Synthetic microfluidic paper
-Towards a novel substrate for lateral flow immunoassay-

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Synthetic microfluidic paper
Towards a novel substrate for lateral flow immunoassay

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Abstract
Paper based diagnostic devices, for example, dipstick pregnancy tests, have received considerable attention due to their rapidity, low cost and portability. To expand the use of such tests to a larger variety of diagnostic applications, higher sensitivity remains a challenge. The higher sensitivity is hard to obtain with conventional paper material, such as nitrocellulose, since there are two limitations, a randomness of the paper structure and opacity. To overcome these limitations, the microscopic structure composing the paper should ideally be controlled to obtain predictable flow and the used material should be transparent. This thesis presents a novel concept of synthetic microfluidic paper made in Off-Stoichiometric-Thiol-Ene (OSTE) which possesses excellent characteristics as photolithographic material and which is transparent. In our design, the synthetic paper comprises slanted and interlocked pillars, which are highly resistant to capillary collapse occurring as a common problem during wet fabrication processes. In experiment, we achieved the slanted and interlocked pillars of the OSTE and explored their geometrical limitation range. Furthermore, dyed water and blood imbibition into the synthetic paper were demonstrated. The synthetic microfluidic paper has the possibility to be integrated with immunoassay system in a point-of-care diagnostic test. We believe the integrated synthetic paper extend instant diagnostic systems based on paper microfluidics into diagnostics for current diseases of interest, such as diabetes, heart diseases or cancer.
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Chapter 1

Introduction

In this chapter, introduction of this thesis is provided. First, background of this research is described. After this, problems remaining in conventional research are summarized and two unique solutions to overcome these problems are suggested. Then, concept and objectives of this thesis are declared. In the end of this chapter, conventional research is summarized to consider important parameters.

1.1 Background

In this section, possibility of pillar forest research as an alternative to the traditional paper microfluidic device is introduced. This introduction is supported by describing necessity of point-of-care testing, fundamental mechanism of lateral flow immunoassay and problems lying in traditional paper material.

Owing to medical advances, world life expectancy has been drastically increased [1]. The advances occurred from various innovations, such as molecular interpretation of physiology, development of pharmacies, occurrence of new diagnostic methods, etc. The diagnostic methods have been extensively developed because they enable early detection and precise identification of various diseases, leading to higher cure rate [2]. For example, combination of x-ray computed tomography (CT) with positron-emission tomography (PET) is a technology to detect lung cancer inside of a human body in early stage [3]. As another example, genetic testing predicts a potential disease by referring to a DNA database [4]. Most diseases could potentially be diagnosed from an internal sample such as blood, urine, or saliva. While the diagnosis of the internal samples traditionally requires time-consuming laboratory treatments, in recent years, development of immediate and on-site diagnosis system known as point-of-care testing has been attempted [5]. The point-of-care testing requires rapidity and portability in addition with basic requirements for diagnosis, such as high sensitivity and low cost [5].

Lateral flow immunoassay (LFI), as typified by the pregnancy test, is one of the point-of-care testing recently used not only for medical diagnostics but also biological studies in laboratories [6]. In LFI, sample liquid containing analytes which has antigenicity are driven through a microfluidic device where the analytes are detected with immobilized antibodies. Filter or nitrocellulose papers have traditionally been used as the microfluidic device since they generate autonomous sample imbibition by capillary force [7, 8]. Although many kinds of LFI research based on the paper have been conducted, two problems
remain; flow irreproducibility and opacity due to their uncontrolled random microscopic structure, which limit sensitivity of measurement.

These days, in order to solve these problems, repetitive vertical pillars in vicinity, termed as pillar forest, has been introduced as an alternative to paper [9] as shown in Figure 1.1. When two structures are located closely, liquid is filled into the inter space by capillary force depending on the surface energy. Repetitive pattern such as pillar forest generates continuous liquid flow into them. Compared with the conventional papers, higher flow reproducibility is expected due to structural controllability during fabrication. In conventional studies, the pillar forest was fabricated of silicon [10], and polymer material such as polymethylmethacrylate (PMMA) [11] or cyclo olefin polymer (COP). Pillar forest of COP [12] has been commercialized in a LFI test [13].

**Figure 1.1. Pillar forest application as an alternative to lateral flow test [14, 15]. A) Example of lateral flow immunoassay device and nitrocellulose paper. B) Microscopic images of filter and nitrocellulose paper. C) A schematic view of pillar forest.**

### 1.2 Problems

Problems that remain in conventional pillar forest to be used for LFI device are summarized and listed below with respect to material. Successful capillary devices based on pillar forest have been achieved but there are still several points to be improved in order to yield higher sensitivity:

- **No transparency (silicon)**  
  Silicon is an opaque material.

- **Complicated and unstable surface modification (polymers)**  
  Surface of polymer material is generally hydrophobic. In order to generate capillary imbibition, the surface has to be treated into hydrophilic. However, there is not simple and stable surface modification.

- **Adsorption of antibodies (COP)**  
  The current method to immobilize antibodies is dependent on adsorption, which potentially has a risk to wash signal molecules away.

- **Limited aspect ratio (pillar height / diameter) (general)**  
  Generally, fabrication of repetitive vertical pillars is prone to collapse by capillary force during liquid fabrication process, which inhibits high aspect ratio.
For all of the above-mentioned problems, we came up with the two solutions, using Off-Stoichiometric-Thiol-Ene to address the opacity, surface modification and adsorption of antibodies, and applying interlocked pillar structure to approach the aspect ratio.

1.3 Overall approach

In this section, two approaches, using Off-Stoichiometric-Thiol-Ene and applying interlocked pillar forest, are suggested in order to address the problems of transparency, surface modification, antibody immobilization and aspect ratio mentioned in the previous subsection.

1.3.1 Off-Stoichiometric-Thiol-Ene

Off-Stoichiometric-Thiol-Ene (OSTE) is a candidate material to address the transparency, surface modification and antibody immobilization problems. OSTE is a transparent polymer system comprising two monomers, one with thiol groups and the other with ene groups, in which the ratio of one monomer over the other is intentionally exceeded. Subsequently, the thiol/ene groups in the excessed monomers are located on the surface of the polymer and grafting can be performed on the surface. This feature gives rise to tunability of hydrophilic/hydrophobic surface and possibility of covalent immobilization of antibodies [16, 17].

1.3.2 Interlocked pillar forest

Interlocked pillar forest stands for a pillar structure where pillars are interlocked and reinforce each other as illustrated in Figure 1.3.2a. Thanks to the interlocked structure, the forest obtains more resistance to collapse or clustering by capillary forces; therefore, the interlocked pillar geometry allows increase in pillar aspect ratio and available surface areas. The pillar forest can be fabricated by photolithography [18]. When the photosensitive polymer is exposed to multi-directional UV through a shadow mask, the fabricated pillars are interlocked where the traces of UV light are superposed as shown in Figure 1.3.2b.

![Figure 1.3.2. Description of interlocked pillar forest. A) A schematic view of interlocked pillar forest. B) Schematic view of fabrication of the structure.](image-url)
1.4 Objectives of this thesis

In this thesis, towards the establishment of novel microfluidic synthetic paper, three goals are determined.

