The Thesis Committee for Kervin Scott Marshall II
Certifies that this is the approved version of the following thesis:

Enabling Scalability of Bio J-FIL Process Using Intermediate Adhesive Layers in Fabricating PEGDA Based Nanocarriers

APPROVED BY
SUPERVISING COMMITTEE:

S.V. Sreenivasan, Supervisor

Li Shi

Enabling Scalability of Bio J-FIL Process Using Intermediate Adhesive Layers in Fabricating PEGDA Based Nanocarriers

by

Kervin Scott Marshall II, B.S.

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Dedication

This work is dedicated to my late Granny, Beverly Anne Hatcher, for first inspiring me to pursue academic achievement and my late Grandmother-in-law Vivian Windell for continuing where my Granny left off. I would also like to dedicate this to my loving parents, Kervin and Kim Marshall, that did not restrain my inquisitive mind, even when it was at their expense, my mother and father-in-law, Lindy and Patti Bull, who have loved and accepted me as if I was their real son, to my friends, especially Cody Weathersby, who have helped to keep me grounded these long years I have been in school, and last but definitely not least, my amazing wife, Tabitha Marshall, without whom I would have not been able to accomplish any of this. Thank you from the bottom of my heart

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I want to express my sincere and heartfelt thanks to my family, who have supported me for what seems like a lifetime of education. Without the love and support of my parents and my family, I would be nowhere near where I am today, not only in my studies but in my life as a whole.

Finally, I would like to acknowledge and thank the National Science Foundation, without whom none of this research would have been possible.

Abstract

Enabling Scalability of Bio J-FIL Process Using Intermediate Adhesive Layers in Fabricating PEGDA Based Nanocarriers

Kervin Scott Marshall II, MSE

The University of Texas at Austin, 2012

Supervisor: S.V. Sreenivasan

The Bio J-FIL process has been demonstrated to be a viable method for manufacturing nanoscale, polymeric drug carriers. The process allows for precise control of the size and shape of the drug carriers. While the original process is sufficient for research scale projects, reliability issues have prevented it from being scalable to levels that could potentially be used for mass-production of the drug carriers.

In this thesis, a detailed root cause analysis has been conducted to determine the cause of the reliability issues limiting the Bio JFIL process. A series of experiments with varying substrate and imprint fluid combinations were conducted to pinpoint the cause of imprint failure in the Bio J-FIL process. Upon determining the cause of failure, an alternative imprint process was investigated that sought to increase the variety of materials used in the process by utilizing an intermediary layer. This process is referred

to in this thesis as the Bio JFIL-*I* process. The results using Bio JFIL-*I* indicated increased reliability over the standard Bio J-FIL process. Further refinement of the Bio JFIL-*I* process could also address additional issues with the Bio J-FIL process unrelated to process reliability. The Bio JFIL-*I* approach presented in this thesis is complementary to other approaches that have been recently pursued in the literature which are discussed in the thesis.

