability.$^{[44]}$ To quantify the decomposition of an absorbed protein on a TiO$_2$-PDMS superhydrophobic surface, a fluorescent protein (Bovine Serum Albumin, Alexa Fluor 488 conjugate), was adsorbed onto TiO$_2$-PDMS and SiO$_2$-PDMS surfaces. The surface was then dried under compressed air. The confocal results show that the TiO$_2$-
PDMS surface (Figure 3.22a) adsorbed significantly more BSA protein than the SiO$_2$-PDMS surface (Figure 3.23a). As seen from the 3D reconstructed surface (Figure 3.22c), the protein adsorption was limited to the upper 90 µm of the post, which is consistent with the descent of the triple contact line. After UV exposure (150 mW/cm$^2$, 2 hours), the integrated fluorescence on the TiO$_2$-PDMS surface decreased by 91.3% (Figure 3.22b,d) whereas the fluorescence remained relatively constant on the SiO$_2$-PDMS surface; the signal degraded by 27.4% (Figure 3.23b). Photo-oxidation of BSA is not expected on a SiO$_2$ surface;[39] the decreased fluorescence intensity on the SiO$_2$-PDMS surface results from photobleaching of the dye during UV exposure. Although Alexa Fluor 488 is resistant to photobleaching,[40] some fluorescence signal degradation is expected upon prolonged irradiation at wavelengths <500 nm.
**Figure 3.22.** Confocal images of protein coated TiO$_2$-PDMS posts before and after UV irradiation. (a) Top view of protein coated TiO$_2$-PDMS tips before UV irradiation, (b) Top view of tips after UV irradiation, (c) Reconstructed 3D image of single post with protein deposition before UV irradiation, (d) 3D image of single post after UV irradiation.

**Figure 3.23.** Confocal images of protein coated SiO$_2$-PDMS posts before and after UV irradiation. (a) Before UV irradiation, (b) After UV irradiation.
These results indicate that the TiO$_2$-PDMS surface exhibits robust superhydrophobic and self-cleaning properties. Not only does the surface remain superhydrophobic when exposed to conjugated dyes, protein and UV light, but the UV light effectively photo-oxidizes absorbed contaminants leading to their removal from the surface.

### 3.4.1.3 Conclusions

In summary, a technique for fabricating robust scalable superhydrophobic surfaces has been demonstrated where catalytic particles can be selectively embedded into the surface of printed PDMS post arrays. By synthesizing surface structures with a significant degree of hierarchical roughness, any arbitrary catalyst particle type, in sizes ranging from tens of nanometers to tens of micrometers, can be incorporated. We show that hydrophilic TiO$_2$ particles, with no surface modification, can cover the PDMS surface without compromising the stability of the plastron layer. Because high oxygen concentrations can be maintained at the gas-liquid-solid interface, and the concentration of TiO$_2$ particles on these superhydrophobic surfaces is large, photooxidation rates of RhB are comparable to hydrophilic TiO$_2$ nanoparticles dispersed in aqueous solutions. Catalytic reactions were studied both in a static gas environment as well as a dynamic environment where the gas flow rate and pressure through the plastron were sufficient to release bubbles into the supported liquid.

These structures provide a unique environment in which photocatalytic mechanistic studies can be conducted as catalyst chemistry, particle size, surface wetting, plastron gas composition and gas flow rate, can be controlled independently of the aqueous solution
composition. In addition, reactions can be conducted where the catalyst particles are proximate to, but isolated from, the aqueous solution. This configuration may be especially important in applications such as water purification and medical devices where gas phase generation of a reactive intermediate or ROS is required but contamination of the fluid by the catalyst must be avoided.

3.4.2 Silicon phthalocyanine-PDMS superhydrophobic surface on porous membrane: generation of singlet oxygen, effect of gas flow and sensitizer wetting on trapping efficiency

In this section, a photocatalytic superhydrophobic surface is prepared with silicon-phthalocyanine (Pc) photosensitizer particles. I studied and discussed physical-organic chemistry principles of singlet oxygen generation and transport into an aqueous solution supported on superhydrophobic surfaces on which silicon-phthalocyanine particles are immobilized. Singlet oxygen (\(1^0_2\)) was trapped by a water-soluble anthracene compound and monitored \textit{in-situ} using a UV-vis spectrometer. By flowing oxygen through the porous superhydrophobic surface, singlet oxygen generated in the plastron (i.e. the gas layer beneath the liquid) is transported into the solution within gas bubbles, thereby increasing the liquid-gas surface area over which singlet oxygen can be trapped. Significantly higher photooxidation rates were achieved in flowing oxygen, as compared to when the gas in the plastron was static. Superhydrophobic surfaces were also synthesized so that the \(Pc\) particles were located in contact with, or isolated from, the aqueous solution to evaluate the relative effectiveness of singlet oxygen generated in solution and the gas phase, respectively; singlet oxygen generated on particles wetted by the solution was trapped more
efficiently than singlet oxygen generated in the plastron, even in the presence of flowing oxygen gas. A mechanism is proposed that explains how Pc particle wetting, plastron gas composition and flow rate as well as gas saturation of the aqueous solution affect singlet oxygen trapping efficiency. These stable superhydrophobic surfaces which can physically isolate the photosensitizer particles from the solution may be of practical importance for delivering singlet oxygen for water purification and medical devices

3.4.2.1 Experimental

3.4.2.1.1 Fabrication of Si-Pc-PDMS surperhydrophobic surface with Si-Pc particles at controlled locations

Similar as the procedures in preparing TiO$_2$-PDMS superhydrophobic surface, the PDMS posts were printed as a 17 × 17 square array with a pitch of 0.5 mm (8 mm × 8 mm array) on a 10 mm × 10 mm membrane surface. Si-Pc were immobilized at controlled locations at the PDMS posts, three types of surface were prepared. For surface A, a layer of Pc particles was spread onto the posts immediately after printing. The viscous and thixotropic properties of the PDMS maintained their shape before cure; the Pc particles became partially embedded into the uncured surface ensuring good adhesion between the particles and the PDMS posts. The surface was cured at 65 °C for 2 h. Excess particles were removed by exposing the surface to high flows of compressed air. Figure 3.24 shows a schematic of the fabrication of surface B. Here, immediately after printing the PDMS posts, the tips of the posts were dipped into a thin layer of Pc particles. The tip-coated posts were cured at 65 °C in an oven with tips facing down.
Figure 3.24. A schematic of the fabrication of surface B.

Figure 3.25 shows a schematic of the fabrication of surface C. The PDMS post surface was printed and coated with Pc particles (as for surface A). After curing and removal of excess Pc particles, the tips were dipped into a thin layer of uncured PDMS approximately 200 µm thick. The surface was then dipped into a thin layer of SiO₂ nanoparticles such that the silica adhered to the uncured silicone. Finally, the post array was cured at 65 °C in an oven with tips facing down.

Figure 3.25. A schematic of the fabrication of surface C.
Figure 3.26 shows the SEM images of surfaces A-C showing the structure of the printed posts with Pc particles embedded on the surface. These surfaces have a coarse-scale primary roughness, which is formed by printing an array of PDMS posts (1 mm tall, 0.50 mm pitch). The Pc particles that are adhered to the uncured PDMS surface produce a secondary roughness, whereas a tertiary roughness is formed from the thin layer of SiO2 nanoparticles (Surface C).

Figure 3.26. SEM images of PDMS posts coated with Pc particles at controlled locations: (A) Surface A with particles coating the entire PDMS surface; (B) Surface B with particles adhered only to the top portion of the PDMS posts; and (C) Surface C where the surface prepared as in Surface A was capped with a layer of silica nanoparticle adhered to a layer of PDMS.

3.4.2.1.2 Design of singlet oxygen photo-reactor

An understanding of the mechanism of \(^1\text{O}_2\) formation in superhydrophobic sensitizers is key to \(^1\text{O}_2\) utilization for various potential applications. To help achieve this mechanistic insight, three types of surfaces were prepared, as shown schematically in Figure 5.4, where the wetting of the particles by the solution was varied. Surfaces were prepared where sensitizer particles were dispersed across the entire surface (Surface A), isolated at the tips of the posts in contact with the liquid layer (Surface B) or physically
separated from the liquid, exposed only in the plastron (Surface C). Surfaces A-C exhibit stable superhydrophobic properties with a fully intact plastron throughout the experiment. Based on results from these surfaces, a photooxidation mechanism is proposed.

![Image](image.png)

**Figure 3.27.** Schematic images of PDMS posts coated with Pc particles at controlled locations. (A) Surface A has Pc particles coating the PDMS posts. (B) Surface B has Pc particles primarily embedded at the PDMS tips. (C) Surface C has Pc coating the PDMS post base, where tips are capped with a layer of PDMS and SiO$_2$ nanoparticles.

The superhydrophobic surfaces were incorporated into an $^1$O$_2$ cuvette photoreactor device as shown schematically in **Figure 3.28.** This design enables control over both the gas composition and gas flow rate through in the plastron (i.e. the gas layer below the liquid). The effect of oxygen concentration at the particle surface was studied systematically and in real time by measuring the concentration of $^1$O$_2$ trapped by 9, 10-anthracene dipropionic acid 1 *in-situ* using UV-vis spectroscopy (**Scheme 3.1**).
Figure 3.28. Geometry of the $^{1}$O$_2$ photoreactor device: a polymethyl methacrylate (PMMA) cuvette was modified to incorporate a superhydrophobic surface embedded with Pc particles printed onto a porous membrane. The printed membrane is held on a plastic support plate that defines the top of the plenum. Holes were drilled through the plate enabling gas to flow from the plenum to the plastron. A gas input needle inserted into the bottom of the plenum is used to introduce a controlled flow of gas.
Scheme 3.1 Singlet oxygen trapping reaction.

To prepare the \(^1\text{O}_2\) photoreactor device, the bottom of a PMMA disposable cuvette was removed to enable connection to the superhydrophobic surfaces A-C. The PDMS posts were printed onto a Millipore membrane (10 mm × 10 mm) with a pore size of 0.5 \(\mu\)m, coated with particles and cured. The printed membrane was then placed on a 1 cm\(^2\) Delrin plastic (3 mm thick) support plate that defined the top of the plenum. Five holes (1 mm diameter each) drilled through the plate enabled gas to flow from the plenum to the plastron. The support plate was inserted halfway into a 1cm\(^2\) custom-molded silicone rubber chamber (3 mm thick), leaving a 1.5 mm deep plenum for gas purging. A 25G 1½” needle was bent and inserted into the bottom of the plenum with the silicone forming a gas-tight seal. The other end of the needle was connected to a regulated gas supply, where a flow rate of 20 cc/min was controlled with a rotameter.

3.4.2.1.3 In-situ measurements of singlet oxygen generation

The cuvette was placed in an Ocean Optics cuvette holder fitted with optical fibers connected to a light source (Mikropack HL2000) and spectrometer (Ocean Optics USB4000) such that the solution absorption of 1 was measured *in-situ* during irradiation from the top opening of the cuvette. In a few cases, absorption spectra were collected using a Perkin Elmer (Lambda 650) spectrophotometer. For the solution presaturation studies,
stock solutions of 1 in D₂O (~20 mL) were purged in N₂ or O₂ for 2 h at 100 cc/min prior to transferring 2 mL portions to the photoreactor.

3.4.2.2 Results and discussions

3.4.2.2.1 Characterizations

The Si-Pc photocatalytic surface has a coarse-scale primary roughness, which is formed by printing an array of PDMS posts (1 mm tall, 0.50 mm pitch) using a robotic printer. After printing, Si-Pc particles were adhered to the uncured PDMS surface forming a secondary roughness. The microstructure and optical images of the surface A are shown in Figure 3.29. As shown in the images, a water droplet is maintained in a Cassie state, which indicates the Pc-PDMS surface exhibits good superhydrophobicity.

Figure 3.29. Microstructure and optical images of Pc-PDMS surface.
3.4.2.2 Effect of plastron gas composition on the trapping of singlet oxygen in water solution

A series of experiments was conducted using Surface A to evaluate the effect of gas flow, gas composition in the plastron, and dissolved oxygen concentration in the fluid on $^1\text{O}_2$ formation. Aqueous solutions form a stable Cassie state on these surfaces owing to the hierarchical roughness. A coarse scale primary roughness is formed by the high aspect ratio of printed PDMS posts. These posts alone would form a superhydrophobic surface, however partial wetting of the PDMS posts can occur.$^{[41]}$ Embedding Pc particles into the PDMS surface, however, increases the stability of the Cassie state owing to the hydrophobic surface of the particles as well as the coarse particle morphology, which adds an additional level of roughness with re-entrant features to the surface. Such hierarchical roughness has been shown to increase the stability of superhydrophobic properties.$^{[42, 43]}$ There was no encroachment of water into the post interstices (i.e. no Wenzel state).$^{[44,45]}$

Data were collected by singlet oxygen trapping with 9, 10-anthracene dipropionic acid 1, a specific $^1\text{O}_2$ reaction developed by Rodgers et al.$^{[46,47]}$ as a facile and convincing $^1\text{O}_2$ reporter compound. By analogy, others$^{[48-51]}$ have detected $^1\text{O}_2$ by trapping with anthracene compounds which lead to endoperoxides that can further decompose to radicals.$^{[52]}$ A $^1\text{O}_2$ mechanism is indicated with Pc sensitizers (Type II process)$^{[53]}$ with a minimal contribution from Type I (radical) photooxidation reactions.$^{[54,55]}$ D$_2$O was used in favor of H$_2$O due to the 20-fold longer lifetime of $^1\text{O}_2$ (65 $\mu$s compared to 3.5 $\mu$s)$^{[56]}$ for rapid and reliable data collection, so that shorter reaction times were required. Figure 3.30 shows the results of experiments with static air, i.e. no gas flowed through the plastron. D$_2$O solutions were presaturated with either $^3\text{O}_2$ or N$_2$ before being filled into the cuvette.
With static air in the plastron, solutions presaturated with $^3\text{O}_2$ produced almost a two-fold higher yield of endoperoxide 2 (51.5 nmol) compared to solutions presaturated with $\text{N}_2$ (22.7 nmol) after laser irradiation for 2.5 h. For the $\text{N}_2$ pre-saturated solutions, $^3\text{O}_2$ was available both from the plastron, as well as from the top of the cuvette which was open to air. Nonetheless, the rates can be seen to slow slightly after the first hour, indicating that oxygen was depleted from the system during the reaction.