1) To fabricate OSTE synthetic paper based on interlocked pillar structures (Figure 1.4).
2) To explore the available fabrication range with respect to pillar diameter, pitch (distance from one pillar to its neighbor) and height.
3) To demonstrate lateral flow of sample liquid, water and blood, into the synthetic paper.

![Figure 1.4](image)

*Figure 1.4. OSTE synthetic microfluidic paper as alternative to a conventional microfluidic paper. A) A photograph of a piece of microfluidic paper. B) Schematic view of synthetic microfluidic paper. C) A SEM image of interlocked pillar forest in a synthetic microfluidic paper.*

1.5 Overview of previous work

In this section, previous studies on capillary imbibition into pillar forest are summarized in terms of different materials: silicon, cyclo olefin polymer (COP), and other polymers. At the end of this section, a summary is shown as table 1.5.

1.5.1 Silicon

In this subsection, examples of pillar forest as capillary pump using silicon are introduced. Silicon is the material intensively used for the pillar forest as capillary pump because there exist well-established fabrication processes, which expectantly induces high reproducibility of flow rate. Moreover, its stiffness enables high aspect ratio of fabricated structures. A number of studies have been conducted using silicon and several important examples are introduced in the table 1.5. In the previous reports, the pillar diameters ranged from several microns to a few hundred micron and the aspect ratio (the height / the pillar diameter) reached 8 in maximum [9, 10 and 19]. The flow rate of capillary pump shown in the literature of [10] had coefficient of variation (C.V.) compared with other studies. The silicon pillar was mainly fabricated by deep reactive ion etching. The opacity and expensive fabrication processes are main disadvantages using silicon.
1.5.2 Cyclo olefin polymer

In this subsection, examples of pillar forest as capillary pump with using cyclo olefin polymer (COP) are introduced. COP has been attracting attention as an excellent candidate for the pillar forest application as capillary pump. Using this material, the commercial product for troponin test has already been achieved [13]. This material is fabricated into pillar forest by means of injection molding or hot embossing. Because natural surface of the polymer is not enough hydrophilic for aqueous liquid imbibition, the surface needs to be treated into hydrophilic in a certain way. In the research of the [12], the surface modification was done with plasma enhanced chemical vapor deposition (PECVD) and COP pillar forest obtained hydrophilicity to withstand practical use. However, the fabrication process limited the pillar to approximately 1 in aspect ratio. In addition to the problem, antibodies are immobilized by covalent bond after the multi-step and complicated process or by adsorption in the commercial device, which potentially induces washing out signal molecules during a measurement.

1.5.3 Other polymers

In this subsection, examples of pillar forest as capillary pump with using other polymer materials are introduced. Pillar forest studies as capillary pump have been performed with using PMMA, PDMS and SU-8.

PMMA was used as the pillar array for capillary pump [11]. The capillary pump application was reported and surface modification was performed by plasma treatment for capillary pump. However, due to short-term viability of hydrophilicity of plasma treated surface of the capillary pump, the PMMA does not seem suitable for lateral flow immunoassay.

PDMS and SU-8 were also attempted to use for capillary pump [20]. But, in the reported research, the hydrophilic surface modification was not performed, that is, the device does not have capability as capillary pump in LFI. In the experiment, isopropanol was used as imbibing fluid.
Table 1.5 Summary of pillar forest research as capillary pump. Flow rate shown on the table is an average value during filling out the whole capillary pump in a device in each study.

<table>
<thead>
<tr>
<th>Ref. no.</th>
<th>Material</th>
<th>Diameter / μm</th>
<th>Height / μm</th>
<th>Pitch / μm</th>
<th>Aspect ratio</th>
<th>Flow rate</th>
<th>C.V.</th>
<th>Fabrication Method</th>
<th>Working medium</th>
<th>Group</th>
<th>Year</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>[9]</td>
<td>Silicon</td>
<td>15-250</td>
<td>30</td>
<td>15-250</td>
<td>&lt;2</td>
<td>0.2-3.7 nl/s</td>
<td>±3%</td>
<td>D-RIE</td>
<td>Water</td>
<td>IBM zurich</td>
<td>2007</td>
<td>PDMS sealing</td>
</tr>
<tr>
<td>[10]</td>
<td></td>
<td>1.3-6.4</td>
<td>8.3-20</td>
<td>4.5-30</td>
<td>4-8</td>
<td>1.54-2.02 μl/s</td>
<td>±3-4%</td>
<td>D-RIE</td>
<td>Water</td>
<td>MIT</td>
<td>2010</td>
<td>Open chamber</td>
</tr>
<tr>
<td>[11]</td>
<td>PMMA</td>
<td>300</td>
<td>15</td>
<td>200</td>
<td>0.05</td>
<td>123 μl/s</td>
<td>±5%</td>
<td>Hot embossing</td>
<td>Water</td>
<td>Univ. Ulster</td>
<td>2011</td>
<td>PMMA sealing</td>
</tr>
<tr>
<td>[12]</td>
<td>Cyclo olefin polymer</td>
<td>70-70</td>
<td>65-70</td>
<td>85(row) 185(colum)</td>
<td>-1</td>
<td>1.55 μl/s</td>
<td>±2%</td>
<td>Injection molding</td>
<td>Dyed water</td>
<td>Dublin City Univ</td>
<td>2009</td>
<td>Surface treatment Open chamber SiOx coat with PECVD</td>
</tr>
<tr>
<td>[13]</td>
<td>PDMS</td>
<td>330</td>
<td>40</td>
<td>320</td>
<td>0.1</td>
<td>3.64 μl/s</td>
<td>N.A.</td>
<td>Molding (SU8)</td>
<td>Isopropanol</td>
<td>Univ. Ulster</td>
<td>2009</td>
<td>Glass sealing</td>
</tr>
<tr>
<td>[14]</td>
<td>SU-8</td>
<td>350</td>
<td>120</td>
<td>300</td>
<td>0.3</td>
<td>18.70 μl/s</td>
<td>N.A.</td>
<td>Lithography</td>
<td>Isopropanol</td>
<td>Univ. Ulster</td>
<td>2008</td>
<td>Glass sealing</td>
</tr>
</tbody>
</table>
1.6 Report structure

In this thesis report, the chapters are constructed as follows.

Chapter 1: Motivation of our study was introduced, followed by conventional studies of pillar forest for capillary imbibition.

Chapter 2: The fundamental chemical mechanism of off-stoichiometric-thiol-ene (OSTE), theoretical inspection of pillar fabrication and capillary pump mechanism in pillar forest are described.

Chapter 3: Fabrication process of interlocked pillar forest and hydrophilic surface modification on the pillar surface are explained.

Chapter 4: Conducted experiments in order to explore the fabrication range and demonstrate liquid imbibition into the interlocked pillar array are summarized.

Chapter 5: Obtained experimental results are discussed.