Table of Contents

List of Tables xi

List of Figures xii

Chapter 1: Nanocarriers for Drug Delivery in Medicine 1

1.1 Role of Nanotechnology 1

1.1.1 Nanopatterning Processes 1

1.2 Use of Nanotechnology in Drug Delivery in Medicine 4

1.2.1 Desired Nanocarrier Properties 6

1.2.2 Top-Down Manufacturing Processes for Nanocarriers 7

1.2.2.1 Particle Replication in Non-wetting Template (PRINT) 8

1.2.2.2 Particle Stretching 9

1.2.2.3 J-FIL for Biological Applications (Bio J-FIL) 11

1.3 Scalable Manufacturing Process for Nanocarriers 13

1.3.1 Suitability of Bio J-FIL for Scalability 13

1.3.2 Challenges of Bio J-FIL 14

1.3.3 Proposed Research in This Thesis 15

1.4 Thesis Outline 16

Chapter 2: Failure Analysis Using Aqueous Resist in Bio J-FIL 17

2.1 Evidence of Substrate Effect on Imprint Failure 17

2.1.1 Imprint Test 17

2.1.2 Discussion of Imprint Test Results 18

2.2 Root Cause Analysis of Bio J-FIL Failure Modes 18

2.2.1 Liquid Phase Failure and Testing Methods 19

2.2.2 Solid Phase Failure and Testing Methods 21

2.3 Results of Bio J-FIL on Transpin Using Water Based Resist 21

2.3.1 Contact Angle and Dissolution Behavior 22

2.3.2 Optical Microscopy of Aqueous Resist 22

2.3.3 SEM of Aqueous Resist 23

2.4 Results of Bio J-FIL on Poly(vynil alcohol) Using Water Based Resist 24
2.4.1 Contact Angle and Dissolution Behavior 24
2.4.2 Optical Microscopy of Aqueous Resist 24
2.4.3 SEM of Aqueous Resist 26
2.4.4 Fluorescence Microscopy of Aqueous Resist 26
2.5 Results of Bio J-FIL on Poly(acrylic Acid) Using Water Based Resist 27
2.5.1 Contact Angle and Dissolution Behavior 27
2.5.2 Optical Microscopy of Aqueous Resist 28
2.5.3 SEM of Aqueous Resist 29
2.5.4 FLM of Aqueous Resist 29
2.6 Results of Bio J-FIL on Poly(methyl methacrylate) Using Water Based Resist 29
2.6.1 Contact Angle and Dissolution Behavior 30
2.6.2 Optical Microscopy of Aqueous Resist 30
2.6.3 SEM of Aqueous Resist 31
2.7 Discussion of Results 31

Chapter 3: Intermediate Adhesive Masking Layer for Imprinting Solvent Versatility in Bio J-FIL 33
3.1 Need for Modified Bio J-FIL Process for Water-Based Imprints 33
3.1.1 Proposed Solution (Bio JFIL-*I*) 33
3.2 Layer Thickness Optimization 35
3.2.1 Optical Microscopy Data of Aqueous Resist Over PMMA 36
3.3 Imprint Results 38
3.3.1 Optical Microscopy Result 39
3.3.2 SEM Result 40
3.4 Etch Selectivity 40
3.5 Water-Based Particle Release Demonstration 41
3.5.1 Etch of RLT and Underlying PMMA Layer 41
3.5.2 SEM of Released PEGDA Nanocarriers 42
3.5.3 FLM of Released PEGDA Nanocarriers 43
3.5 Results and Conclusion 44

Chapter 4: Conclusions and Future Work.....46
4.1 Conclusion 46
4.2 FUTURE WORK.....46
4.2.1 Backside Etch Process for Bio JFIL-*I*.....47
4.2.2 Challenges of Back-Side Etch Process 49
4.2.3 Benefits of Back-Side Etch Process.....50
References.....51

List of Tables

Table 2-1: Imprint run results with varying substrate and solvent 18

Table 2-2: Contact angle measurement (in degrees) over various substrates compared to fused silica with release monolayer. 20

Table 3-1: Preliminary Drop Dispense Results of DI Water Over Substrates Masked with Varying Molecular Weight PMMA 35

List of Figures

Figure 1-1: Basic Photolithography Process (see Section 1.1.1 for details on process) 2

Figure 1-2: Figure of the Jet and FlashTM imprint process (see Section 1.1.1 for details on process).....4

Figure 1-3: Particle Replication In Nonwetting Templates process flow (see Section 1.2.2.1 for details on process) (DeSimone Group) 8

Figure 1-4: Various shapes produced using the PRINT Method (Roland 2005).....9

Figure 1-5: Particle stretching process flow (see Section 1.2.2.2 for details on process) (Champion 2007).....10

Figure 1-6: SEM of particles formed by particle stretching (Scale Bars: 2 μm) (Champion 2007) 11

Figure 1-7: Bio J-FIL process flow (see Section 1.2.2.3 for details on process)...12

Figure 1-8: Particles produced using the Bio J-FIL process (Glangchai, 2008)....12

Figure 2-1: Optical microscopy of imprint region (containing cylinders with diameter of 150nm not seen in the micrograph) over Transpin using PEGDA-water based resist. 22

Figure 2-2: Top down SEM image of imprinted cylinders with diameter of 150nm23 over Transpin using PEGDA-water based resist. 23

Figure 2-3: Cross-section SEM image of imprinted cylinders with diameter of 150nm over Transpin (2nm) using PEGDA-water based resist.....23

Figure 2-4: Optical microscopy image showing peel-off (yellow boxes in image) at original drop dispensed area of first PEGDA water based imprint on PVA.....25