![Figure 3.30](image.png)

**Figure 3.30.** Endoperoxide 2 yield in static experiments where D$_2$O solutions were presaturated with O$_2$ or N$_2$. There was no gas sparging through the plenum of the device. Error bars were obtained from 3 measurements.

Introducing a gas flow through the plastron significantly affects the yields of endoperoxide. **Figure 3.31** shows optical images of the photoreactor equipped with surface A, in which a bubble forms at the plastron-liquid interface. The air bubbles grow
from the plastron then release and rise through the 2 mL D₂O solution. After bubble release, the plastron reforms and the bubble growth and release cycle repeats continuously. The solution remains in the Cassie state throughout the experiment. Formation of bubbles increases the surface area and time over which the gas can dissolve into solution.

**Figure 3.31.** Optical images of surface A, showing: (i) plastron with a planar and reflective air-water interface, (ii) gas bubble forming over the surface, and (iii) gas bubble releasing from the surface.

**Figure 3.32** shows the results of experiments where gas was purged through the plastron; two significant effects were observed. First, the rate of endoperoxide formation is significantly enhanced by the flow of ³O₂. The yield of endoperoxide 2 increased by >40% (from 51.5 nmol to 68.3 nmol after 2.5 hours) in flowing ³O₂ compared to when static air was maintained in the plastron. Second, the oxygen concentration in solution has a significant effect on ¹O₂ trapping. Presaturating the D₂O solution with N₂ gas results in higher initial rates of ¹O₂ trapping with an endoperoxide yield of 97.6 nmol, compared with a yield of 68.3 nmol in a solution presaturated with ³O₂. Although ³O₂ is necessary for ¹O₂ formation, solutions presaturated with ³O₂ exhibit lower yields of endoperoxide 2. When N₂ was bubbled through a solution presaturated with N₂, little endoperoxide 2 was formed (2.3 nmol after 2.5 hours); the small amount of endoperoxide observed may be due to leaks.
or residual oxygen in solution. These observations are in good agreement with the results of previous work, where nitrogen-purged solutions also yielded the highest rates of $^1\text{O}_2$ trapping as evidenced either by endoperoxide 2 yield$^{[57]}$ or \textit{E. coli} deactivation.$^{[58]}$ The lower rate observed with $^3\text{O}_2$ purged solutions is attributed to a reduced transport of $^1\text{O}_2$ across the gas/liquid interface into the $^3\text{O}_2$ saturated solution.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.32.png}
\caption{Endoperoxide 2 yield in bubbling experiments where O$_2$ or N$_2$ gas was sparged through the plenum into the D$_2$O solution. Error bars were obtained from 3 measurements.}
\end{figure}

\textbf{Figure 3.32}. Endoperoxide 2 yield in bubbling experiments where O$_2$ or N$_2$ gas was sparged through the plenum into the D$_2$O solution. Error bars were obtained from 3 measurements.

\textbf{Table 3.2} summarizes the static and sparging gas flow $^1\text{O}_2$ photoreactor experiments. The highest rate of endoperoxide 2 formation (0.96 nmol/min) occurs when $^3\text{O}_2$ is bubbled through a D$_2$O solution presaturated with N$_2$. This experimental scenario produces the highest rates as the $^1\text{O}_2$ produced has both the greatest surface area over which
to contact the solution (owing to the bubbles rising through the solution) as well as the greatest solubility in the solution (owing to the depleted $^3\text{O}_2$ concentration resulting from $\text{N}_2$ presaturation). Purging the solution with oxygen before irradiation lowers the initial rate to 0.60 nmol/min. Once the solution becomes saturated in $^3\text{O}_2$, the rates drop significantly and become similar, regardless of the initial condition. This lower rate is similar for all oxygen-saturated systems including the final rate for solutions presaturated with either $\text{N}_2$ or $\text{O}_2$ (0.22 and 0.21 nmol/min respectively) as well as the static solution purged with $^3\text{O}_2$ (0.21 nmol/min). When $^3\text{O}_2$ is excluded, essentially no $^1\text{O}_2$ is produced (0.007 nmol/min for the last 30 minutes of the system with $\text{N}_2$ flow through a $\text{N}_2$ purged solution).

**Table 3.2. Singlet Oxygen Trapping Experiments.**

<table>
<thead>
<tr>
<th>gas flow</th>
<th>solution presaturated</th>
<th>percent decrease in anthracene 1 after 2.5 h (%)$^a, c$</th>
<th>endoperoxide 2 formed (nmol)$^c$</th>
<th>initial rate (nmol/min)$^{b, c}$</th>
<th>final rate (nmol/min)$^{b, c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{O}_2$ purging</td>
<td>$\text{N}_2$</td>
<td>48.8 ± 2.7</td>
<td>97.6 ± 5.3</td>
<td>0.96 ± 0.03</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>$\text{O}_2$</td>
<td>34.2 ± 2.4</td>
<td>68.3 ± 4.8</td>
<td>0.60 ± 0.04</td>
<td>0.21 ± 0.07</td>
</tr>
<tr>
<td>static</td>
<td>$\text{N}_2$</td>
<td>11.4 ± 1.4</td>
<td>22.7 ± 2.8</td>
<td>0.20 ± 0.03</td>
<td>0.17 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>$\text{O}_2$</td>
<td>25.8 ± 2.3</td>
<td>51.5 ± 4.6</td>
<td>0.36 ± 0.05</td>
<td>0.21 ± 0.04</td>
</tr>
<tr>
<td>$\text{N}_2$ purging</td>
<td>$\text{N}_2$</td>
<td>1.2 ± 0.6</td>
<td>2.3 ± 1.1</td>
<td>0.07 ± 0.01</td>
<td>0.007 ± 0.001</td>
</tr>
</tbody>
</table>

$^a$ Experimental conditions: Under subdued light, solutions were presaturated with $\text{N}_2$ for 2 h. Samples were then illuminated at 669 nm with either $\text{O}_2$ bubbling (20 mL/min), static (no gas bubbling), or $\text{N}_2$ bubbling (20 mL/min) for 2.5 h, where $^1\text{O}_2$ is generated and
detected by trapping with 1 (0.10 mM, pH=10). The concentration of anthracene 1 was measured by monitoring the decrease of its absorption at 378 nm. Initial and final rates were calculated over the first and last 30 min of the reaction, respectively. The numbers shown here are averages of 3 measurements.

3.4.2.2.3 Effect of Si-Pc particle location on singlet oxygen yield

To examine the effect of particle location on \(^1\)O\(_2\) trapping, a series of surfaces (A-C) was prepared where the location of the Pc particles was controlled such that the particles covered the entire surface of the posts (Surface A), were restricted to the tops of the posts such that the particles were near or in contact with the solution (Surface B), or were exposed only in the plastron and isolated from direct contact with solution (Surface C). In this way, the relative effectiveness of \(^1\)O\(_2\) generated in solution vs. in the plastron could be evaluated.

The endoperoxide 2 yields for surfaces A-C were compared to each other in experiments that flowed oxygen continuously through the plastron using solutions presaturated with N\(_2\). Table 3.3 shows that \(^1\)O\(_2\) was trapped more effectively (factor of > 2.3) when the Pc particles were located on the top of the posts in or near contact with the solution (86.3 nmol, surface B) as compared to when particles were located only in the plastron, isolated from the liquid phase (37.3 nmol, surface C). Surface A (with Pc particles coating the entire surface) resulted in the highest overall yield of trapped \(^1\)O\(_2\) (97.6 nmol) as \(^1\)O\(_2\) was generated at both the gas-solid interface in the plastron as well as at the liquid-solid interface in solution. The results clearly show that direct Pc particle contact with the solution is not required for trapping \(^1\)O\(_2\), however the yield of endoperoxide 2 is
reduced by more than 60% for Surface C. Unlike heterogeneous sensitizers in direct contact with the solution,[59,60] surface C may be promising for extended photolysis applications where the photodegradation of the sensitizers can be lessened since it is not in contact with solution.

Table 3.3. Effect of Sensitizer Location on $^1$O$_2$ Trapped in D$_2$O Solution $^a$.

<table>
<thead>
<tr>
<th>Pc particle location</th>
<th>decrease of anthracene 1 (%)</th>
<th>endoperoxide 2 produced (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>surface A (Pc located over entire surface)</td>
<td>48.8±2.7</td>
<td>97.6±5.3</td>
</tr>
<tr>
<td>surface B (Pc located at the tip of the posts)</td>
<td>43.1±1.2</td>
<td>86.3±2.7</td>
</tr>
<tr>
<td>surface C (Pc isolated from the liquid phase)</td>
<td>18.6±0.6</td>
<td>37.3±2.7</td>
</tr>
</tbody>
</table>

$^a$Experimental conditions: the solution was presaturated with N$_2$ for 2 h prior to introducing 2.0 ml of solution into the reactor. Samples were then illuminated at 669 nm with O$_2$ bubbling (20 mL/min) for 2.5 h. Generated $^1$O$_2$ was detected by trapping with 1 (0.10 mM, pH=10). The numbers shown here are averages of 3 measurements.

3.4.2.2.4 Mechanism of single oxygen trapping reaction in a triphasic photoreactor

The cuvette-based photoreactor is useful for elucidating the mechanism of singlet oxygen photooxidation as it enables the use of solutions presaturated with different gases as well as independent control of the gas phase composition, flow rate, and specific location of the sensitizer particles on the surface relative to the gas-liquid interface. Figure 3.33 shows the proposed mechanism. Singlet oxygen can be generated either at the gas-solid interface in the plastron and then transported to the solution, or can be generated at the liquid-Pc interface directly in solution. In the plastron, $^3$O$_2$ reacts on the Pc particle
surface to generate $^{1}\text{O}_2$. When there is no gas flow through the cuvette, $^{1}\text{O}_2$ must diffuse across the plastron until it enters the plastron-liquid interface where it can react with 1. Because of the limited lifetime of $^{1}\text{O}_2$ only a fraction of the $^{1}\text{O}_2$ generated will be solvated and react with 1 before it decays to $^{3}\text{O}_2$. A flow of $^{3}\text{O}_2$ through the plastron increases the yield of $^{1}\text{O}_2$ trapped in solution by two mechanisms. First, oxygen gas flow increases the concentration of $^{3}\text{O}_2$ on the catalyst surface thereby increasing the overall quantity of $^{1}\text{O}_2$ formed. Higher concentrations of $^{3}\text{O}_2$ are known to increase the yields of $^{1}\text{O}_2$ in the presence of a photosensitizer.\cite{61} Second, the $^{1}\text{O}_2$ generated in the plastron will be transported more efficiently into contact with solution due to the gas flow which creates bubbles that rise through the solution, thereby increasing the liquid-gas interfacial area. This increases the opportunity for $^{1}\text{O}_2$ to become solvated and react with 1 before it decays. Indeed, we observe the highest rates of endoperoxide 2 formation in the presence of flowing $^{3}\text{O}_2$. Results from Surface C demonstrate that this mechanism, alone, can result in singlet oxygen trapping.
Figure 3.33. Mechanism of singlet oxygen generation via O$_2$ flowing through the plastron of a superhydrophobic sensitizer surface.

When Pc particles are in contact with the solution (surfaces A and B), a second mechanism for generating $^1$O$_2$ is operational. Oxygen ($^3$O$_2$) dissolved in solution will react at the particle-solution interface to generate $^1$O$_2$ directly in solution where it can diffuse and be trapped by reacting with 1. Increasing the $^3$O$_2$ concentration dissolved in solution would again increase the yield of $^1$O$_2$ trapped. This was observed for static solutions presatured with $^3$O$_2$.

For flowing $^3$O$_2$ experiments, however, presaturation of the solution with N$_2$ proved to be more effective than presaturation with $^3$O$_2$. Initial rates of endoperoxide 2 formation were higher for N$_2$ vs $^3$O$_2$ presaturation (0.96 vs 0.60 nmol/min). In this condition, the solubility and diffusion of O$_2$ into solution will be more rapid when the concentration of dissolved $^3$O$_2$ is lower (Fick’s Law).[62] As the concentration of dissolved oxygen in
solution increases, singlet oxygen solubility from the bubble into solution will decrease, slowing the overall trapping rate; reactions of singlet oxygen present in bubbles would ultimately be limited to the gas-liquid interface. This was reflected by the lower final rates of endoperoxide formation (~0.21 nmol/min regardless of presaturation level). We observed a similar effect of solution presaturation (i.e. higher rates with N$_2$ vs $^3$O$_2$ solution presaturation) using a very different type of singlet oxygen generator.$^{[57]}$

The generation of singlet oxygen in solution at the Pc particle-solution interface accounts for the majority of the trapped $^1$O$_2$. This higher rate on the particle surface (vs. the plastron) will not be reduced by presaturation of the solution with N$_2$, since $^3$O$_2$ can rapidly diffuse from the plastron to this interface. Only when the dissolved $^3$O$_2$ content is reduced will the rate of $^1$O$_2$ generation in solution be significantly reduced. This was observed for N$_2$ presaturated solutions in static air, especially at the end of the 150 min experiment when $^3$O$_2$ would have been depleted from the plastron.