Chapter 6: Conclusion of the present project is described.
Chapter 2

Theory and Background

This chapter provides theory and background to understand phenomena and mechanisms of chemistry and physics in this project. First, chemistry of OSTE is described from mechanism of polymerization to method of hydrophilic surface modification. Then, geometrical limitation on pillar fabrication is described in terms of capillary collapse. Finally, mechanisms of liquid imbibition into narrow channel and pillar forest are described.

2.1 Off‐stoichiometric‐thiol‐ene (OSTE)

In this section, fundamental mechanisms of photolithography and surface modification of off‐stoichiometric‐thiol‐ene (OSTE) are described. OSTE is a negative photoresist and was used for manufacturing of the interlocked pillar in this project.

2.1.1 Polymerization mechanism of OSTE

In this subsection, polymerization mechanism of OSTE is described. The OSTE is fundamentally a negative photoresist comprising two monomers with thiol groups and ene (allyl) groups, respectively. The two monomers can be covalently bonded into polymer by initiator and UV exposure, which is called as "click" chemistry (Figure 2.1.1A). The term of off‐stoichiometry indicates intentional excess of one of the monomers in the OSTE. In Figure 2.1.1B, \( m \) is the molar concentration of the ene monomer, \( x \) is the number of ene group in the monomer, \( n \) is the molar concentration of the thiol monomer, and \( y \) is the number of thiol groups in the monomer. If \( n^x \) is not equal to \( m^y \), the polymer system is off‐stoichiometric. When the unpolymerized OSTE is exposed to the UV light, the OSTE is covalently bonded into the polymer, leaving exceeded and unreacted functional groups. The unreacted functional group remains on the surface of the polymerized OSTE. The unreacted thiol groups are located on the surface when thiol excessed OSTE is used. The thiol group on the surface can be functionalized as hydrophilic or hydrophobic [17].

2.1.2 Photolithography of OSTE

In this subsection, an interesting and useful photolithographic mechanism in OSTE is described. Compared with stoichiometric thiol-ene, OSTE enables higher aspect ratio structures using photolithography [28]. Figure 2.1.2 shows schematic view of photolithography of OSTE. When prepolymer of OSTE is exposed to collimated UV through shadow patterns, OSTE is cured along the patterns. As a general problem during polymerization of photoresist, unwanted polymerization induced by light leakage or scattering likely occurs, here termed as over curing. OSTE reduces the risk of the over curing as follows. During UV exposure, OSTE polymerization is initiated under UV exposure. As OSTE does not include appropriate ratio of the two monomers for polymerization, the polymerization always occurs “incompletely”. However, in the vicinity of the edge of the exposed area, green area in the Figure 2.1.2, the polymerization occurs by absorbing the ene monomer under the shadow areas, purple area, to compensate for the off-stoichiometry. As a result, the purple area includes less amount of ene monomer than other regions. Thus, the prepolymer in the purple area cannot be polymerized due to the very high off-stoichiometry. Thus, the OSTE photolithography enables higher aspect structures.
Figure 2.1.2 Mechanism of photolithography of thiol-excessed OSTE. Off-stoichiometry in area 1, 2 and 3 become different during UV exposure by ene monomer diffusion. Area 1 contains the initial off-stoichiometric ratio of thiol and ene monomers. Area 2 contains the very high off-stoichiometric ratio to the extent to prevent polymerization. Area 3 contains the low off-stoichiometric ratio compensated for by ene monomer diffusion.

2.1.3 Surface modification of OSTE

In this subsection, the mechanism of surface modification on OSTE with hydroxyethylmethacrylate (HEMA) is introduced. HEMA is a molecule that includes both one ene and one hydroxyl groups. On the surface of OSTE with excess of thiol groups, unreacted thiol groups remain. The ene functional group in HEMA molecule is able to bind to the thiol group on the surface. When HEMA is exposed to UV close to the OSTE surface, the ene group is covalently bonded to the thiol group in the presence of proper initiator similar to the OSTE polymerization, as photo-grafting. After the surface modification, the surface of OSTE gets more hydrophilic than natural because of the hydroxyl group originally included in the HEMA.
2.2 Pillar collapse

In this section, the relation of pillar collapse with geometrical and physical parameters during fabrication is described. Pillar structure in a pillar forest is subject to capillary collapse when it is fabricated in liquid and the liquid is being dried. The capillary force applied to the pillar is described as

\[
F_{\text{capillary}} = \frac{\pi y d^2 \cos^2 \theta}{2} \left( \frac{2}{\sqrt{(p - 2\delta)^2 - d^2}} + \frac{1}{2(p - 2\delta)^2 - d^2} \right) \tag{2.2.1}
\]

where \(d\) is diameter, \(p\) is pitch, \(\delta\) is deflection of a pillar, \(\theta\) is contact angle and \(\gamma\) is surface energy [23]. Against the capillary force, the elastic restoring force depending on the deflection is described as

\[
F_{\text{elastic}} = \frac{3\sqrt{2} \pi E d^4 \delta}{64 h^3} \tag{2.2.2}
\]

where \(h\) is height and \(E\) is young's module [23]. When \(F_{\text{elastic}} \geq F_{\text{capillary}} (\exists_1 \delta_c; E_{\text{elastic}} = F_{\text{capillary}})\), the critical young's modulus is obtained as

\[
E_{\text{crit}} = \frac{32\sqrt{2} y \cos^2 \theta h^2}{3d^4 f(r)} \tag{2.2.3}
\]

in the literature of [23, 27]. Here, the function \(f\) of \(r\) (= \(p/d\)) is described as

\[
f(r) = \frac{1}{r - k} \left( \frac{2}{k^2 - 1} + \frac{1}{2k^2 - 1} \right) \tag{2.2.4}
\]

\[
r = \frac{1}{k} \left( \frac{\sqrt{2}(k^2 - 1)^{\frac{1}{2}} + (2k^2 - 1)^{\frac{1}{2}}}{\sqrt{2}(k^2 - 1)^{\frac{3}{2}} + 2(2k^2 - 1)^{\frac{3}{2}}} \right) + k \tag{2.2.5}
\]

According to Figure 2.2A, the equation (2.2.5) is simplified to a linear function, where \(r > 1\). Assuming \(r < 10\) by taking practical pillar conditions into consideration, the equation is approximated as \(r = 2.208 k - 1.208\) by connecting points at \(k = 1\) and 5. This approximated function is substituted for (2.2.3) and (2.2.4) and then the following equation is obtained.

\[
E_{\text{crit}} = \frac{32\sqrt{2} y \cos^2 \theta h^2}{3d^4} \frac{1.8278}{r - 1} \left( \sqrt{\frac{2}{0.2992r^2 + 0.4955r - 0.7949}} + \frac{1}{0.5984r^2 + 0.9909r - 0.5898} \right) \tag{2.2.6}
\]

Using the equation (2.2.6), geometrical limitations are drawn in Figure 2.2B with respect to different heights, 50, 150, 300 and 450 \(\mu\)m. The limitations are
indicated as a line and pillars are supposed to collapse when the point of pillar diameter and pitch is located under the line.