Figure 2-5: Optical microscopy image showing peel-off (yellow boxes in image) at original drop dispensed area of 5th PEGDA water based imprint on PVA.....25
Figure 2-6: SEM image showing stretching of imprint field of cylinders with diameter of 150nm over PVA using PEGDA-water based resist. 26
Figure 2-7: FLM of PEGDA water-based imprint over PVA.....27
Figure 2-8: Optical microscopy image showing peel-off (yellow boxes in image) at original drop dispensed area of first PEGDA water based imprint on PAA.....28
Figure 2-9: SEM image showing stretching due to insufficient adhesion of imprint field of cylinders with diameter of 150nm and height of <80nm over PAA using PEGDA-water based resist.....29
Figure 2-10: Optical microscopy of imprint field over PMMA using PEGDA-water based resist. 30
Figure 2-11: Cross-section SEM image of imprinted cylinders with diameter of 100nm and height of <80nm over PMMA using PEGDA-water based resist. 31
Figure 3-1: Bio JFIL-*I* Process Illustration showing the Intermediate Adhesive Masking Layer (see Section 3.1.1 for details on steps A~E).....34
Figure 3-2: Water dispense location on 495kDa PMMA Masked PVA demonstrating partial dissolution of the substrate. 36
Figure 3-3: Water dispense location on 950kDa PMMA masked PVA. Drop was dispensed to the right of the physical mark and the wafer. No dissolution evident upon physical inspection. 37

Figure 3-4: Profilometer Measurement of Material Thickness Variation of Drop Dispense Area, 495kDa PMMA Masked PVA.....37
Figure 3-5: Profilometer Measurement of Material Thickness Variation of Drop Dispense Area, 950kDa PMMA Masked PVA.....38
Figure 3-6: Optical Microscopy Image of Corner of Imprint on PMMA/PVA Dual Layer 39
Figure 3-7: Optical Microscopy Image of Drop Merge Area on PMMA/PVA Dual Layer 39
Figure 3-8: Cross-Section SEM of Imprints on PMMA Masked PVA Layer.....40
Figure 3-9: Cross-section SEM of 120nm diameter PEGDA pillars on PMMA/PVA dual layer prior to etching. 42
Figure 3-10: Cross-section SEM of 120nm diameter PEGDA pillars on PMMA/PVA dual layer post etching. 42
Figure 3-11: SEM of Particles Released From PMMA/PVA Dual Layer.....43
Figure 3-12: FLM of Particles Released From PMMA/PVA Dual Layer (select particles highlighted). 44
Figure 4-1: Back-Side Etch Process flow to improve the Bio JFIL-*I* process (see Section 4.2.1 for details on steps A ~ I).....48

Chapter 1: Nanocarriers for Drug Delivery in Medicine

1.1 Role of Nanotechnology

Nanotechnology generally refers to the understanding and manipulation of materials at the nanoscale that results in structures, devices, and systems with unique properties enabled by the nano-scale. By controlling matter on such a small scale, engineers and scientists are exploring the ability to greatly increase the effectiveness of past technologies, as well as develop new technologies in areas such as electronics, healthcare, security, and energy.

The effect of nanotechnology on society is most easily seen in the semiconductor industry. The industry has long been observed to follow what is known as Moore's Law, which states that the transistor density of integrated circuits doubles roughly every two years. This has led to not only increases in stationary computing power that have allowed for supercomputers capable of simulating processes as complicated as global weather patterns and nuclear weapons test, but also the ability to pack more computing power into smaller chips has led to mobile computing and communication technologies that were once considered farfetched science fiction. In the semiconductor manufacturing process, a key processing step in micro- and nano-patterning that allows a substrate (say silicon) to have regions that are masked to allow for the selective doping and deposition of materials on the substrate needed to form a functional electronic device. The patterning process is described next.

1.1.1 Nanopatterning Processes

The precise patterning and structuring of different materials is of key importance in many nanotechnology applications. High speed patterning processes include photolithography which is the dominant commercial patterning approach today.

Nanoimprint lithography has demonstrated great potential to provide a high throughput nanopatterning solution with sub-10nm resolution and is being explored for a variety of sub-100nm manufacturing applications (Sreenivasan, 2008; Sreenivasan, 2009).