By controlling the location of particles on the surface, illustrated by the synthesis of surfaces A-C, the mechanistic significance of these two non-equivalent $^1$O$_2$-generating regions is revealed. The observations of our experiments are consistent with the short lifetime of $^1$O$_2$ and the transport mechanisms involved. When generated at the top of the PDMS posts, the distance over which singlet oxygen must diffuse to encounter 1 is relatively short as $^1$O$_2$ is either generated in solution, or can easily diffuse to the liquid-gas interface. When generated in the plastron, however, $^1$O$_2$ is first transported via a bubble into the liquid layer where it must be solvated and diffuse to and encounter 1 before decaying back to the ground state. Flowing gas enhances the transport of $^1$O$_2$ into solution, and thus the yield of 1, as compared to an environment in which, for example an individual
droplet of solution\textsuperscript{[63]} rests on a superhydrophobic surface embedded with Pc particles in static air.

\textbf{3.4.2.3 Conclusions}

Physical-organic studies were used to investigate superhydrophobic sensitizer surfaces coupled into a photoreactor device so that the layer of gas trapped between the surface and the liquid could be enriched with oxygen. This superhydrophobic surface device is especially well suited for such studies as it enables control of the sensitizer particle location relative to the solution as well as provides facile access to the plastron. Because of the hierarchical texture on the printed surface, a stable Cassie state was maintained throughout the experiment; the Pc particles partially embedded into the PDMS posts required no special surface treatment to maintain superhydrophobicity. Fabrication of these surfaces is inexpensive, scalable and easily adaptable to a wide range of catalyst particles.

The endoperoxide \textbf{2} is the sole product from the reaction of singlet oxygen with anthracene \textbf{1}. By flowing oxygen continuously through the plastron, the efficiency of singlet oxygen trapping increased significantly as compared to the static plastron environment. By contrast, flowing N\textsubscript{2} through the plastron essentially precluded endoperoxide formation. By studying surfaces where the catalyst particles are proximate to, or isolated from, the aqueous solution, (surfaces A-C) the relative efficiency of \textsuperscript{1}O\textsubscript{2} trapping was determined. Singlet oxygen generated directly in solution is trapped more efficiently resulting in >60\% higher yields. However, we clearly demonstrated that \textsuperscript{1}O\textsubscript{2} can be trapped in solution with reasonable yields (~46 nmol/min) even when the sensitizer Pc
particles are physically separated from the solution.
Chapter 4. Reaction in individual droplets on a superhydrophobic surface, effect of convection

4.1 Introduction

A sessile droplet placed on a superhydrophobic surface can act as a micro-reactor that allows studying reactions in small scale, and the droplet is easily to be removed from the surface for further analysis due to the superhydrophobic property. However, when the size of the reactor is smaller the mixing in the reactor becomes problematic since conventional mixing methods do not work anymore and the mass transfer in the reactor only occurs through diffusion.\(^1\)\(^,\)\(^2\) To overcome this shortcoming, different methods were studied to introduce mixing into a sessile droplet on superhydrophobic surface, such as adding magnetic particles to the droplet;\(^3\) heating the surface to create a temperature gradient;\(^4\) and mixing two separate droplets by control the electrowetting on a superhydrophobic surface\(^5\). These methods are either requiring a sophisticated design or changing the temperature of system, which are increasing the complexity of the reaction. So I planned to develop a method which can control the mixing in a droplet based micro-reactor without the disadvantages mentioned above.

Experiments were designed to show that convection can be induced by causing a droplet to evaporate and that convection can accelerate the rate of a reaction that occurs at the droplet-surface interface. Firstly, functionalized superhydrophobic surfaces were fabricated by a modified 3-D printing method. Arrays of conical polydimethylsiloxane (PDMS) posts (~1mm tall × 0.5mm pitch) were coated with reactive particles that became partially embedded into the surface upon curing. These surfaces have a hierarchical roughness and exhibit stable superhydrophobic properties. On these surfaces, individual
aqueous droplets can be formed that act as micro-reactors. Two types of reactions were studied: species generated at the liquid-solid interface that react with solute in the droplet and solutes in the droplet that react with the solid surface. In the first case, the reaction rate is dependent upon dispersing the concentration of reactive species away from the liquid-solid interface; in the second case, the rate is dependent upon maintaining the concentration of solute at the interface. To conduct these reactions, two types of particles were partially embedded into arrays of polydimethylsiloxane (PDMS) posts to create superhydrophobic (SH) surfaces. In one case, catalytic particles were embedded to generate singlet oxygen at the solid-liquid interface that could react with a selected trapping agent dissolved in the droplet. In this case, convection could accelerate distribution of a reactant generated at the liquid-solid interface throughout the droplet. In the second case, biotinylated particles were embedded that could selectively bind NeutrAvidin dissolved in the droplet. In this case, convection could accelerate the frequency with which reactants distributed in the droplet would be brought into contact with the reaction surface. The kinetic rate constant of protein binding (NeutrAvidin and biotin) was calculated from the experimental data and compared with literature values.

Both reactions were studied as a function of convection within the droplet. Convection was suppressed by creating a static, humid, environment in which the droplet volume was stable, thus limiting the reaction to diffusion of molecules away from (singlet oxygen) or towards the SH surface. Alternatively, convection could be induced by flowing a stream of gas (oxygen, nitrogen or air) through the environmental chamber that caused evaporation and so induced mixing within the droplet. Higher reaction rates were achieved when convection was induced, due to the faster transport of the singlet oxygen trapping
agent or from NeutrAvidin to the solid-liquid interface. The increase in concentration of the trapping agent that occurs during evaporation was shown not to affect the reaction rates.

4.2 Experimental

4.2.1 Design of environmental chamber

A sealed environmental chamber (Model 100-07 from ramé-hart Instrument Company) was modified to conduct the experiments as shown in Figure 4.1. Two sides of the chamber were mounted with clear glass windows, the convective motion within the droplet can be monitored through the window. Light was introduced from top of the chamber. A gas inlet and outlet were located on one side of the chamber. In the experiments, the gas flow was turned on 5 minutes before placing the droplet onto the functionalized superhydrophobic surface to achieve equilibrium in the chamber.

![Figure 4.1 Schematic of environmental test chamber for studying the effect of convection on reactions in micro-droplets on a multifunctional superhydrophobic surface.](image)
4.2.2 Fabrication of multifunctional superhydrophobic surfaces

Superhydrophobic surfaces were fabricated where silicon-phthalocyanine (Pc) particles, a known sensitizer for the generation of singlet oxygen (\( ^1\text{O}_2 \)), were partially embedded into polydimethylsiloxane (PDMS) printed posts creating superhydrophobic surfaces on which \( ^1\text{O}_2 \) can be trapped in both single, spherical droplets as well as bulk solution suspended in the Cassie state on the surface. Our study focused on placement of particles at the solid-liquid interface.

The process for printing PDMS posts was reported previously.\[^6\] Briefly, the PDMS posts were printed as a 17 × 17 square array with a pitch of 0.5 mm (8 mm × 8 mm array, 1mm tall) on a 25 mm × 25 mm × 1mm glass slide surface. Immediately after printing the PDMS posts, the tips of the posts were dipped into a thin layer of Pc particles. The tip-coated posts were cured at 65 °C in an oven with tips facing down. The viscous and thixotropic properties of the PDMS maintained their shape throughout curing. After curing, excess Pc particles were removed by rinsing the surface with deionized water and exposing the surface to high flows of compressed air.

4.2.3 Generation of singlet oxygen on a photocatalytic superhydrophobic surface

A 100 μL droplet containing anthracene dipropanate dianion (0.5 mM-1.5 mM) was placed on the photocatalytic superhydrophobic surface and illuminated with the 669nm laser. Gas flow rate was controlled by a rotameter. A series of flow rates were used to control the convection: 0 cc/min (static), 75 cc/min and 150 cc/min. The 100 μL droplet was dispensed onto the superhydrophobic surface by using a precision pipette. Then the environmental chamber was sealed and gas flow was started. After 5 minutes, the humidity and evaporation equilibrium was established in the chamber. Then the laser was turned on.
After 60 minutes of illumination, the droplet was collected by using a 100 μL Hamilton syringe, benefitting from the superhydrophobicity of surface, the entire droplet was picked up without any liquid left behind. The volume of the collected droplet was also measured by the Hamilton syringe, and then the droplet was diluted into 800μL for UV-vis spectroscopy analysis by adding deionized water. Reaction of singlet oxygen with 9,10 anthracene dipropionic acid (1) results in the formation of the endoperoxide (2) as shown in Scheme 4.1. The efficiency of \( ^1\text{O}_2 \) trapping was examined by the decrease in (1) concentration by UV-vis spectroscopy by measuring the absorbance at 378nm.

In order to track the convective motions, TiO\(_2\) particles (individual nominal size is 100 nm) were added in deionized water at a concentration of 0.05 wt%. Particle paths were visualized using a Phantom V7.3 high-speed camera at 300 fps. Image-J software was used to process the images.

![Scheme 4.1 Reaction of singlet oxygen with 9,10 anthracene dipropionic acid (1)](image)

### 4.2.4 Pretreatment of glass particles

Glass particles (~75μm in diameter) were purchased from U.S. Silica (SIL-CO-SIL® 250). The particles were washed in hydrogen peroxide and ammonium hydroxide mixture solution (4% H\(_2\)O\(_2\) and 4% NH\(_4\)OH mixture, volume ratio 1:1) at 80 °C for 15
minutes, rinsed with DI water and dried in an oven at 60°C. After drying, the glass particles were washed in a hydrogen peroxide and hydrochloric acid solution (4% H₂O₂ and 0.4M HCl, volume ratio 1:1 mixture) at 80 °C for 15 minutes, following by rinsing with DI water and drying at 60 °C for 30 minutes.

Silane-PEG-biotin (50 mg) was dissolved in 2.5 mL of solvent (ethanol and DI water, 95%: 5%, w:w) to form a solution concentration of 20 mg/mL. Cleaned glass particles were added to this solution and the mixture was shaken at 450 rpm for 1 hour to covalently bind the silane-PEG-biotin to the glass surface. Unreacted sites on the glass particles were treated with excess amount of silane-PEG at a concentration of 50 mg/mL to prevent non-specific binding of NeutrAvidin on the glass surface. The modified glass particles were washed with DI water and dried at room temperature.

**4.2.5 NeutrAvidin binding with biotin modified superhydrophobic surface**

Arrays of PDMS posts were prepared as described for superhydrophobic surfaces with Pc particles *(Chapter 3, Section 3.3)*. Hydrophobic silica nanoparticles (TS-530) were partially embedded into the PDMS. The “dusted” surfaces were cured at 60 °C for 2 hours, loose particles were removed by rinsing with DI water and exposing the surfaces to high flows of compressed air. To selectively anchor the hydrophilic biotinylated glass particles on the tips of the surface, the TS-530 coated surfaces were dipped into a thin layer (200 μm thick) of silicone prepolymer (Dow Corning 3140) such that only the upper 200 μm of the post tips were coated. This surface was then dipped into a thin layer of the biotinylated glass particles such that only the uncured silicone came into contact with the
particles. The surface was then cured again (surface upside down during curing) at 60 °C for 2 hours. This cured superhydrophobic surface had the upper portion of the posts (about 200 μm) covered by biotin modified glass particles and the lower portion covered by hydrophobic SiO₂ nanoparticles.

A 20 μL droplet containing 1.67×10⁻⁸ M NeutrAvidin-FITC was placed on the biotin modified superhydrophobic surface in the environmental chamber. The gas flow was controlled by a rotameter at 75 cm³/min or 150 cm³/min; a static experiment was performed without flowing gas. The droplet was left in contact with the surface for a certain incubation time (5 min, 10 min, 15 min, 20 min, 40 min, 60 min, or 120 min), before being removed by a pipette. The NeutrAvidin dissolved in the droplet diffused to the modified surface and bound to the biotin molecules as illustrated in Figure 4.2. Due to superhydrophobicity of the surface, the entire droplet was easily removed without leaving any liquid behind. The surface was then rinsed with deionized water to wash out unbound NeutrAvidin. Confocal images were taken using an excitation wavelength of 488 nm and emission filters between 500 nm and 530 nm, and a Z-stack scanning of 200 μm of the post tips. The scanning area was 3 mm × 3 mm. IMARIS imaging software was used to calculate the fluorescence intensity. At each incubation time, the fluorescence intensity was measured three times and averaged. A freshly prepared biotinylated surface was used as control, and measured with the confocal microscope using the same parameters.
4.3 Results and discussion

4.3.1 Convection within droplet on a superhydrophobic surface

A side-view and a perspective view of 10 μL droplet sitting on the superhydrophobic photocatalytic surface are shown in Figure 4.3. The water contact angle (CA) was measured to be 163.3° ± 3.5°, and the sliding angle was 3.6° ± 1.6°. The SEM image (Figure 4.4) shows that only the top portion of the posts were coated with photocatalytic particles.
Figure 4.3. A 10 µL water droplet on the superhydrophobic photocatalytic surface.

Figure 4.4. SEM image of the superhydrophobic photocatalytic surface.

Twenty-one frames captured with the high speed camera were concatenated to form the images shown in Figure 4.5, illustrating convective motion within the droplets. The movement of particles at a distance of 2.5mm from the convention center was measured. The convection rate can be controlled by tuning the flowing gas rate. Flowing gas accelerated evaporation within the droplet and hence increased convection. The velocity of particles at outer part of droplet was calculated to be 0.351 mm/s when the gas flow rate was 150cm³/min, which is a 14 times higher compared to the velocity without gas flow (0.025 mm/s). When flowing gas at 75cm³/min, the velocity of convection was measured to be 0.202mm/s.
Figure 4.5. Convective motion of particles in a droplet.