*Figure 2.2* A) A plot of \( r \) over \( k \). The red line indicates an approximated linear function of equation (2.2.5). B) Plots of theoretical limitations of geometrical parameters, diameter and pitch in \( \mu \text{m} \), on the vertical pillar fabrication with different height. In the region under the line, vertical pillars are supposed to collapse.

### 2.3 Capillary pump

In this section, the fundamental mechanisms of capillary phenomenon are described. When liquid flows in narrow channels by capillary force, it can be described as a capillary pump. The capillary pump has traditionally been used for microfluidic devices [21].

#### 2.3.1 General description of capillary pump

In this subsection, a general description of capillary pump is introduced. All of the equations are according to [22 and 23]. Figure 2.3.1A schematically shows capillary imbibition in a narrow channel in liquid-gas system. The flow rate \( Q \) of the imbibition liquid is described in the following equation:

\[
Q = \frac{\Delta P}{R} \tag{2.3.1.1}
\]

where \( \Delta P \) is the pressure drop over the interface of the liquid and gas and \( R \) is the flow resistance that occurs at the interface of the liquid and channel. Pressure drop at a meniscus is described as

\[
\Delta P = \gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right) \tag{2.3.1.2}
\]

where \( \gamma \) surface tension and \( R_i \) \((i=1,2)\) radius of water front meniscus which has two dimensions due to the geometry of the internal channel surface. Considering channel dimensions, the pressure drop for Figure 2.3.1A is rewritten as follows.
\[
\Delta P = 2\gamma \cos \theta \left( \frac{1}{H} + \frac{1}{W} \right) \tag{2.3.1.3}
\]

where \( \theta \) is the contact angle of the liquid to the channel surface as shown in Figure 2.3.1B. When liquid flows into a narrow channel as shown in Figure 2.3.1A, it is known that there is a flow resistance, which is described as below.

\[
R = \frac{12\mu L}{HW^3(1 - 0.63W/H)} \tag{2.3.1.4}
\]

where \( \mu \) is the viscosity of the flowing liquid.

Figure 2.1.1. A) Schematic model of capillary pump. Liquid injected from the inlet flows through channel and reaches the outlet. \( L \) is the length from the inlet to the liquid front. \( W \) is the width of the channel. \( H \) is the height of the channel. B) Definition of contact angle. The contact angle is defined as an angle formed by two interfaces; the liquid/solid and the liquid/gas interfaces at the point where the liquid/gas interface meets the solid surface.

### 2.3.2 Capillary pump with pillar forest

In this subsection, liquid imbibition into pillar forest is introduced. An array of repetitive pillar structures, termed as pillar forest, is capable of generating autonomous liquid flow into the forest due to capillary forces. Despite many studies about the capillary pumping with pillar forest, general modeling and prediction of imbibition in the pillar forest have not been achieved yet [24]. However, there have been several approximation based on Darcy’s law [25]. Darcy’s law is described as

\[
v = -\frac{\kappa \Delta P}{\mu \Delta x} \tag{2.3.2.1}
\]

where \( v \) is the velocity of the liquid imbibition, \( \kappa \) the permeability, \( \mu \) the viscosity of imbibed liquid, and \( \Delta P/\Delta x \) the pressure drop [25]. The permeability and pressure drop are approximated by interaction of pillar diameter, pitch, height, and surface tension [10]. Obtained flow result behaves as liquid propagating based on Washburn’s equation, described as

\[
x^2 = \frac{\gamma Dt}{4\mu} \tag{2.3.2.2}
\]

where \( x \) is the distance of the liquid imbibition, and \( D \) the pore diameter, and \( t \) the imbibition time [26].
Chapter 3

Fabrication

In this chapter, fabrication processes of the present synthetic microfluidic paper, including interlocked pillar forests and surface modification protocol, are described. Subsections of 3.1.1 and 3.1.2 introduce the used OSTE formulation and the parameters of the photomasks, respectively. Figure 3 shows a flowchart of the fabrication processes.

**Base preparation**: This process prepares the substrate supporting the interlocked pillar structures. Uncured OSTE was poured on a transfer film (Xerox Sverige AB, Kista, Sweden) on which two slides of glass (VWR, USA) was placed to define the height of the substrate. Then, the uncured OSTE was covered with a slide of glass (VWR, USA). Subsequently, the OSTE was exposed to UV light using a 12mW cm⁻² near UV mercury lamp (OAI, Milpitas, USA).

**Multidirectional UV exposure**: This process is used to fabricate interlocked pillar forests. A detailed fabrication process is described in subsection 3.1.3.

**Development**: This process is used to develop the interlocked pillar structure after the UV exposure. A detailed process is described in subsection 3.1.4.

**Flood curing**: This process is used to cure interlocked pillar structured OSTE completely. A detailed fabrication process is described in subsection 3.1.5.

**Drying**: This process is used to dry OSTE before surface modification. After the flood curing, the fabricated pillar forest was kept in a fume hood until it dried.

**Surface hydrophilic treatment**: This process is used to make the OSTE hydrophilic in order to enable the pillar forest to imbibe liquid. A detailed fabrication process is described in following section 3.2.

*Figure 3. Fabrication flowchart overview*
3.1 Fabrication of pillar forest

In this section, material, mask design and photolithographic fabrication procedure of pillar forest are described. First, used OSTE formulation is introduced with description of procedure of mixing monomers. Second, principal of the photomask design based on the pillar parameters of interest is explained. Then processes of pillar forest fabrication are sequentially described.

3.1.1 OSTE preparation

In this subsection, the used OSTE composition is described. Prior to the fabrication, OSTE40, where OSTE40 indicates 40% excess of thiol groups, was prepared as follows. Tetraeneoxyethane (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and a photoinitiator; Irgacure (BASF Corp., USA), were mixed such that the amount of the Irgacure was 0.5 wt% of the final mixture. The mixture was kept in 75°C oven for 10 minutes to dissolve the initiator into the mixture. After that, it was cooled down in 5°C refrigerator for 5 min, to avoid unintended oligomerization or polymerization when the other monomer was added. Then, tris 2–(3-mercaptopropionyloxy) ethyl isocyanurate (BOC Science, NY, USA) were added to the mixture. Finally, Q1301 (Wako Chemical Inc., USA) saturated in toluene (Sigma-Aldrich, USA) solution was added such that the amount of Q1301 is 1 wt%, in order to inhibit autonomous oligomerization in the OSTE40. The Young’s modulus of the cured OSTE40 was measured as 5.5 MPa by DMA and contact angle of butylacetate (VWR, USA) to the surface of the cured OSTE40 was approximately 5.8°.