4.3.2 Effect of convection on trapping of singlet oxygen

On the photocatalytic superhydrophobic surface, the water contact angle (WCA) was $163^\circ \pm 5^\circ$ and the slip angle was measured to $5^\circ \pm 1^\circ$. Singlet oxygen was generated at the solid-liquid interface upon light irradiation and reacted with 1 to form the endoperoxide 2. Since the lifetime of singlet oxygen is short ($3.5 \mu s$ in water), the diffusion length is only about 150 nm in static water before it decays to the triplet state. The reaction between singlet oxygen and (1) in the aqueous droplet produces (2) and results in a decrease in the UV-vis absorption of (1) at 378 nm. At static air conditions (i.e. no air flow), essentially no convection was observed (convection velocity $\sim 0.025 \text{mm/s}$). The reaction between 1 and singlet oxygen resulted in a decrease of 1 by $22.2\% \pm 1.1\%$. When introducing an air flow of $75 \text{cm}^3/\text{min}$ into the environmental chamber, a convection at a velocity of $0.202 \text{mm/s}$ was observed, and the percentage decrease of 1 increased to $29.8\%$, which was $34.2\%$ higher than under static conditions. By increasing the air flow rate to $150 \text{ cm}^3/\text{min}$, higher convection in the droplet ($0.351 \text{mm/s}$) was observed. As a result, $35.1\%$ of (1) reacted with singlet oxygen; the rate of reaction is about 58% faster than the reaction under static conditions. Changing the gas to nitrogen, decreased reaction rate to only $6.7\%$ which was due to the depletion of oxygen.
Flowing air through the environmental chamber increased convection and the chemical reaction rate within the droplet. The increased convection was due to faster evaporation, however evaporation of the solvent (water) also caused the anthracene concentration to more than double over the course of the experiment. To determine the effect of increasing the anthracene dipropionate concentration on the reaction rate with singlet oxygen, droplets with different concentrations of \((1)\) were studied in static air, as well as in flowing air and flowing oxygen conditions. As shown in Table 4.2, increasing the initial concentration of \((1)\) did not result in a large change of the reaction rate. Tripling the starting concentration of \((1)\) from 0.5 mM to 1.5 mM resulted in only a small increase in singlet oxygen reaction with the percent decrease of \((1)\) increasing from 22.2% to 25.1%, respectively, under static air conditions. Similar results were observed under flowing air and flowing oxygen conditions (Table 4.1). Higher convection resulted in faster reaction rates (40.0% in flowing air vs. 25.1% in static air, with anthracene concentration of 1.5 mM).

At higher concentrations of \((1)\), the trend of greater convection on increasing reaction rates remains in effect as shown in Figure 4.6. Even at the highest concentration (1.5 mM) in static air, the rate is slower than at the lowest concentration (0.5 mM) under flowing air at 75 cc/min (25.1% and 35.1%, respectively). This suggests that convection is the primary mechanism for accelerating the reaction, not higher concentrations.
<table>
<thead>
<tr>
<th>Ambient</th>
<th>Gas flow rate (cm³/min)</th>
<th>Convection rate (mm/s)</th>
<th>Percent decrease in (1) after one hour (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air 0</td>
<td>0.025±0.008</td>
<td>22.2±1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75 0.202±0.015</td>
<td>29.8±1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150 0.351±0.018</td>
<td>35.1±1.7</td>
<td></td>
</tr>
<tr>
<td>Oxygen 150</td>
<td>0.351±0.018</td>
<td>37.7±1.2</td>
<td></td>
</tr>
<tr>
<td>Nitrogen 150</td>
<td>0.351±0.018</td>
<td>6.7±1.3</td>
<td></td>
</tr>
</tbody>
</table>

Experimental conditions: a 100 µL droplet containing 0.5mM (1) was placed on the superhydrophobic photocatalytic surface and then illuminated at 669nm for 1 hour with static air (no air flow), flowing air (75 cm³/min, or 150 cm³/min), flowing oxygen (150 cm³/min), or flowing nitrogen (150 cm³/min)
**Table 4.2.** Effect of initial concentration on singlet oxygen trapping.

<table>
<thead>
<tr>
<th>Ambient</th>
<th>Initial concentration of (1) (mM)</th>
<th>Gas flow rate (cm³/min)</th>
<th>Percent decrease in (1) after one hour (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0.5</td>
<td>0</td>
<td>22.2±1.1</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0</td>
<td>22.9±1.8</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0</td>
<td>25.1±1.7</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>150</td>
<td>35.1±1.7</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>150</td>
<td>36.8±1.8</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>150</td>
<td>40.0±0.6</td>
</tr>
<tr>
<td>Oxygen</td>
<td>0.5</td>
<td>150</td>
<td>37.7±1.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>150</td>
<td>39.2±0.7</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>150</td>
<td>41.9±2.3</td>
</tr>
</tbody>
</table>
**Figure 4.6.** Effect of convection and concentration on the decrease of (1) after 1 hour of reaction.

### 4.3.3 Effect of convection on interaction between NeutrAvidin and biotin

The surface fabricated with biotinylated glass particles has good superhydrophobicity, which shows a water contact angle of $158.5° \pm 3.6°$, as seen in **Figure 4.7**. The sliding angle of water on the surface was measured to be $15.6° \pm 2.8°$, this relative high sliding angle is because the biotinylated glass particles are hydrophilic. The SEM image (**Figure 4.8**) shows only the top portion of the PDMS posts were coated with the biotinylated glass particles.
Figure 4.7. A 10 µL water droplet on the superhydrophobic surface fabricated with biotinylated glass particles.

Figure 4.8. SEM image of the biotinylated superhydrophobic surface.

Fluorescence images (Figure 4.9) taken by confocal microscopy shows bound NeutrAvidin (labeled with fluorescein isothiocyanate) on the biotinylated superhydrophobic surface. Higher fluorescence intensity indicates more NeutrAvidin was bound to biotin on the surface. The fraction of biotin sites occupied (θ) was plotted as a function of time (Figure 4.10), assuming the surface concentration of occupied biotin (bound with NeutrAvidin) is linear with the fluorescence intensity. The fraction of occupied biotin sites can be defined by Equation 4.1, where Γ_t is the surface concentration of occupied biotin at time t, Γ∞ is the maximum concentration of biotin that can bind, I_t is the fluorescence intensity at time t, and I_max is the maximum fluorescence intensity when all available biotin sites are bound with NeutrAvidin. In the experiment, the increment of fluorescence intensity became very small when the incubation time was increased from 60 minutes to 120 minutes, so the fluorescence intensity at 120 minutes was used as I_max.
The kinetics of NeutrAvidin binding to the biotinylated surface can be described by the generalized Langmuir Equation 4.2:

\[
\frac{d\theta}{dt} = k_a c (1 - \theta) - k_d \theta
\]  

(4.2)

Here, \(c\) is the concentration of NeutrAvidin, \(k_a\) is the association rate constant, \(k_d\) is the disassociation rate constant and \(\theta\) is the fraction of biotin sites occupied. The binding between NeutrAvidin and biotin is highly selective and has a very high affinity (\(K = k_a/k_d = 5.5 \times 10^{11} \text{ M}^{-1}\)). Thus the binding is essentially irreversible. To simplify the kinetic equation, we neglected the disassociation and assumed the concentration of NeutrAvidin was unchanged over time. Equation 4.2 can be solved with the above assumptions, and the solution can be described as Equation 4.3:

\[
\theta = 1 - e^{-k_a c t}
\]  

(4.3)

In Figure 4.10, the \(\theta\) values obtained from experiments at different conditions (convection and static) were plotted as a function of time. By fitting the curves with Equation 4.3, \(k_a\) values were obtained. We find a rate constant of \(k_a(c) = 6.01 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}\) (\(R^2 = 0.9978\)) when the binding reaction was performed under convection, and a rate constant of \(k_a(s) = 1.52 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}\) (\(R^2 = 0.9828\)) for the static experiment. A kinetic curve using a \(k_a\) value reported by Maldarelli\(^{[13]}\) (7.63 \times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}) is also plotted in Figure 2.10 for comparison. The value of \(k_a(s)\) is a factor of 4 smaller than \(k_a(c)\), this is because of the depletion of NeutrAvidin near the biotin modified surface. Figure 4.11 shows the theoretical concentration profiles of NeutrAvidin at different times under static conditions.
when the reaction is diffusion limited. **Equation 4.4**[^13,^14] was used to plot the curves in **Figure 4.11**, where $C$ is the local concentration of NeutrAvidin at a height above the surface, $h$, where $C_0$ is the initial concentration of NeutrAvidin in the droplet, and $D$ is the diffusion coefficient of NeutrAvidin, error function $\text{erf} (\xi) = \frac{2}{\sqrt{\pi}} \int_{0}^{\xi} e^{-\xi^2} d\xi$. The boundary conditions for equation 4 are $C(h,0) = C_0$ and $C(0,t) = t$.

$$C(h,t) = C_0 \text{erf} \left( \frac{h}{\sqrt{4Dt}} \right) \quad (4.4)$$

The concentration of NeutrAvidin in the droplet at $h=200 \, \mu m$ is plotted as a function of time as shown in **Figure 2.12**, which illustrates the depletion of NeutrAvidin near the surface as the reaction proceeds. The significant difference of binding rate constants (about 4 times greater with convection) indicates that convection significantly increases the rate at which NeutrAvidin is bound to the biotinylated surface. Convection helps to maintain a nearly stable NeutrAvidin concentration near the biotin modified surface, whereas the concentration of NeutrAvidin near the surface decreases in the diffusion-limited case.

The binding rate constant we obtained under convection ($6.01 \times 10^4 \, \text{M}^{-1} \cdot \text{s}^{-1}$) is close (within 20% difference) to the value reported by Maldarelli *et al* ($7.63 \times 10^4 \, \text{M}^{-1} \cdot \text{s}^{-1}$).[^13] They measured the rate constant $k_a$ of NeutrAvidin-biotin binding on the surface of microbeads sequestered in wells in a microfluidic cell, with a Peclet number of 5600. The Peclet number is defined as $Pe = \frac{Uh}{D}$, in which $U$ is the flow velocity, $h$ is the height of microfluidic cell, and $D$ is the diffusion coefficient of target molecule. The Peclet number in our convection experiment is calculated to be $Pe=5888$ using an average convection
velocity as $U$ and the height of droplet as $h$. Generally speaking, the Peclet number in mass transfer relates the rate of advection (i.e. flow) to the rate of diffusion, which means that a smaller Peclet number indicates that the reaction tends to be diffusion limited whereas a larger Peclet number indicates that the reaction is dominated by flow (i.e. less diffusion limiting). The Peclet number in our convection experiment is comparable to that in Maldarelli’s study\textsuperscript{[13]}, however the rate constant we calculated is 20% smaller. This may because we assumed a stable concentration of NeutrAvidin and did not take the change of concentration during binding into account when doing the calculation. In the static experiment, we have a much smaller Peclet number of 438, which means the reaction at static condition is much more diffusion limited.

To better understand the effect of experimental conditions on the observed rate constant, we compared our rate constant values with other values reported in the literature. The rate constant reported by Wayment and Harris in their single molecule imaging experiment\textsuperscript{[12]} is much higher ($2.1 \times 10^8 \text{M}^{-1} \cdot \text{s}^{-1}$) than the value I calculated using the value reported by Maldarelli. There may be several reasons: 1) they were using a very dilute concentration (picomolar) of NeutrAvidin and a very small density of biotin ($<10^{-6}$ of a protein monolayer), so they were measuring the intrinsic rate constant rather than the apparent rate constant; 2) each NeutrAvidin molecule has four binding sites for biotin, however fluorescence only measures the bound NeutrAvidin and cannot distinguish between single binding and multiple binding; 3) at the higher concentrations of NeutrAvidin and biotin used in my experiment, the bound NeutrAvidin can overlap and cover unbound biotin and make it unavailable for binding.

The apparent rate constant calculated in this experiment is comparable (difference
within 20%) to the apparent rate constant value stated in literature.\cite{13} The binding reaction in this experiment is a diffusion limiting reaction, so the calculated rate constant was much smaller than the intrinsic rate constant which describes the binding affinity.

Figure 4.9 Confocal images of biotinylated superhydrophobic surface after binding with fluorescent tagged NeutrAvidin: with induced convection for a. 20 min; b. 40 min; c. 60 min, and in static air for d. 20 min; e. 40 min; f. 60 min.
Figure 4.10 Fraction of biotin coverage (θ) plotted as a function of time at different conditions and comparison with reference\textsuperscript{[13]}: with convection (blue circle and blue solid line); static (pink triangle and pink solid line); plotted using the $k_a$ value from reference (red dash line). Experimental values are shown as points; trend lines are calculated using Equation 4.3
**Figure 4.11** Concentration profiles plotted as a function of height above the superhydrophobic surface at different times for a static droplet.

**Figure 4.12** Concentration of NeutrAvidin at a height of 200 μm above the surface plotted as a function of time
4.4 Conclusion

In this Chapter, a functionalized superhydrophobic surface was fabricated on which individual droplets were formed that acted as micro-reactors. Two types of reactions were studied. We studied the effects of convection and also ambient gas composition on singlet-oxygen trapping rate. Under static air conditions, convection was suppressed; only 22% of the trapping agent reacted with singlet oxygen. However, flowing gas accelerated evaporation and induced convection within the droplet. With convection, we observed that about 50% more trapping agent reacted with singlet oxygen in one hour compared to the static environment. Similar results were achieved with flowing oxygen. When changing the ambient gas to N₂, the trapping of singlet oxygen was reduced, this was due to the absence of oxygen. We also studied the effect of trapping agent on concentration. The increased concentration of the trapping agent had a relatively small effect on the reaction rate. This indicates that convection is the mechanism by which the reaction is accelerated, not increasing the concentrating.

Binding between NeutrAvidin and biotin at the liquid-solid interface was studied as a function of time and convection rate using fluoresce microscopy. Binding rate constants were calculated by fitting the data with Langmuir kinetic equation. Convection is shown to significantly enhance the rate of NeutrAvidin binding to the surface.
Chapter 5. Controlled precise dispensing of nanoliter droplets for detecting biomolecules with high sensitivity

5.1 Introduction

Precise dispensing of nanoliter droplets is necessary for the development of sensitive and accurate assays,[1-5] especially when the availability of the source solution is limited[6]. To address the need for precise and economical dispensing of nanoliter volumes, we developed a nanoliter droplet virtual well microplate (nVWP) where the volume of liquid transferred is defined by the chemistry and geometry of the surface. We can precisely dispense 25 nL ± 0.5 nL droplet on glass pedestals that are bonded to the tops of polydimethylsiloxane conical posts. By using the nVWP dispensing platform we were able to achieve sensitive detection of proteins and peptides by MALDI-TOF. Functionalization of the glass pedestal surface enabled the selective adsorption of specific peptides and proteins from synthetic and natural biomolecule mixtures such as venom.

5.2 Experimental

5.2.1 Fabrication of the nVWP

The fabrication of virtual well microplates is shown schematically in Figure 5.1. An array of polydimethylsiloxane (PDMS) posts was printed onto a glass substrate using a robotic dispensing system (Janome 2203N and EFD Performus syringe dispenser) as previously described.[8,9] Briefly, PDMS silicone resin with thixotropic properties (ELASTOSIL® LR 3003 50A/50B, Wacker) was loaded and degassed in 10 cc syringes fitted with a 22 gauge tapered tip mounted to the robot. The robot was programmed to bring the tip to the first location at a controlled height of 300 µm above the glass
microscope substrate. The robot triggers the syringe dispenser to deposit a controlled amount of PDMS (85 psi and 0.3 seconds) and then lifts vertically from the surface. This forms a single cylindrical cone of PDMS with a base diameter of 700 μm and a height of 1.2 mm. An array is created by repeating this procedure on 1.2 mm pitch. After printing, the conical posts were planarized during cure (165°C for 5 min) by contacting the tips with a flat, Teflon-coated plate, which was mounted on a stage that could move up and down in the Z direction. The height of the posts was controlled by a 1 mm spacer attached to the Teflon coated plate. Since the printed silicone posts can flow easily upon contact with the Teflon plate, no additional pressure was required. After curing, the plate was easily released, exposing the tapered posts with flat tops. The diameter of the flat top was 150-200 μm as shown schematically in Figure 1b. Glass pedestals measuring 500 μm x 500 μm x 100 μm (diced from glass coverslips by Valley Design Corp) were mounted onto the flat tops using a room-temperature vulcanizing) hydroxyl-terminated dimethyl siloxane (DAP, Dow Corning) adhesive, as shown schematically in Figure 5.1. The glass pedestals were first placed into a 3D printed alignment fixture with square well arrays. The adhesive coated PDMS posts were aligned and brought into contact with these glass pieces under a microscope with the aid of an x,y,z stage. As these glass pedestals are larger than LED and other commonly available semiconductor die, automatic pick-and-place tools can be used for practical large scale manufacturing.

Figure 5.1. Process schematic for the fabrication of nVWPs.
5.2.2 Glass pedestal preparation

Glass coverslips, were diced into 0.5mm squares (500 μm × 500 μm ×100 μm) by Valley Design Corp. The glass pedestals were dismounted from the carrier substrates by soaking in acetone solution for 1h in an ultrasonic bath (Fisher Scientific Inc.). The dismounted glass pedestals were rinsed twice in DI water and dried. The glass pieces were then thoroughly cleaned using the following procedure: 1) Washed in an aqueous solution of 4 wt% hydrogen peroxide and 4 wt% ammonium hydroxide at 80 °C for 10 min; 2) Rinsed with DI water three times; 3) Washed with an aqueous solution consisting of 4 wt% hydrogen peroxide and 1.5 wt% HCl at 80 °C for 10 min.; 4) Rinsed with DI water three times; 5) Dried in an oven for 12 h at 60 °C. The glass pieces were then ready to assemble onto the PDMS post arrays, or the surfaces were further modified as described below.

5.2.3 Dispensing of nanoliter droplets onto the nVWP

Manual dispensing of microliter droplets: Large droplets with volumes between 0.5-2 μL were deposited onto the 500 x 500 μm glass pedestals using a hand-held Eppendorf pipette. The droplet was first formed at the tip of the adjustable pipet and the bottom of the droplet was brought into contact with the top of the glass pedestal. The pipet was manually lifted up, transferring the droplet to the glass pedestal.

Manual dispensing of nanoliter droplets: A drop of 10 μL source solution was loaded onto the tip of an “L” shaped polystyrene rod, using an Eppendorf pipette. The droplet was brought into contact with the glass pedestals and lifted-up by hand to transfer nanodroplets onto the nVWP surface.
Automated dispensing of nanoliter droplets: Automated dispensing was conducted using a robot (Janome-2203N) with a positioning accuracy of ± 10 μm. A 2.5 mm diameter polymer rod was mounted on the robotic arm. A fixed volume (3 μL) of the source solution was placed onto the flat bottom of the rod using an Eppendorf pipette. The robotic dispensing process consists of three steps as illustrated in Figure 5.2: the rod with source solution was aligned to a glass pedestal; the rod was then moved downward at a preset speed (2 mm/s) enabling the source solution to contact the glass pedestal at fixed height such that the bottom of the droplet was brought 200 μm below the top surface of the glass pedestal; lastly, the rod was lifted-off from the surface at a pre-determined speed (15 mm/s unless otherwise specified) and translated to the next dispensing location. The dispensing process was monitored using a high speed camera (Vision Research, Phantom V7.3) with Mitutoyo 5X Plan APO objective coupled to a Tokina AT-X 100mm f/2.8 macro lens operated at 5000 frames per second. The images/videos were analyzed with PCC 2.5 software.

![Schematic of the automated dispensing process.](image)

**Figure 5.2.** Schematic of the automated dispensing process.

Measurement of dispensed droplet volume: An initial source volume \( V_i \) of 3μL of DI water was placed on the flat tip of the cylindrical dispensing rod (2.50 mm diameter)
using an Eppendorf pipette. The volume of this spherical cap-shaped drop was confirmed by calculating the volume using the equation \( V = \pi h \left( \frac{3a^2 + h^2}{6} \right) \), where \( h \) is the height of the drop and \( a \) is the radius of the water drop base. Values of \( a \) and \( h \) were measured from the optical image profile of the drop acquired using the high speed camera system with a resolution of 7 \( \mu \)m/pixel.

The average volume of droplets dispensed on the nVWP surface was determined by calculated the change in volume of the source droplet after dispensing ~ 10 droplets onto the nVWP surface. Five different lift-up velocities (0.5 mm/s, 5 mm/s, 15 mm/s, 50 mm/s, and 100 mm/s) were evaluated. For each velocity, 9-11 drops were dispensed onto different pedestals on an nVWP surface. After dispensing, the volume remaining on the dispensing rod (\( V_t \)) was determined by measuring the source drop width and height and calculating the volume of the spherical cap as described above. To account for evaporation, the volume of an initial 3 \( \mu \)L drop was calculated after the time required for dispensing (\( V_{te} \)) using measured values of the height and radius; the volume lost to evaporation (\( V_e \)) was determined by subtraction (\( V_i - V_{te} \)). The average dispensed volume (\( V_d \)) was determined by \( V_d = (V_i - V_t - V_e)/n \) where \( n \) is the total numbers of dispensed droplets. Plotting \( V_d \) vs \( h \) reveals a linear relationship that fits the Equation 5.1 with \( R^2 = 0.979 \).

\[
V_d = 173.4 \times h - 8.4 \quad \text{(5.1)}
\]

This linear relationship was used to determine droplet volumes from height measurements of manually dispensed nanoliter droplets.

To assess the precision of \( V_d \), the height of nL droplets dispensed on pedestals was measured using a Centrimax long distance microscope with MX-5 lens and Pixilink PL-
B681C USB camera with a resolution of 2 µm/pixel on 10 sample droplets. The standard deviation of height values was converted to a volume standard deviation using (5).

5.2.4 MALDI-TOF detection of proteins on nVWP surfaces deposited from nanoliter droplets

An nVWP was directly fabricated onto a stainless steel MALDI plate (MSP 96 target, Bruker Daltonics). The MALDI plate was machined to create a recess 1.0 mm deep onto which the PDMS posts were printed (Figure 5.3). In this way, the top surface of the glass pedestals was at the same height as the MALDI plate surface. To deposit protein onto the glass pedestals, an ultrathin-layer sample preparation technique was used. The glass pedestals were first coated with 0.5 µL of a matrix solution (α-Cyano-4-hydroxycinnamic acid (4-HCCA) saturated in 1:2:1 0.1% trifluoroacetic acid : acetonitrile : water) diluted 1:4 into isopropanol and dried. Droplets of a mixture of NeutrAvidin and a second matrix solution (4-HCCA saturated in 3:1:2 formic acid:water:isopropanol) in a 1:10 ratio were dispensed onto the coated glass and dried. MALDI-TOF spectra were recorded on a Bruker Microflex MALDI-TOF instrument.
5.2.5 Detection of selectively adsorbed peptides on nickel-chelated treated glass nVWP surfaces

Nickel chelate resin coated glass pedestals (500 µm x 500 µm x 180 µm) were used to anchor KcsA ion channel proteins to selectively adsorb TX7335 peptide from snake venom. The KcsA ion channel, modified to contain a 6-his tag, was prepared in buffer containing 50 mm Tris pH 7.5, 150 mM KCl, and 10 mM of the surfactant n-decyl-β-D-maltopyranoside (DM) to form ion channel micelles. The 6-his tagged KcsA micelles were anchored onto the Ni-chelate-glass pedestals by depositing 1 µL droplets of the KcsA solution onto the pedestals, incubating for 5 min and removing the droplet with vacuum. After anchoring, excess ion channels were washed away by rinsing two times with DM buffer. Crude lyophilized venom from the Eastern green mamba snake (*Dendroaspis angusticeps*) was redisolved in the same buffer at a concentration of 2 mg/mL and pre-depleted of most non-specifically binding toxins by passing it over a Ni²⁺ affinity column.
(GE Life Sciences Ni Sepharose 6). A 1 μL droplet of prepared venom solution was deposited onto the nickel-chelate coated glass pedestal of an nVWP and incubated for 2 min. Excess venom was washed away by rinsing with DM buffer for 6 times. A 1 μL droplet of matrix solution [4-HCCA saturated in 3:1:2 formic acid: water: isopropanol] was then added onto the surface and allowed to dry for MALDI-TOF detection. A schematic of this selective adsorption process is illustrated in Figure 5.4.

![Figure 5.4](image)

**Figure 5.4.** Schematic of selective adsorption of peptides on nickel-chelated treated glass pedestals.

5.3 Results and discussions

5.3.1 nVWP fabrication

Nanoliter droplet virtual well microplates (nVWPs) were fabricated on standard microscope slides as shown in Figure 5.5. The glass pedestals, measuring 500 μm x 500 μm in area and 100 μm thick, were aligned and placed onto the printed PDMS posts. The height of the glass surface is 1.1 mm above the surface of the microscope slide and the pitch between adjacent pedestals is 1.2 mm, unless otherwise noted. The fabrication
process is flexible; depending upon the dispensing need, the area of each glass pedestal can be modified by dicing glass coverslips to different dimensions. The height and pitch of the posts can be easily modified to accommodate different sizes of glass adjusting the printing parameters.

![Image 1](image1.png)

**Figure 5.5.** Optical image of fabricated nVWP

### 5.3.2 Dispensing Process

The dispensing process was monitored using a high speed camera (5000 fps); still images from one video are shown in **Figures 5.6**. Initially, the dispensing rod, with a 10 \( \mu \text{L} \) drop pinned to the bottom of the rod, descends and contacts the glass pedestal. The source drop quickly wets the top surface of the glass and the solid-liquid-vapor TCL spreads to the edge of the glass pedestal as shown in **Figure 5.6b**. The TCL is strongly pinned at this edge preventing the wetting of the side wall of the glass pedestals. As the source drop is retracted from the surface, its shape changes; it becomes progressively more elongated because the TCL is immobilized at the edge of the hydrophilic glass. Eventually the drop necks down sufficiently that it breaks, leaving a droplet with a specific volume and contact angle of \(~80^\circ\) remaining on the surface as shown in **Figure 5.6d**. The volume of the dispensed droplet is determined by the glass perimeter and retraction velocity of the source drop. If the droplet is allowed to evaporate after dispensing, the height of the droplet
decreases, thereby decreasing the apparent contact angle. The liquid-solid contact area does not change because the TCL remains pinned at the edge of the glass pedestal throughout the entire evaporation process.

**Figure 5.6.** Images taken by a high-speed camera illustrating the dispensing process. A 30 nanoliter droplet was dispensed onto a glass pedestal-PDMS superhydrophobic surface.

### 5.3.3 Detection sensitivity of proteins on nVWP by MALDI-TOF

nVWPs fabricated on standard MALDI-TOF plates can be used to measure the molecular weight of small quantities of biomolecules deposited onto the glass pedestal surfaces after evaporation of aqueous solutions. After the initial matrix solution was dried, aliquots of a NeutrAvidin solution in PBS (0.5 mg/mL) were mixed with matrix solution and manually deposited on the glass pedestals forming 32 nL droplets that were allowed to dry. MALDI-TOF spectra obtained from the nVWP have the same peak positions compared with the sample deposited directly on the standard MALDI-TOF plate. The quantity of NeutrAvidin in the 30 nL droplets was varied from 288 to 0.144 pg (4,800 to 2.4 attomoles). As shown in **Figure 5.7**, peaks at 14,300 and 7,090 which correspond to the singly and doubly charged NeutrAvidin monomer molecular ion (14,298 m/Z)\(^9\), were detected at quantities ranging from \(4.8 \times 10^{-15}\) to \(4.8 \times 10^{-17}\) moles, with a concomitant decrease in peak intensity. Each concentration was measured three times. The singly charged peak remains visible down to 4.8 attomol, but is no longer observed at 2.4 attomol.
This sensitivity is comparable to the best values reported for MALDI-TOF\textsuperscript{[10,11]} and demonstrates the high label-free sensitivity that can be achieved with nVWP using a standard, low-cost MALDI-TOF instrument.