3.1.2 Shadow mask

In this subsection, the shadow mask design is described. Figure 3.1.2 shows the shadow masks which were used for pillar fabrications. Interlocked pillar structure has several parameters of interest during photolithography; cross-sectional shape of pillar, representative dimensions (ex. diameter in circle or side length of square), pitch between two pillars (P), height (H) of the pillar and angle (θ) of pillar slanting. In this project, the cross-sectional shape was already defined as circular and thus the shadow masks determined diameter (D) and pitch (P) of the pillars. The shadow mask was prepared and purchased from JD photo, UK. The mask was diced into 25 mm x 6.25 mm which included circular patterns. In order to facilitate UV-exposed OSTE peeling off from mask, the mask was coated with thin Teflon layer as follows; Teflon AF (DuPont, USA) was spin-coated on the masks with 2000 rpm for 60 seconds. Then, the masks were kept at 180°C for 15 minutes for evaporating the solvent.
3.1.3 Multidirectional UV exposure

In this subsection, UV exposure process is described. Figure 3.1.3A is a setup that enabled four-directional and slanted photolithography. As shown in figure 3.1.3B, the setup consisted of a base, two blocks 1, 2 attached with Al-coated silicon wafer and a roof having four legs. Figure 3.1.3C shows schematic image of the setup. Uncured OSTE was prepared in the space between OSTE substrate and a shadow mask. The height of fabricating interlocked pillar was determined by piles of 150 μm height of cover glass for 150, 300 and 450 μm height of the interlocked pillar. When pillar height was 50 μm, spacer tape of 50 μm height was used and the defined space was prepared in the Base preparation process, because handling of the tape was difficult in this process. The space to fabricate pillar forest of 50 μm height was prepared by means of molding of the tape. 60° slanted mirror reflected collimated UV so that the OSTE was exposed to the reflected UV at 30°. The exposing UV from the four mirrors is superposed in the uncured OSTE. Suitable time during the exposure depended on pillar diameter, pitch and height each time and it was optimized each time based on the results of microscopic observation.
Figure 3.1.3. Image and schematics of multi-directional UV exposure. A) An image of multi-directional UV exposure setup. B) Schematic of decomposed setup. The setup was composed of four blocks to support mirror, a base and a roof. C) Mechanism of slanted UV exposure at one mirror. A mirror was made of silicon wafer coated with Aluminum. Roof was fixed in order not to prevent the slanted UV exposure.

3.1.4 Development

In this subsection, the way in which the device was developed is described. After the UV exposure, the pillar forest was peeled off from the shadow mask and, then, soaked in developer as shown in Figure 4.1.3. Butylacetate was used as developer for the pillar forest. The pillar forest was kept in the developers for at least 3 minutes during ultrasonication in order to fill the developer into interspace of pillars and eliminate all of uncured OSTE out of the structure. After the development, the device was dried in a fume hood.
3.1.5 Flood curing

In this subsection, the procedure of flood curing is described. The schematic view of flood curing is shown in Figure 3.1.5. The optimized exposure time for photolithography was not always enough to polymerize OSTE completely. If the exposure time was not sufficient for internal polymerization of OSTE, the stiffness was lower than completely polymerized OSTE and unpredictable collapse of pillar structure was induced. In order to avoid this, the flood curing procedure was introduced as follows; during the development, internal initiator of pillar forest seemed to be leaked out into the solvent (butylacetate or acetone). Thus, although the device was exposed to UV again in new solvent, further polymerization could not effectively occur. After development and rinsing, the pillar forest was soaked into the same solvent which contained same percentage of photo-initiators as OSTE bulk (0.5% of Irgacure). Then it was exposed to UV for 60 seconds with keeping it soaked, i.e. flood curing.

![Figure 3.1.5 Schematic view of flood curing. The device taken out from the developer was rinsed with pure solvent. Before drying the device, it was soaked into the same solvent with photo-initiator for flood curing. Then, the device was exposed to UV in the solvent as the flood curing.](image)

3.2 Hydrophilic surface modification

In this subsection, the procedure of hydrophilic surface modification is explained. For aqueous liquid imbibition, the surface of the interlocked pillars has to be hydrophilic. To achieve a hydrophilic surface, surface modification was performed with 2-hydroxyethyl methacrylate (HEMA) (Sigma-Aldrich Co., USA). OSTE40 has thiol groups on the surface owing to the off-stoichiometry. Under the existence of proper initiator, the unreacted thiol group on the OSTE40 surface can be bonded to the HEMA, as described in section 2.1.4. After the photolithographic processes, pillar forest was filled with solvent containing HEMA (5 wt%) and two kinds of initiators, TPO-L(0.1 wt%) (BASF Corp., USA) and benzophenone (0.1 wt%). A slide of glass was placed on the synthetic paper such that the distance from the top surface of pillar to the slide became 150 μm in order that UV exposure was homogeneously applied. Then, they were exposed to UV for 300 seconds. After the exposure, the pillar forest was rinsed and incubated in a bare isopropanol bath for 300 seconds. After this procedure, the synthetic paper was ready for aqueous liquid imbibition.
Figure 3.2 Procedure of surface hydrophilic treatment. The OSTE was soaked in the mixture of HEMA and two kinds of photo-initiators, TPO-L and Benzophenone in isopropanol. Glass slide was placed to perform homogeneous UV exposure on the surface of the OSTE. OSTE was exposed to UV for 300 seconds and incubated for 300 seconds.
Chapter 4

Experiment

This chapter describes conducted experiments. In the first part, experiment in order to evaluate available fabrication range of pillar forest is described. In the second part, experiment in order to demonstrate a function of the synthetic paper as capillary pump is described.

4.1 Evaluation of fabrication

In this section, an experimental series to evaluate available fabrication range is described. As shown in Table 4.1A and B, different parameters of pillar forest were prepared in shadow masks with respect to the pillar diameter and pitch/diameter or width, respectively. Each parameter was implemented in a 2.5 mm x 2.5 mm square area on the shadow mask. Four different height (50, 150, 300 and 450 μm) of interlocked pillar forest were fabricated. The mask presented on table 4.1B was used only for the 150 μm in the height. In addition to the interlocked pillars, vertical pillars were also fabricated using the same masks. Fabricated pillar forest was classified into three groups, non-collapse, collapse or over-curing caused by nonoptimization of UV exposing time, as shown in Figure 4.1. When the pillars greater than 50% in the square could be classified as one group, the classification for the forest was determined to be the group. For example, the pillar forest was classified as “collapse” when pillars greater than 50% in the square collapsed. Optimized UV exposure time was empirically determined since it depends on the pillar diameter, pitch and height. In order to optimize the exposure time, each pillar forest fabrication was attempted with at least three different UV exposing time. The fabricated pillars were inspected with microscope (Leica, Wetzlar, Germany) and scanning electron microscope (SEM) for several geometries. Pillar forest was observed from top and the cross section was also observed to verify over-curing by splitting the pillar forest laterally.
Table 4.1A Parameters of shadow masks of fabrication range evaluation in terms of diameter and ratio of pitch over diameter. Rows indicate diameters in µm and columns indicate the ratio. Numbers in elements indicates pitch in µm.

<table>
<thead>
<tr>
<th>Diameter [µm]</th>
<th>r = Pitch/Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
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<td>45</td>
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<tr>
<td>50</td>
<td>75</td>
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<tr>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 4.1B Parameters of shadow masks of fabrication range evaluation in terms of diameter and width. Rows indicate diameters and columns indicate the width (distance between contiguous pillars) in µm. Numbers in elements indicates pitch in µm.

<table>
<thead>
<tr>
<th>Diameter [µm]</th>
<th>Width [µm]</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>5</td>
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<tr>
<td>5</td>
<td>10</td>
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<td>35</td>
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<td>50</td>
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</table>

Figure 4.1 Schematic view of classification of fabricated pillar forest. Non-collapse is a group in which pillars stand straightly and no vending structure is observed. Collapse is a group in which pillars are fallen down to the bottom or cluster each other. Over-curing is a group in which pillars looks standing without collapse but interspaces of pillars are filled with cured OSTE.
In this section, experimental procedure of capillary imbibition test and evaluation of the liquid imbibition into synthetic paper are described. The synthetic papers were fabricated by following the fabrication process as described in the section 3. Three geometries of the synthetic papers were chosen as (50, 150, 150), (50, 100, 150), and (20, 40, 150) = (Diameter, Pitch, Height in \( \mu \text{m} \)). The size of the synthetic papers was 5 mm in the orthogonal direction to flow and 50 mm in the parallel direction.

Figure 4.2A shows the setup for the capillary imbibition test. Two acryl sheets which had approximately 90’ of contact angle to water were stuck on a glass slide and the glass slides were placed such that the distance between them was 3 mm. 5% of dyed water was injected and filled in the distance between the sheets. A tested synthetic paper was placed horizontally and pushed towards the meniscus of the dyed water. The liquid imbibition was filmed at 25 fps with using digital camera (EOS 500D, Canon, Japan). Three different synthetic papers were tested at the same time to calculate the distribution of liquid imbibition volume over time.

Additionally, a blood imbibition test was also performed in order to demonstrate possibility as LFI device. Here, one of the tested synthetic papers for the dyed water imbibition was used for the blood imbibition test. As shown in Figure 4.2B, 30 \( \mu \text{l} \) of blood was placed onto the one edge of the paper by a pipette (VWR, USA) and the blood filling was recorded at 25 fps using the digital camera.

All the data of liquid filling was analyzed in Matlab using a threshold algorithm for one image per second of the recorded movie.
Figure 4.2 Schematic views of capillary imbition tests of dyed water and blood. A) A Schematic view of the dyed water imbition test. The tested synthetic papers were manually placed to the liquid meniscus. B) A Schematic view of the blood imbition test. Blood was placed at one end by using a pipette.
Chapter 5

Result and Discussion

This chapter describes and discusses the experimental results. The first part describes and discusses results of the experiment to evaluate the fabrication of interlocked pillar arrays and the limitations. The second part describes and discusses results of the experiment to demonstrate a function of the synthetic paper as capillary pump.

5.1 Evaluation of fabrication range

5.1.1 Result of Pillar fabrication

In this subsection, experimental results to evaluate fabrication and its limitations of vertical and interlocked pillar forests are summarized. Experimental results of 150 µm height pillar fabrication are plotted in Figure 5.1.1.1A, classifying each fabricated pillar forest into one of the three groups: Non-collapsed, collapsed and over cured, as described in Figure 4.1. Figure 5.1.1.1A also shows a line of the geometrical limitation on the fabrication of vertical pillar forests when the pillar height is 150 µm, as introduced in the section 2.2. The pitch must always be larger than the diameter and thus the region where the pitch is smaller than the diameter is ignored. Examples of SEM pictures of both vertical and interlocked pillar forests are shown in Figure 5.1.1.1B to I. Where the diameter of the pillars was 10 µm and the pitch was 50 and 60 µm, we observed that collapsing interlocked pillar forests spontaneously recovered to the non-collapsing state as the solvent was evaporated. As shown in Figure 5.1.1.1J-L, the gradual self-recovery was observed for 10 µm diameter pillars with 50 µm pitch. Fabrication results obtained by using the mask defined by Table 4.1A are shown in Figure 5.1.1.2. Figure 5.1.1.2 A, B, C and D shows results of heights of 50, 150, 300 and 450 µm. Examples of optical microscope pictures of both vertical and interlocked pillar forests are shown in Figure 5.1.1.2 a1-d3.
Figure 5.1.1.1 Result of pillar forest fabrication of 150 µm height. A) Plots of vertical and interlocked pillar fabrication results. Red triangles imply vertical pillars while blue squares imply interlocked pillars. The solid diagram indicates non-collapse and the hollow diagram indicates collapse. The black circle indicates over-curing, which was observed in the case of interlocked pillar forests. The theoretical geometrical limitation, as defined by Chandra et al. (equation 2.2.6), on the vertical pillar fabrication is drawn as a green line. Overlapping indicates the region where the pitch is smaller than the diameter B-I) SEM images. The scale bar shown in the images is 50 µm. B) Vertical, non-collapsed, (30, 105) = (Diameter, Pitch in µm). C) Vertical, collapsed, (20, 60). D) Vertical, collapsed, (10, 310). E) Interlock, collapsed, (10, 110). F) Interlock, non-collapsed, (50, 250). G) Interlock, over cured, (50, 55). H) Interlock, non-collapsed, (30, 130). I) Interlock, non-collapsed, (10, 30). J-L) Images during pillar self-recovery of 20 µm diameter and 100 µm diameter. J) An image during pillar self-recovery. K) An image during pillar self-recovery 10 seconds after the state in B. L) An image during pillar self-
recovery 20 seconds after the state in B.

Figure 5.1.1.2 Result of pillar forest fabrication of height 50, 150, 300 and 450 μm. All fabrication was conducted using the mask in Table 4.1A. A) Plot of a height of 50 μm and typical images from optical microscope. The interlocked pillar forests of 100 μm diameter could not be fabricated since the height was lower than the diameter. B) Plot of a height of 150 μm and typical images. C) Plot of a height of 300 μm and typical images. D) Plot of a height of 450 μm and typical images.
5.1.2 Discussion

This subsection discusses the experimental result of fabrication tests; especially effects and improvements by using interlocked pillar arrays are discussed compared to vertical pillar arrays.

Based on the result on Figure 5.1.1.1A, the graph in Figure 5.1.2 was divided into several regions in terms of the pillar classification to visualize the improvement of pillar fabrication by using interlocked pillar arrays. For interlocked pillars, pillar forests were successfully fabricated without collapse beyond the limitations of vertical pillars as shown in Figure 5.1.1.1A for height 150 μm and 5.1.1.2A-D for height 50, 150, 300 and 450 μm. This indicates that the interlocked pillars are more resistant to capillary collapse and enable higher height without collapse than the vertical pillars, as shown in the purple region in Figure 5.1.2. Moreover, compared to previous studies in Table 1.5, it was possible to achieve the interlocked pillar forests with higher aspect ratio than vertical pillar forest in previous studies.