![MALDI-TOF results for NeutrAvidin-matrix solutions deposited as 30 nL droplets on nVWP substrate with 500 μm diameter glass pedestals. The minimum detectable quantity of NeutrAvidin required for detection was 4.8 attomoles.]

**Figure 5.7.** MALDI-TOF results for NeutrAvidin-matrix solutions deposited as 30 nL droplets on nVWP substrate with 500 μm diameter glass pedestals. The minimum detectable quantity of NeutrAvidin required for detection was 4.8 attomoles.

### 5.3.4 Selective binding of a snake venom peptide by an ion-channel protein anchored on nVWP

Ion channels are attractive targets for drug discovery and the venom of scorpions, spiders and snails provide a wide range of ion channel modulating peptides.\textsuperscript{[8,12-16]} To
illustrate the power of the nVWP platform, we designed an experiment that highlights the advantages of working with nanoliter droplets of difficult to obtain natural products. Glass pedestals coated with nickel chelate resin were cut to size and fabricated into an nVWP surface directly on a MALDI-TOF plate. An ion channel protein, KcsA, modified to include a 6-his tag, was incorporated into a micelle and bound to the surface of the Ni-chelated resin coated glass pedestals through the 6-his tag. MALDI-TOF analysis confirmed the presence of the ion channel on the glass surface through the presence of the doubly-and triply-charged channel peaks at 9550 and 6400 m/Z (green trace in Figure 3.8). A droplet (~30 nL) of pre-depleted snake venom was manually dispensed onto the glass pedestal modified with the ion channel micelle and incubated for 2 min in air, followed by 6 rinses with DM buffer. A 1 μL droplet of 4-HCCA matrix solution was added and allowed to dry. Results of the MALDI-TOF analysis are shown by the blue trace in Figure 5.8. Three peaks are observed: 6400 m/Z, which is due to the ion channel protein; 6680 m/Z, which is attributable to a non-specifically bound peptide and; 7335 m/Z, which is attributed to a peptide that selectively binds to KcsA. This peptide is a toxin that has been shown to modify the function of the KcsA ion channel. To verify the non-specific vs specific binding of venom peptides, a control experiment was conducted where a droplet (~30 nL) of pre-depleted venom was deposited on the Ni-chelated resin coated glass directly, without first binding the ion channel micelle. The MALDI-TOF spectrum (red trace, Figure 5.8) shows a strong peak at 6680 m/Z demonstrating that this peptide is non-specifically adsorbed on the Ni-chelate resin. Only a trace of TX7335 can be seen in this control.

Thus ion channel functionalized nVWPs provide an especially effective means for rapidly screening peptides from biologically important natural products, such as venoms,
that are found in limited volumes. About 30 nL was required to identify the peptide that specifically binds to the KcsA ion channel. No labeling of the peptide was required.

![MALDI-TOF spectra of solutions deposited on Ni-chelate coated glass pedestals](image)

**Figure 5.8.** MALDI-TOF spectra of solutions deposited on Ni-chelate coated glass pedestals: (green) KcsA ion channel micelle bound to the Ni-chelate resin coated pedestals; (blue) snake venom incubated on KcsA ion channel micelles bound to Ni-chelate pedestals; (red) depleted snake venom on Ni-chelate resin coated pedestals (no ion channel micelles).

### 5.4 Conclusion

A new type of surface, the nVWP, was fabricated that enables the dispensing of nanoliter volumes of aqueous solutions with a precision of ±0.5 nL. The nVWP substrate is composed of an array of small (500 µm x 500 µm) glass pedestals adhered to an array of PDMS posts printed onto a glass substrate. The sharp edges and hydrophilic properties of the glass cause the triple contact line (TCL) to be pinned, which is essential for precisely controlling the dispensed volumes. The local energy barrier at the sharp edges effectively retains biofluids on the top of each glass pedestal without wetting the sidewalls, thus effectively concentrating the biomolecules for detection by fluorescence or MALDI-TOF.
Because all solute molecules in the nanoliter droplets are confined to the top surface of the pedestals, a detection limit of 530 attomole was achieved using standard fluorescence microscopy (not shown). The detection sensitivity could be further improved to 4.8 attomole when MALDI-TOF was used. The glass pedestal surface could be coated with a nickel chelate resin onto which an ion channel protein (KcsA), incorporated into a micelle, was bound. A nVWP so prepared could selectively bind the peptide toxin found in snake venom (TX7335) that reacts with this ion channel. This peptide was detected by MALDI-TOF using only a ~30 nL drop of pre-depleted snake venom.

Precise control of dispense volume, coupled with chemical functionalization of the glass surface, provides an effective platform for detection of biomolecules as well as ELISA assays and label-free MALDI-TOF screening tests. The nVWP device may prove to be especially effective when sample volumes are limited to nanoliter quantities.
Chapter 6. Single particle dispensing, effect of surfactant chemistry on the distribution of hydrophobic microbeads

6.1 Introduction

Precisely dispensing an individual particle on a pre-determined location is desired for many actual applications, ranging from single cell studies to ultra-small integrated circuit chips packaging. Recently the nano-droplet Virtual Well Microplate (nVWP) was developed by the Lyons’ group, which is capable of placing a large array of nanoliter sized aqueous droplets of precise volume on pre-determined locations, has shown great potential for biological applications.\(^\text{[1]}\) This dispensing technology is compatible with any type of aqueous mixture. In addition to dispensing solutions of biomolecules, small particles (~50 \(\mu\) m) can be dispensed on a precise location from a source drop containing a suspension of particles. Therefore, the nVMP precise dispensing system could be useful in particle manipulation, such as dispensing single particles of cells and potentially miniature integrated circuits (ICs).

High-throughput single cell analysis provides an important way to understand the behavior of an individual cell from an isogenic cell population.\(^\text{[1,2]}\) Traditional cell studies such as gene expression, response to external stimuli, etc., rely on average measurements from a large cell population (i.e. \(10^3-10^6\) cells) assuming the purified cell samples are homogeneous. However, recent studies indicate that cells could be heterogeneous in one population, even cultured under the same conditions.\(^\text{[1-3]}\) For example, the study from Mathies’ group\(^\text{[1]}\) suggested that when measuring siRNA knockdown of the GAPDH gene in individual Jurkat cells, two distinct populations of cells with moderate (50%) or
complete (0%) gene silencing could be generated. This difference in gene expression and silencing between individual cells cannot be found by conventional bulk measurements.

At present, commercial technologies to separate single cells from a suspension are available based on fluorescence-activated cell sorting (FACS), such as the MoFlo XDP (Bechman Coulter, Brea, CA) and FACS Aria (BD BioScience, San Jose, CA). However, there are still several limitations. A minimum of several hundred microliters of sample volume are required, which is not suitable when sample volume is limited. Fluorescence labeling of cells is required, which increases complexity of sample preparation and might adversely affect cell viability. The laser radiation and electrical charging of cell solutions may also have negative effects on cell viability.

A second approach to study single cells uses microfluidic devices, which enable the high-throughput trapping and analysis of individual cells.\cite{4-8} However, the challenge for these devices is how to create an in situ incubation environment for culturing the individual cells and allowing access for retrieval and analysis. Other types of single cell dispensing, based on drop-on-demand technologies, have also been developed, such as modified inkjet printing,\cite{9-12} and single-cell printer.\cite{13,14} The small volume droplet, only slightly larger than a typical cell, is generated by a jetting system. Single cell detection is realized by an optical camera microscope system.\cite{13} However, the shear forces generated during jet printing can adversely affect cell viability.

Another potential application of single particle dispensing is for integrated circuit (IC) chip placement. The traditional methods for IC chip placement uses a vacuum gripper, often called a “pick-and-place” tool. The standard process used for IC chip placement is shown schematically in Figure 6.1.\cite{15} The first step in IC assembly is adhesive deposition
onto the target position on substrate (Figure 6.1a). Figure 6.1b depicts aligning of the pick-and-place tool head with the target die. Visual methods are used for exact alignment. The diced wafer is mounted on a carrier tape surface where all dies are face up. The target die is subsequently picked up by a vacuum tool with ejection from underneath, which is shown in Figure 6.1c. Then the pick-and-place tool is moved and aligned to the designated location on the substrate and placed on the adhesive, following by a curing step (Figure 6.1d-f).
Figure 6.1. Schematic process of direct die placement. (a) Adhesive application, (b) die presentation, (c) die eject and pickup, (d) alignment before placement; (e) die placement; (f) adhesive curing.\textsuperscript{[15]}

The traditional assembly methods are designed for thicker wafers and dies as small as $\sim 200 \, \mu m$ on a side, but cannot be scaled for assembly of ultra-small, ultrathin chips. In some chip-in-paper applications, such as papers used for banknotes and security documents, ultrathin semiconductor dies ($< 30 \, \mu m$ in thickness) are embedded in a thin paper substrate with a total thickness of about $120 \, \mu m$.\textsuperscript{[16,17]} The complications with die packaging increase exponentially as the chip thickness falls below $50 \, \mu m$. When the chip size is small, the forces at the surface such as van der Waals, surface tension, and electrostatic forces are not negligible and become problematic. The pickup nozzle can be scaled to fit the size of small chips of several hundred microns, however the surface forces can cause chips to “jump” to the nozzle before it is in precise position and it becomes difficult to release the chips as the gravitational force is comparable to the surface forces. As a result, the precision of placement becomes poor.\textsuperscript{[18]} The small chips can tend to stick together and thus become difficult to disperse in the atmosphere in which traditional pick-and-place process takes place.\textsuperscript{[19]} The vacuum suction force from the pickup tool can damage delicate thin chips. All these problems make the traditional placement process unfeasible.

Techniques have been developed for assembly of ultrathin chips, including fluidic self-assemblely (FSA),\textsuperscript{[20]} micro vacuum tweezers,\textsuperscript{[19]} laser-induced forward technique (LIFT),\textsuperscript{[21,22]} and laser-enabled advanced packaging (LEAP).\textsuperscript{[23]} In the FSA process, the bare die with a typical size of several hundred micrometers and a flattop pyramidal shape are suspended in liquid and flowed over the receiving substrate which has correspondingly
shaped receptor holes into which the dice settle and self-align.\textsuperscript{[20]} Micro vacuum tweezers are used to pick up individual chips suspended in aqueous solution and place them on the desired position.\textsuperscript{[19]} In LIFT and LEAP processes, a sacrificial layer of material, which can be vaporized is embedded in the release layer and can generate gases upon a short pulse of laser irradiation to release the attached chips.\textsuperscript{[22,23]} Figure 6.2 shows a general process for die placement using LEAP. These techniques are alternatives to conventional pick-and-place method and can be used to precisely control the placement of ultrathin chips, however either the substrate is required to be precisely shaped to accommodate the chips,\textsuperscript{[20]} or the pickup head needs a complicated design and fabrication.\textsuperscript{[21-23]} The size of chips described in the literature\textsuperscript{[19-23]} is of the order of hundred micrometers, the usability for smaller chips placement, i.e. less than 50 µm, were not tested.

Our previous study\textsuperscript{[24]} showed the nVWP dispensing system could precisely dispense nanoliter sized droplets onto pre-determined positions and is compatible with a wide range of aqueous solutions. So dispensing particles from a particle suspension using a single particle dispensing system is a promising approach for ultra-small IC chips packaging.\textsuperscript{[25]}

\textbf{Figure 6.2.} A schematic illustration of die placement using LEAP\textsuperscript{[23]}
In this paper, I report on the dispensing of single, hydrophobic particles onto arrays of surface features. Nonionic, Pluronic block copolymer surfactants were used to insure uniform distributions of particles in solution. A new technique was developed to insure that the particles were uniformly distributed in water. Critical to the success of this approach is the use of the nano-Droplet Array Plate (nDAP) surface that insures precise, uniform liquid droplets are dispensed on an array of surface features.

Nonionic Pluronic surfactants have been widely used for particle dispersions in aqueous solutions, such as carbon black particles,[26] graphene sheet,[27] carbon nanotubes,[28] and polyaniline particles.[29] The hydrophobic block of the surfactants are adsorbed on the hydrophobic surface of the particles such that the hydrophilic blocks are solvated by the aqueous solution. This reduces the surface energy of the particles to enable them to disperse in the aqueous solution. For dispersing single particles, two aspects are critical: the concentration of particles must be such that, on average, only a single particle is contained in the volume of a single dispensed droplet (obviously the volume of the droplet must be precise and reproducible); the other factor is that the particles are uniformly distributed through the liquid. The uniformity and stability of a particle suspension is usually examined and quantified by methods such as UV transmittance and sedimentation ratio tests.[27,29] These techniques work well for high particle concentrations. However, at the low concentration needed for single particle dispensing, as well as the necessity of a perfectly uniform dispersion, an improved method for quantifying the uniformity of particle dispersions is required. In this chapter, I studied the effect of Pluronic surfactants on microbeads dispersion uniformity by a new quantitative method in which the uniformity
of the particle dispersion in the source drop can be quantitatively characterized by the number of particles dispensed in each nanoliter droplet.

The nano-Droplet Array Plate (nDAP) consists of an array of PDMS posts on which a precise volume of nanoliter droplets can be dispensed using a microliter source drop suspended from a robot. Since the volume of the droplet dispensed was precisely controlled by the geometry of post and the dispense parameters (e.g. lift-up velocity), the number of microbeads dispensed is related to the relative distribution of beads within the source droplet. So we are able to use the number of microbeads dispensed (i.e. transferred to the surface in a single droplet) to infer the uniformity of the bead dispersion in the source drop. The polyethylene microbeads are hydrophobic, so surfactant is required for dispersing microbeads in aqueous solution. When poorly dispersed, the microbeads will prefer the vapor-liquid interface; on the other hand, when the microbeads are uniformly dispersed in water, the distribution of beads in a droplet are uniform. Thus a larger number of microbeads will be dispensed in a droplet when the dispersion is poor; and a smaller, but consistent, number of microbeads will be dispensed if they are better dispersed in the water. The goal of this study is to control the distribution of microbeads in a water droplet and dispense single microbead onto individual nDAP posts.

6.2 Experimental

6.2.1 Fabrication of nDAP

The nDAPs were fabricated by printing an array of polydimethylsiloxane (PDMS) posts onto a glass substrate using a robotic dispensing system as previously described for nVMP fabrication in Chapter 5, section 5.2.1. These nDAP posts were cured by heating the
substrate to 165°C for 5 minutes. The fabricated nDAP posts are shown in Figure 6.1. The diameter of the flat top was about 850 µm.

![Figure 6.1. Optical image of nDAP.](image)

6.2.2 Preparation of microbeads dispersion in surfactant solution

Fluorescent polyethylene microbeads were purchased from Cospheric (UVPMS-BG). The microbeads have a density of 1.002 g/cm³ and a diameter of 32-38 µm. A series of concentrations of Pluronic surfactants were prepared in de-ionized water (18 MΩ, purified using a Millipore Quantum filtration system with a 0.22 µm filter). Fluorescent polyethylene microbeads were added to the surfactant solution to prepare a suspension at specific concentrations. The suspension was centrifuged for 5 minutes to settle the microbeads down to the bottom of the centrifuge tube and ensure the wetting of the microbead surface by surfactants. Then the microbeads were re-suspended by vortexing at 1500 rpm for 15 minutes.

6.2.3 Dispensing of a nanoliter droplet onto nDAP

Automated dispensing was conducted using a robot. A 2.8 mm diameter polystyrene rod was mounted on the robotic arm. A fixed volume (10 µL) of the source solution was placed onto the flat bottom of the rod using an Eppendorf pipette, as shown in Figure 6.2. The rod with the source drop was aligned to a post; the rod was then moved...
downward at a preset speed (2 mm/s) enabling the source drop to contact the post surface at fixed height such that the bottom of the drop was brought 200 μm below the top surface of the post; lastly, the rod was lifted-off from the surface at a pre-determined speed (10 mm/s) and translated to the next dispensing location. Two types of experiments have been performed: single dispense and multiple dispense. For single dispense, after dispensing one droplet onto an nDAP post, the source drop was removed and a fresh 10 μL source drop was placed on the dispensing rod. For multiple dispense, a 10 μL source drop was used for 8 continuous droplet dispenses, and the consistency of number of beads dispensed was recorded. The dispensing process was monitored using a high-speed camera (Vision Research, Phantom V7.3) with a Mitutoyo 5X Plan APO objective coupled to a Tokina AT-X 100 mm f/2.8 macro lens operated at 5000 frames/s. High-speed videos were recorded with PCC 2.5 software.

When the dispensing was finished, the nDAP was kept at room temperature for 10 minutes until the solvent evaporated and only microbeads remained on the post surface. The number of microbeads dispensed was counted using an optical microscope (Figure 6.3).

The volume of the spherical cap-shaped droplet was calculated using the Equation 6.1:

\[ V = \frac{1}{6} \pi h(3a^2 + h^2) \quad (6.1) \]

where \( h \) is the height of dispensed droplet, and \( a \) is the radius of the nDAP post surface. Values of \( h \) and \( a \) were measured from the optical image of droplet, which was extracted
from the video recorded with the high-speed-camera system with a resolution of 7 µm/pixel. ImageJ software was used for the measurements.

Figure 6.2 nDAP dispensing system. A 10 µL source droplet was placed on the polystyrene dispensing rod.

Figure 6.3. A 130 nL droplet containing 2 mg/mL microbeads was dispensed on an nDAP post. a. side view, before drying; b. top view, after drying.

6.3 Results and discussions

6.3.1 Effect of ethylene oxide/propylene oxide chain length on dispersion quality

Three BASF Pluronic nonionic surfactants were used to prepare suspensions of microbeads. Pluronic surfactants are a series of nonionic triblock polymers composed of
a central hydrophobic chain of poly(propylene oxide) (PPO) and two side hydrophilic chains of poly(ethylene oxide) (PEO). The general structure of Pluronic copolymers is shown in **Scheme 6.1**, which is \( EO_{x/2}PO_yEO_{x/2} \). The sizes of hydrophilic and hydrophobic blocks of Pluronic surfactants are shown in **Figure 6.4**.

![Scheme 6.1 General structure of Pluronic surfactants.](image)

**Figure 6.4.** HLB grid of Pluronic surfactants (color code: physical state of copolymers under ambient conditions: green = liquid; red = paste; orange = flake). The surfactants used in this study are circled.

The properties of the three Pluronic surfactants, P103, F108 and P123 studied in this work are summerized in **Table 6.1**. The hydrophilic-lipophilic balance (HLB) of a
surfactant is a value that measures the degree of hydrophilicity, i.e. the relative strength of the hydrophilic portion to the lipophilic portion in a surfactant molecule. The definition and application of HLB were introduced in Chapter 1. Generally speaking, a surfactant is more hydrophilic if it has a larger HLB value. The critical micelle concentration (CMC) of a surfactant is the concentration at which micelles start to form. CMC is an important characteristic of a surfactant because only the unassociated or free surfactant molecules (unimer) contribute to lowering surface tension and the concentration of unimer will never exceed the CMC.

**Table 6.1.** Properties of Pluronic® surfactants used in this study.

<table>
<thead>
<tr>
<th>Pluronic surfactant</th>
<th>Average molecular weight</th>
<th>Average number of EO units (x)</th>
<th>Average number of PO units (y)</th>
<th>HLB</th>
<th>CMC (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P103</td>
<td>4950</td>
<td>38</td>
<td>56</td>
<td>9</td>
<td>6.1×10⁻⁶</td>
</tr>
<tr>
<td>F108</td>
<td>14600</td>
<td>258</td>
<td>56</td>
<td>27</td>
<td>2.2×10⁻⁵</td>
</tr>
<tr>
<td>P123</td>
<td>5750</td>
<td>39</td>
<td>69</td>
<td>8</td>
<td>4.4×10⁻⁶</td>
</tr>
</tbody>
</table>

*a* The average molecular weights were provided by the manufacturer (BASF, Wyandotte, MI)

*b* The average number of EO and PO units were calculated using average molecular weights

*c* HLB values were determined by the manufacturer

*d* CMC values were reported in the literature [31]

The dispensing process is illustrated in **Figure 6.5.** A 10 µL source drop containing microbeads, which is attached to a dispensing rod, approaches an nDAP post (**Figure 6.5a**). After initial contact with the surface of the nDAP post, the rod descends 200 µm more to insure that the drop fully wets the surface. The sharp edge of the post surface pins the triple contact line (TCL) at the droplet/post/air interface preventing wetting of the side of the
post, as shown in Figure 6.5b. As the source drop is retracted from the post surface, there is a necking deformation of the drop because the TCL is pinned at the sharp edge of nDAP post (Figure 6.5c). Eventually, the drop breaks at the narrowing neck, leaving a droplet of specific volume containing a microbead suspension on the post surface (Figure 6.5d). After evaporation of the solvent (i.e. water), a certain number of microbeads remained on the surface. Since the volume of dispensed droplet is predefined by the area of the nDAP post and the retraction velocity of the source drop, the number of microbeads dispensed is related to the dispersion quality of microbeads in source drop. Without surfactant, the hydrophobic microbeads are not dispersible in water and will migrate to the surface of the source drop (Figure 6.5e). As a result, a large number of microbeads will be dispensed in the first liquid transfer step. By adding a surfactant to the solution, the hydrophobic portion of the surfactant molecule is adsorbed onto the microbead surface, rendering the particle more hydrophilic and more dispersible in water.
Figure 6.5. Images taken with a high-speed camera illustrating the dispensing process. A 10µL source drop containing 20mg/mL microbeads and 40 µM P103 was used for the dispensing (a-d). The arrows indicate the movement direction of the source drop. (a. source drop approaching the nDAP post; b. source drop fully contacted with the nDAP surface; c. source drop retracting and stretching; d. a 130 nanoliter droplet dispensed onto the nDAP surface.) e. A 10µL source drop containing 20mg/mL microbeads without surfactant. The scale bar is 250 µm.
The volume of the dispensed droplet was measured using a source drop with two different concentrations of surfactant, as well as pure water. Fifteen measurements were performed for each concentration on five different posts. The average dispensed volume was calculated using Equation 6.1 and listed in Table 6.2. The average diameter of the five nDAP posts was measured to be 867±18 µm. The surface tension of an aqueous solution decreases with added surfactant, however the dispensed volume does not change and the variance is comparable with the variation of nDAP post size. The difference of dispensed volume measured on the largest post (884 µm ±3 µm) and on the smallest post (850 µm ±2 µm) was 15 nL. So we can conclude that the surfactant in solution does not have a significant affect the dispensed volume; the dispensed volume is related to the size of source drop and the lift-up velocity of source drop, which were discussed in Chapter 5. When the size and lift-up velocity of the source drop are fixed (10 µL and 10 mm/s), the dispensed volume remains consistent between each dispense (with a precision of ±9 nL for all solutions).

Table 6.2. Measurements of dispensed volume.

<table>
<thead>
<tr>
<th>Concentration of F108 (µM)</th>
<th>Surface tension (mN/m) (^a)</th>
<th>Volume of droplet (nL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>72</td>
<td>130±8</td>
</tr>
<tr>
<td>1.37</td>
<td>46</td>
<td>132±6</td>
</tr>
<tr>
<td>13.7</td>
<td>42</td>
<td>132±7</td>
</tr>
</tbody>
</table>

\(^a\) The values of surface tension were extracted from reference article.\(^{[29]}\)
To determine the effect of the hydrophilic chain length (EO) and hydrophobic chain length (PO) of surfactants on dispersion quality of microbeads, two sets of surfactant were compared: 1. P103 and F108, which have same PO chain length, and 2. P103 and P123, which have same EO chain length. All surfactant solutions were prepared at the same concentration of 13.7 µM. The concentration of microbeads was varied from 0.05 mg/mL to 0.50 mg/mL.

The theoretical number of microbeads contained in a 132 nL droplet with an ideal uniform dispersion can be calculated with the Equation 6.2:

\[
N = \frac{6CV_d}{\rho\pi R^3}
\]  

(6.2)

where \(C\) is the concentration of microbeads, \(V_d\) is the volume of droplet (132 nL), \(\rho\) is the density of polyethylene microbeads (1.002 g/cm\(^3\)), and \(R\) is the diameter of microbead (35 µm). The calculated results are listed in Table 6.3. The uniformity of microbeads dispersed in surfactant solutions can be quantified by measuring the number of microbeads dispensed on an nDAP post compared to the theoretical value. With a more uniform microbead suspension, the number of microbeads dispensed will approach the theoretical value, since fewer microbeads are accumulated at the vapor-liquid interface.
Table 6.3. Calculation of the number of beads in droplet assuming an ideal dispersion.

<table>
<thead>
<tr>
<th>Concentration of beads (mg/mL)</th>
<th>Theoretical number of beads in a 132 nL droplet</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>3.0</td>
</tr>
<tr>
<td>0.30</td>
<td>1.8</td>
</tr>
<tr>
<td>0.20</td>
<td>1.2</td>
</tr>
<tr>
<td>0.10</td>
<td>0.6</td>
</tr>
<tr>
<td>0.05</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Figure 6.6. Effect of hydrophilic PO chains on microbeads dispensing. The green squares indicated the theoretical number of microbeads in the dispensed droplet, if the microbeads were uniformly distributed in the source drop.

As shown in Figure 6.6, in all three cases the number of microbeads dispensed increases with increasing the concentration of microbeads. In all cases, the number of microbeads dispensed is larger than with an ideal uniform dispersion (green squares). This is because the microbeads were not dispersed uniformly throughout the source drop, because insufficient surfactant was present. More microbeads diffused to the vapor-liquid interface than under ideal conditions. When the concentration of microbeads decreased from 0.50 mg/mL to 0.05 mg/mL, the difference between the number of actual dispensed
Microbeads and the theoretical numbers became smaller, as shown in Table 6.4. This trend can be explained as the total hydrophobic microbead surface area became smaller, thus the relative concentration of surfactant to microbeads was larger, which means more surfactant was adsorbed on each microbead and the microbeads were more hydrophilic compared to when a higher concentration of microbeads was used.

**Table 6.4.** Comparison of experimental results with theoretical values.

<table>
<thead>
<tr>
<th>Concentration of beads (mg/mL)</th>
<th>Theoretical number of beads in dispensed droplet</th>
<th>Experimental data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average number to theoretical dispensed (%)</td>
<td>Average number to theoretical dispensed (%)</td>
</tr>
<tr>
<td></td>
<td>P103</td>
<td>P123</td>
</tr>
<tr>
<td>0.05</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>0.10</td>
<td>0.6</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>0.20</td>
<td>1.2</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>0.30</td>
<td>1.8</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.6</td>
</tr>
<tr>
<td>0.50</td>
<td>3.0</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.8</td>
</tr>
</tbody>
</table>
When comparing the results from the experiments of P103 (red triangles) and P123 (purple triangles), we found that P123 is a better surfactant for the microbeads suspension, since fewer microbeads were dispensed. P103 and P123 have similar length of EO chains (Table 6.1), but P123 has a longer PO chain (y=69) than P103 (y=56). The adsorption of surfactant on a solid surface is related to two factors: the stability of the surfactant in the liquid phase; and the interaction between the surfactant and the solid surface. The hydrophilic EO chains are of similar size in P123 and P103, however, P123 has a longer hydrophobic PO chain which reduces its solubility in the aqueous phase. At the same time, the longer PO chain interacts more strongly with the hydrophobic surface of the microbeads. These two effects cause more P123 surfactant molecules to be adsorbed on the microbeads surface. As a result, the microbead surfaces became more hydrophilic and more uniformly dispersed resulting in fewer microbead dispensed.