Interlocked pillar fabrication is limited by two failure mechanisms. The first limitation by capillary collapse and adhesion is observed when the pitch is over 50 μm for 10 μm diameter, which may be explained by ground collapse [29] caused by adhesive force between the pillars and the bottom surface. Interestingly, for some geometry, it was observed that the interlocked pillar forest collapsed during solvent evaporation and then self-recovered after the evaporation was complete. Initially, the capillary force causes part of the pillar forest to structurally collapse down towards the substrate. Once the solvent is evaporated, the energy stored in the buckled pillars restores the structure to its original non-collapsed geometry. The second limitation was over-curing during photolithography. For example, as shown in Figure 5.1.1.1, interlocked pillars of 50 μm diameter and 55, 60, 65 and 70 μm pitch were over cured whereas the vertical pillars of the same height, diameter and pitch were successfully fabricated. Since the OSTE is exposed to UV at an angle of 60° in four directions, it is suspected that the interlocked pillars are more affected by UV light diffraction than vertical pillars. The occurrence of over-curing depends on the light diffraction and thus, tuning the balance between UV exposure time for photolithography and flood curing will improve the over curing.

The green line on Figure 5.1.1.1A indicates the fabrication limit above which vertical pillars theoretically are not supposed to collapse. In the experiment, however, the fabricated vertical pillar forest also collapsed for conditions above the line, that is, the theory did not always fit our experiments. For instance, pillar forest with 30 μm in the diameter and 60 μm in the pitch was not predicted to collapse but the fabricated pillar forest collapsed. It seems that this discrepancy between theory and reality was due to the influence of the solvent on the geometrical and physical characteristics of the pillars. The Young’s modulus, shown in section 3.1, was measured with cured and dried OSTE40. Experiment shows that when the OSTE40 fragment had been soaked overnight in butylacetate, it revealed 8% weight increase and a decrease in E-modulus of the pillars when exposed to the solvent (Chapter Appendix). The exact Young’s modulus of the pillars during evaporation is difficult to measure; however, the effect of the solvent can be expected to be more severe for small pillar diameters
due to their increase surface to volume ratio. Indeed, as pillar diameter decreased, the discrepancy was larger. This phenomenon was observed in other heights of vertical pillar forest as shown in Figure 5.1.1.2.

Vertical pillar forests with pillar diameter < 20 μm always collapse regardless of pitch. This phenomenon is explained by ground collapse. The limitation due to ground collapse is observed to be between 10 μm and 20 μm in diameter for own experimental conditions.

The obtained result in Figure 5.1.1.1 can be explained in terms of mechanical nature of pillar forest. As shown in Figure 5.1.1C, vertical pillars collapsed by bending [23]. As described in equation 2.2.2, the elastic force induced by bending is proportional to $d^4/h^3$. In contrast, interlocked pillars collapsed by buckling (shown in Figure 5.1.1.1H). The interlocking points partition each pillar in shorter sections, which are more difficult to deform, and which have as section length $L$

$$L = (p - d)/(2 \sin \theta). \quad (5.1.2.1)$$

According to fundamental mechanics, the restoring elastic force by buckling of pillar which are fixed at both ends is proportional to $I/L^2$, where $I$ is area moment of inertia of the cross section of the pillar. Therefore, taking into consideration that $I \propto d^4$ when the cross sectional shape is circular, the elastic force is proportional to $d^4/(p - d)^2$. As described in equation 2.2.1, the capillary force applied to pillar structure is proportional to $d^2/(p - d)$. The three relations between force and parameters can be summarized as:

$$
\begin{align*}
F_{\text{vertical}} & \propto d^4/h^3 \\
F_{\text{interlocked}} & \propto d^4/(p - d)^2 \\
F_{\text{capillary}} & \propto d^2/(p - d).
\end{align*}
(5.1.2.2)
$$

For example, with decreasing interpillar distance, p-d, the capillary force, $F_{\text{capillary}}$, increases linearly; the mechanical strength of vertical pillars against bending, $F_{\text{vertical}}$, remains unaltered; but the critical buckling strength of interlocked pillars, $F_{\text{interlocked}}$, increases quadratically. Consequently, vertical pillar forest collapse with decreasing interpillar distance, whereas interlocked pillar forests become increasingly more collapse resistant. The predicted phenomena by (5.1.2.2) can be applied to obtained results shown in Figure 6.1.
Figure 5.1.2 Plots of vertical and interlocked pillar fabrication results with divided and visualized regions in terms of the classification of pillar fabrication at 150 μm height. Yellow region indicates that both vertical and interlocked pillars were non-collapsed, purple indicates that only the interlocked pillar was non-collapsed, red indicates only the vertical pillar was non-collapsed, and white region indicates that both pillars were collapsed or over cured.
5.2 Capillary imbibition test

5.2.1 Result of capillary imbibition test

In this subsection, result of capillary imbibition tests with synthetic papers for dyed water and blood are summarized.

Successful imbibition of dyed water was observed. The imbibition volume over time was plotted in Figure 5.2.1.1A and B for device with (50, 150, 150) and (50, 100, 150) (= (Diameter, Pitch, Height in μm)), respectively. The error bar was obtained from three devices which were prepared using the same fabrication conditions. The average coefficient of variation (C.V.) of flow rate is 6.43% for device with (50, 150, 150) and 33.71% for that with (50, 100, 150). The synthetic paper of (20, 40, 50) was also tested but the imbibition data was not plotted since liquid flooding caused the experiment to fail.

Figure 5.2.1.2 shows example images of dyed water imbibition into synthetic paper of (50, 100, 150). Figure 5.2.1.2A shows an even liquid front and B shows an uneven liquid front in the same synthetic paper. As similar as the Figure 5.2.1.2, in most of synthetic papers, the liquid front of dyed water imbibition was even in the some parts, while it was uneven in other parts.

Blood successfully flowed into the synthetic paper as shown in Figure 5.2.1.3A. The results of imbibition volume over time by the liquid imbibition are approximately in proportion to the square root of time, as expected by the Washburn equation (see Equation (2.3.2.2)). Figure 5.2.1.3B shows the imbibition volume over time into the synthetic paper. Least mean square regression fitting shows that the blood liquid front propagation scales approximately (R² = 0.99, 0.98, and 0.97, respectively) with t¹/₂, as expected from the Washburn equation, with liquid front velocities of 1.7 mm s¹/₂, 1.8 mm s¹/₂, and 2.0 mm s¹/₂, respectively.
Figure 5.2.1.1 Result of capillary imbibition of dyed water into synthetic paper using three different devices of same diameter, pitch and height. A) Plot of imbibition volume over time by capillary imbibition using synthetic papers of (50, 150, 150) (\(=\) Diameter, Pitch, Height in \(\mu\)m)). B) Plot of imbibition volume over time by capillary imbibition using synthetic papers of (50, 100, 150).

Figure 5.2.1.2 Images of dyed water imbibition into synthetic paper of (50, 100, 150) (\(=\) Diameter, Pitch, Height in \(\mu\)m)). A) A image of the dyed water imbibition with even liquid front at \(t = t_0\). B) A image of the dyed water imbibition with uneven liquid front at \(t = t_0 + 8\) seconds.
Figure 5.2.1.3 Results of blood imbibition tests. A) Images of blood imbibition into the synthetic paper of \((50, 100, 150)\) \(= (\text{Diameter, Pitch, Height in } \mu\text{m})\) at 10 and 20 seconds after the blood application. B) Plot of imbibition volume of blood over time by capillary imbibition using synthetic papers of \((50, 150, 150)\), \((50, 100, 150)\) and \((20, 40, 150)\). Their least mean square regression fittings, assuming Washburn behavior, are also shown as solid line.
5.2.2 Discussion

In this subsection, results of liquid imbibition into synthetic papers are discussed.

The synthetic papers allowed successful imbibition of the dyed water, as shown in Figure 5.2.1.1. The synthetic papers also allowed successful imbibition of blood, similar to that of the dyed water. The filled blood volume over time differed with structural parameters, such as pillar diameter, pitch and height. The flow behavior indicates that the synthetic paper can be used as a capillary pump for biological samples such as blood. The pumping rate makes it a potential alternative to other porous microfluidics, such as filter paper, nitrocellulose or vertical pillar forest.

In the results of the dyed water imbibition, variation of flow speed into synthetic microfluidic papers of (50, 100, 150) (= (Diameter, Pitch, Height in μm)) is larger than that of (50, 150, 150). It can be suspected that the variation difference occurs due to larger variation of fabricated pillar structures and surface wettability in devices of (50, 100, 150) compared with (50, 150, 150). The flow rate of capillary filling depends on geometrical parameters and wettability of microfluidic device, as described in section 2.3. Thus, such structural variation is likely to affect reproducibility of flow rate. The structural variation of pillar geometries and surface wettability probably originates from partial over curing or the remnant of uncured OSTE around pillar after fabrication. As shown in Figure 5.1.2, interlocked pillar fabrication is subject to over curing when its pitch is small. It may occur that synthetic papers of (50, 100 150) contain partial over curing or the remnant in the entire substrate of 5 mm x 50 mm, even though they are not apparent to microscope observation. During photo-grafting surface treatment, the partial over curing or the remnant conceivably induce heterogeneous UV exposure, such as uncontrollable scattering or refraction, hence causing unevenness of hydrophilicity on the surface of the synthetic paper. The effect of the variation seems to appear as the variation of flow speed in liquid imbibition into the synthetic paper as shown in Figure 5.2.1.2. The front meniscus of the liquid flow was not a straight line in some parts of almost all synthetic papers.

The flow rate reproducibility can be improved in future works. As an example of the improvement for the reproducibility, automated production can be applied to fabricate the synthetic paper. Since our process depends on hand making, error occurs with higher probability than in automated production. In addition with the aspect of the production environment, surface treatment not depending on UV initiation could also be used to improve the reproducibility. As mentioned in the previous paragraph, the surface treatment with UV initiation is highly affected by the unsuccessful fabrication, the partial over curing or the remnant, which leads to the irreproducibility.
Chapter 6

Conclusions

In this chapter, this project is summarized and the outlook of the project is presented. The summary includes discussion of the accomplished goals which were listed in the chapter 1 and brief suggestion towards further development of this work. After the summary of this work, an outlook of the present synthetic paper is introduced not only as diagnostic application but also self-assembling or folding material.

6.1 Summary

In this section, the project is summarized. In the thesis report, the novel synthetic microfluidic paper has been presented. The originalities of this work are fabricating interlocking pillar structures and using them in microfluidic applications. Accomplishments of the three goals determined in chapter 1 are summarized.

1. To fabricate OSTE synthetic paper based on interlocked pillar structures
   The fabricated synthetic paper was successfully fabricated with interlocked pillar structure and the whole size was 5 mm x 50 mm. This feature provides the synthetic paper with high resistance to capillary collapse and thus it is possible to fabricate larger height of macroscopic structure compared with vertical pillar forest which had same pillar diameter.

2. To explore available fabrication range in terms of geometries, pillar diameter, pitch (distance from a pillar to another) and height
   The available fabrication range in terms of diameter, pitch and height was extended by interlocked pillar structures compared with vertical pillar structures. The fabrication of the synthetic paper is limited by two events; over curing and ground collapse. The ground-collapsing interlocked pillar structure was recovered by stored energy in the structure on certain parameters.

3. To demonstrate lateral flow of sample liquid, water and blood, into the synthetic paper
   The surface of the interlocked pillar forest was covalently treated with HEMA. The surface modification enabled the synthetic paper to imbibe both dyed water and blood into the structure, hence functioning as a capillary pump. The distribution of liquid flow among similarly fabricated devices was larger in a synthetic paper with smaller pitch. This can be explained by geometrical and wettability variation over the whole device occurring during photolithographic manufacturing and surface modification. As future work, the variation can be improved by applying automated
production instead of hand fabrication. It is likely that surface modification not depending on photo-grafting can also improve the flow reproducibility.

6.2 Outlook

In this subsection, outlook of this synthetic paper is discussed in terms of not only diagnostic application but also another future possible application.

Our synthetic paper was proved as an alternative highly resistant to capillary collapse to conventional microfluidic paper for LFI. In addition to the proved abilities as a capillary pump, the synthetic paper has several potential novelties. First thing is immobilization of antibodies. As we demonstrated, covalent surface modification based on photo grafting can be applied onto the surface of the OSTE. Such ability for surface modification is straightforward to biofunctionalization for lateral flow immunassays. Lafleur et. al. [30] performed photo patterning of biotin on the surface of thiol excessed OSTE and demonstrated streptavidin binding to the functionalized surface by observing the fluorescence. The result indicated the possibility of antibody immobilization to which similar bonding protocol could be applied. Second, the interlocked pillar forest could work as a mechanical filter for micro particles owing to their porous structure. If the above-mentioned functions are successfully integrated, the synthetic paper becomes an autonomous diagnostic system. For example, it could be possible to filter human blood, propagate it to the antibody part and detect an analyte which shows antigenicity.

The interlocked pillar forest developed in this thesis could also find other interesting applications outside microfluidics. One such area is self-assembling and self-folding structures. During this work we have found that the synthetic paper can be shrunken slightly by heating the structures in an oven. Altering of the geometrical dimensions of the structures, such as the cross-sectional shape, diameter, pitch, and tilting angle of pillars, can be expected to modulate the extent of this shrinking phenomena.
Acknowledgement

This master thesis work has been performed in the research environment of the department of Micro and Nanosystems, at KTH Royal Institute of Technology in Stockholm. At the same time, my whole study in Sweden was a part of the double-degree program at KTH and Keio University, Japan. It was, therefore, the competent collaboration and support from not only many different members of the Micro and Nanosystems but also cooperators of Keio university that enabled the successful completion of this project, a few of which shall be mentioned more specifically for their contribution and help:

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Wouter van der Wijngaart, Professor at Micro and Nanosystems, for accepting me to the group and giving a number of opportunities.
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Chapter 7

Appendix – Volume increase of OSTE in butylacetate

The OSTE40 fragment was soaked in butylacetate and kept overnight. After the soaking, the fragment revealed 8% weight increase and a decrease in E-modulus of the pillars when exposed to the solvent.

Table A1. Weight increase before and after butylacetate soaking

<table>
<thead>
<tr>
<th>Before soaking</th>
<th>After soaking</th>
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<tbody>
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<td>0.232 g</td>
<td>0.249 g</td>
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