The number of microbeads dispensed using F108 was the smallest among the three surfactant solutions at each concentration of microbeads (blue triangles). F108 molecules have the same length of central PO block as P103, but a much larger molecular weight and 6 times longer EO block than P103. When a polymer surfactant is adsorbed on the surface of the microbeads, the hydrophilic EO chains stay in the aqueous phase and form random coils\(^\text{[32]}\). The size of the coils is determined by the radius of gyration and proportional to the molecular weight. Because a longer EO chain has a larger radius of gyration (\(R_g\)), it will cover a larger area when adsorbed on the solid surface (Figure 6.7). As a result, when using F108, the microbeads were more hydrophilic and more uniformly dispersed, the number of microbeads dispensed was smaller.
Since the EO chains in F108 molecules are also much longer than those in P123 (258 EO units vs. 39 EO units), the number of microbeads dispensed from F108 solution was smaller than using P123, although F108 has a shorter PO chain than P123 (56 PO units vs. 69 PO units). Among these three Pluronic surfactants, F108 is the most efficient one in making a uniform microbeads suspension.

**Figure 6.7.** Schematic illustration of surfactants on the surface of microbead. a. F108 (longer EO block) adsorbed on the surface of microbead; b. P103 (shorter EO block) adsorbed on the surface of microbead.

### 6.3.2 Effect of surfactant concentration

To study the effect of surfactant concentration on microbead dispensing, F108 solutions with different concentrations of surfactant (0.4 µM, 1.37 µM, 4 µM and 13.7 µM) were used. As shown in **Figure 6.7**, when the concentration of microbeads was 0.5 mg/mL, fewer microbeads were dispensed with increasing surfactant concentration. The effect is similar to the effect of microbead concentration (**Figure 6.6)**, at higher concentration of surfactant, the microbeads are better covered by the surfactant molecules and became more hydrophilic, and more uniformly dispersed throughout the droplet. When the concentration of F108 is held at 1.37 µM (**Figure 6.8**, blue dots) or 13.7 µM (**Figure 6.8**, blue triangles)
as the concentration of microbeads decrease, the number of microbeads dispensed per droplet also decreases and approaches the theoretical value for an ideal dispersion.

**Figure 6.8.** Effect of surfactant concentration on the number of microbeads dispensed in a 132 nL droplet.

### 6.3.3 Universal curve of microbeads dispensing

As discussed previously, the number of microbeads dispensed is related to several factors: concentration of surfactant, concentration of microbeads, as well as the length of EO/PO chain in the molecule of surfactant. To compare the number of microbeads dispensed from different surfactant solutions, a universal curve was obtained by plotting the ratio ($R_d$) of actual microbeads dispensed to the theoretical number of microbeads in a
droplet of uniformly dispersed solution \( R_d = \frac{N_{\text{actual}}}{N_{\text{theoretical}}} \) as a function of EO chain coverage on the microbead surface. The EO chain coverage was calculated using the Equation 6.3a:

\[
S_{EO} = \frac{x \cdot C_s}{C_b \cdot \rho V_b \cdot S_b}
\]  

\[ (6.3a) \]

\[
S_{EO} = \frac{x \cdot C_s}{6C_b} \times \rho R
\]  

\[ (6.3b) \]

where \( x \) is the number of EO units per surfactant molecule, \( C_s \) is the concentration of surfactant in moles per liter, \( C_b \) is the concentration of microbeads, \( \rho \) is the density of a microbead \( (1.002 \text{g/cm}^3) \), \( V_b \) is the volume of a microbead, \( V_b = \frac{4}{3} \pi \left( \frac{R}{2} \right)^3 \), \( S_b \) is the surface area of a microbead, \( S_b = 4\pi \left( \frac{R}{2} \right)^2 \), \( R \) is the diameter of microbead \( (35 \mu \text{m}) \). By plugging \( V_b \) and \( S_b \), Equation 6.3a can be expressed as Equation 6.3b.

The concentration of microbeads has an effect on two properties of the suspension: the theoretical number of microbeads in dispensed droplet and the total surface area of microbeads. The normalizations in the universal curve cancel the effect of microbead concentration, thus making it possible to have all the experimental data plotted on one graph, as shown in Figure 6.9. Increasing the EO chain coverage makes the microbead surface more hydrophilic and creates more uniform dispersions in water. Thus the number of microbeads dispensed is closer to the theoretical number of microbeads in an ideally uniform dispersion (the green line indicating \( R_d = 1 \)). As we can conclude form Figure 6.9, the microbeads migrate to the vapor-liquid interface when the surface coverage of EO chains is low, resulting in a larger number of microbeads dispensed on the nDAP post.
compared to the theoretical value. The value of $R_d$ decreases rapidly as the EO chain coverage increases from 0.001 mol/m$^2$ to 0.01 mol/m$^2$. $R_d$ continues to decrease with increasing EO chain coverage, and approaches 1, as the EO chain coverage approaches 1 mol/m$^2$. At this surfactant concentration, the dispersion of microbeads approaches an ideal uniform dispersion and the surface of microbeads would become saturated with surfactant molecules.

![Figure 6.9. Universal curve of dispensing property.](image)

6.3.4 Multiple dispensing

From the universal curve, we learn that Pluronic F108 is the most efficient surfactant among the three surfactants tested. So F108 solutions were used to test the
uniformity of microbeads distribution within the source drop. Multiple dispensing experiments were performed with two concentrations of F108 solution (1.37µM and 13.7µM). If the microbeads were uniformly distributed in the source drop, the same number of beads would be dispensed in each droplet on an nDAP post. If insufficient surfactant was present, the number of beads dispensed in each droplet would decrease as the beads with lower surfactant coverage would come to the vapor-liquid interface and be dispensed first, leaving a progressively lower concentration of particles dispersed in the droplet.

Multiple dispensing experiments were performed using one 10 μL source drop with a microbead concentration of 0.5 mg/mL that was used to dispense droplets onto eight consecutive nDAP posts. The number of microbeads on each post was counted and plotted in Figure 6.10. At lower surfactant concentrations (1.37 μM), more beads were dispensed on the first two posts than with the higher surfactant concentration (compare Figure 6.10a and Figure 6.10b). This is because the microbeads were not uniformly covered by surfactant; as a result, more beads migrated to the vapor-liquid interface of the droplet. However, after the relatively hydrophobic microbeads were dispensed onto the posts, the remaining beads were more uniformly dispersed within the source drop. As a result, the number of beads dispensed onto the nDAP became more uniform and smaller in number. When the surfactant concentration was higher (13.7 μM), the number of beads dispensed on each nDAP post was stable throughout the multiple dispensing experiments as shown in Figure 6.10b, the average numbers of microbeads dispensed were between 3.5 to 4.5 with a standard deviation of 0.44. These results indicate most of the microbeads were dispersed uniformly in the source drop and did not migrate to the vapor-liquid interface. The same trends were observed when the microbead concentration was decreased to 0.3
mg/mL, as shown in Figure 6.11. The numbers of microbeads dispensed also decreased and became more uniform, ranging between 2.6 to 3.0 with a standard deviation of 0.17. By further decreasing, the concentration of microbeads to 0.1 mg/mL, the average numbers of microbeads dispensed from the 1.37 µM F108 solution decreased to 1.8 ± 0.8, which indicated the surfactant was sufficient to cover most microbeads (Figure 6.12a). A more uniform microbeads suspension was obtained, when using 13.7 µM F108. The average number of microbeads dispensed was 0.9 ± 0.1 (Figure 6.12b), which is very close to single particle per post.

The multiple dispensing experiments provided results to quantify the uniformity of microbeads dispersion, which can be used to select optimum conditions for single microbeads dispensing.

**Figure 6.10.** Number of microbeads dispensed in multiple dispensing experiments. Microbeads concentration was 0.5 mg/mL (3.0 microbeads per droplet, theoretically, blue lines). a. concentration of F108 is 1.37 µM; b. concentration of F108 is 13.7 µM.
**Figure 6.11.** Number of microbeads dispensed in multiple dispensing experiments. Microbeads concentration was 0.3 mg/mL (1.8 microbeads per droplet, theoretically, blue lines). a. concentration of F108 is 1.37 μM; b. concentration of F108 is 13.7 μM.

**Figure 6.12.** Number of microbeads dispensed in multiple dispensing experiments. Microbeads concentration was 0.1 mg/mL (0.6 microbeads per droplet, theoretically, blue lines). a. concentration of F108 is 1.37 μM; b. concentration of F108 is 13.7 μM.
6.3.5 Single microbeads dispensing

As discussed in the multiple dispensing experiments, the F108 solution with a concentration of 13.7 µM can make the microbead suspension similar to an ideal uniform suspension. From the results of the multiple dispensing (Figure 6.10 to Figure 6.12), the probability of dispensing a single microbead onto an nDAP post was calculated using the Equation 6.4:

\[
P = \frac{N_s}{N_{\text{total}}}
\]

(6.4)

where \(N_s\) is the number of nDAP posts which have one microbead, \(N_{\text{total}}\) is the total number of nDAP posts. The results are listed in Table 6.5.
Table 6.5. Probability of dispensing a single microbead.*

<table>
<thead>
<tr>
<th>Concentration of microbeads (mg/mL)</th>
<th>Theoretical number of microbeads in 132 nL droplet</th>
<th>Average of number of microbeads dispensed</th>
<th>Probability of finding an nDAP post with one microbead</th>
<th>Ideal Poisson distribution**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>0.3</td>
<td>0.5</td>
<td>0.40</td>
<td>0.30</td>
</tr>
<tr>
<td>0.1</td>
<td>0.6</td>
<td>0.9</td>
<td>0.56</td>
<td>0.37</td>
</tr>
<tr>
<td>0.2</td>
<td>1.2</td>
<td>1.3</td>
<td>0.49</td>
<td>0.35</td>
</tr>
<tr>
<td>0.3</td>
<td>1.8</td>
<td>2.7</td>
<td>0.10</td>
<td>0.18</td>
</tr>
<tr>
<td>0.5</td>
<td>3.0</td>
<td>4.2</td>
<td>0.05</td>
<td>0.063</td>
</tr>
</tbody>
</table>

*13.7 µM F108 solution was used to prepare microbeads suspension.

**based on average number of microbeads dispensed.

When using Pluronic F108 with a concentration of 13.7 µM, the highest probability of finding one microbead on an nDAP post is 0.56.

The number of beads dispensed from a uniformly dispersed source drop can be estimated using a Poisson distribution, in which the probability of dispensing \( x \) beads is given by Equation 6.5[32]:

\[
P(X = x) = \frac{e^{-\lambda} \lambda^x}{x!} \quad (6.5)
\]
with \( \lambda \) representing the average number of microbeads in the volume of each dispensed droplet. The experimental results from dispensing with 13.7 \( \mu M \) F108 solutions were plotted in Figure 6.13 and compared with the Poisson distribution.

![Figure 6.13](image)

**Figure 6.13.** Probability of a single microbead dispensed plotted as a function of the measured average number of microbeads in each droplet.

In the Poisson distribution, the highest possibility of finding single bead is 0.37 when the average number of beads in the dispensed droplet is 1. In the experimental data, the highest probability of finding a single bead on a post was 0.56, which is higher than the probability predicted by the Poisson distribution. In an ideal Poisson distribution, the size
of source drop is considered to be large enough and the microbeads can be taken out from anywhere in the source drop, so the curvature of source drop and the interactions between microbeads and vapor-liquid interface are not taken into account. However, in the nDAP dispensing system, the source drop is small (10 µL) and microbeads are only dispensed from the surface of the source drop, so the interactions at the vapor-liquid interface have an important effect on dispensing. The microbeads have a tendency to migrate to vapor-liquid interface, thus the possibilities of single bead dispensing are higher than the ideal Poisson distribution. The peak in the experimental distribution results was found at a microbead concentration equivalent to 0.9 microbeads per droplet. The true optimal concentration could be between 0.5 and 1.3 microbeads per droplet. Further experiments are needed to define the optimal concentration more precisely.

6.4 Conclusion

A convenient platform (nDAP) was used for precisely dispensing nanoliter sized droplets to pre-determined locations. By using the nDAP system, the quality of dispersions of hydrophobic microbeads using three different Pluronic non-ionic surfactants was studied. The effect of hydrophobic chain was studied by using P123, which has same hydrophilic chain length as P103 but a longer hydrophobic chain. The longer hydrophobic chain increases the adsorption of the surfactant at the microbead surface and makes the microbead better dispersed in water. At the same surfactant concentration, microbeads tended to disperse more uniformly in water by using F108, the surfactant with the longer hydrophilic chain block. F108 showed the best performance in making the hydrophobic microbeads uniformly dispersed in aqueous solution.
When insufficient surfactant concentration was present, microbeads became poorly dispersed in water and tended to migrate to the vapor-liquid interface of the source drop. These beads at the interface are preferentially transferred during the dispensing process onto the nDAP posts. A universal curve was developed by plotting the ratio of actual microbeads dispensed to the theoretical number of microbeads in a droplet of uniformly dispersed solution as a function of EO chain coverage on the surface of the microbeads. This universal curve can be used to estimate the concentration of surfactant required for any concentration and size of particles. As observed from the universal curve, the microbeads would be uniformly dispersed in a surfactant solution when the coverage of EO chains is larger than 0.1 mol/m².

By dispensing multiple droplets from a single source drop, trends in particle dispersion could be readily observed. When insufficient surfactant was present, the first few droplets transferred contained a larger number of microbeads, but this number decreased with subsequent droplets. However, when a sufficient concentration of surfactant was present, the number of microbeads transferred in droplets remained constant over 8 consecutive dispenses.

By using the nDAP precise dispensing system, single microbeads were dispensed on the post surfaces with a high probability of 0.56.

Single particle dispensing is a desired technique for the biomedical and semiconductor industries. The studies in this Chapter provide a method to effectively evaluate the quality of particle dispersions as well as dispense a specific number of particles at a precisely defined location.
Bibliography

Chapter 1


Chapter 2
Chapter 3

Chapter 4

Chapter 5


Chapter 6