Photolithography has long been used by the semiconductor industry to transfer patterns to materials for device fabrication. In photolithography, a resist material is first placed on a substrate material. This material is then selectively masked and exposed using a series of lenses and suitable wavelength light source. Depending on the type of resist, the exposed or unexposed resist material is then removed leaving behind a patterned substrate that then goes through further processing. This procedure is repeated several times to form a completed device. Current manufacturing scale photolithography techniques are capable of patterning features as closely spaced as 40nm using 193nm immersion lithography.

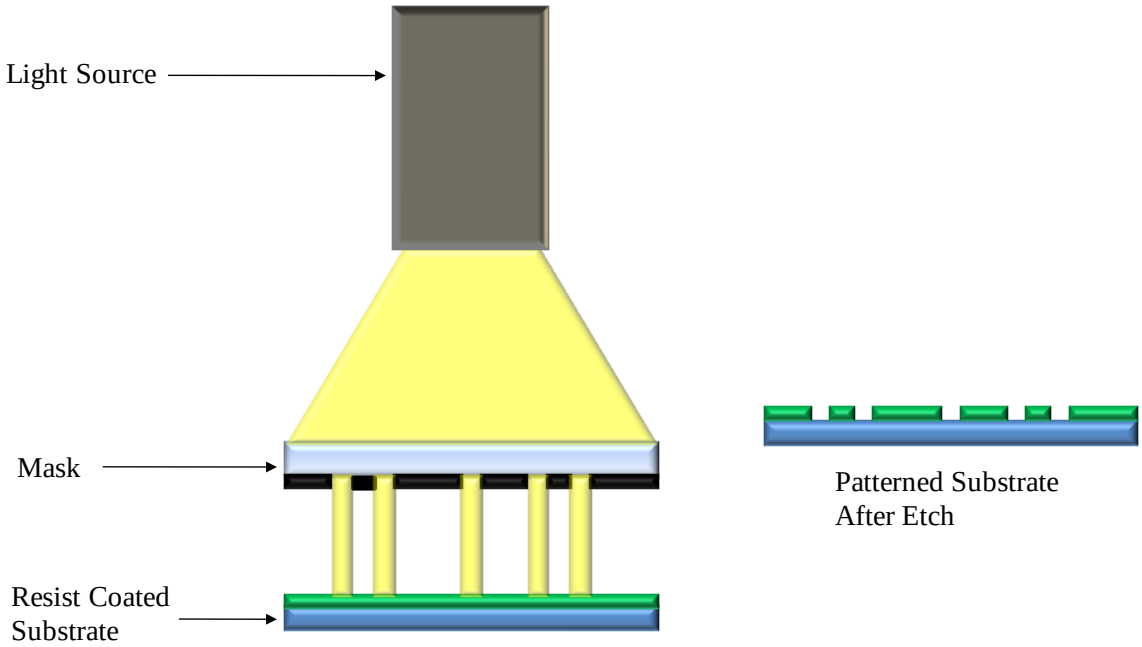

Figure 1-1: Basic Photolithography Process (see Section 1.1.1 for details on process)

Nanoimprint lithography uses proximity patterning techniques to fabricate nanoscale features on a substrate. It is not subject to the same optical constraints as photolithography, though it requires a 1:1 patterned template that can be costly to produce (ITRS, 2009). One of the more commercially successful nanoimprint technologies is Jet and Flash™ Imprint Lithography (J-FIL™). In the J-FIL process first, a low viscosity, UV curable monomer solution is dispensed on a substrate coated with a specialized adhesion layer using an inkjet that allows for selective placement of drops, reducing waste and allowing for variable density patterns. A fused silica template with nanoscale features patterned is then brought into contact with the still liquid monomer. The low viscosity of the monomer allows the template features to be filled without the need for high pressure or high temperature associated with other imprint lithography techniques. This process also prevents direct solid contact between the template and the substrate. The monomer is polymerized upon exposure to UV light, successfully imprinting the template pattern in the polymer. The template is then removed leaving behind the imprinted substrate. Figure 1-1 shows the J-FIL™ process steps. J-FIL™, along with other imprint lithography techniques, has been included in the International Technology Roadmap for Semiconductors as a candidate for lithography in the sub-22nm semiconductor generation and is currently being explored by the hard disk industry to create patterned media with densities of >1 TB/in^2.

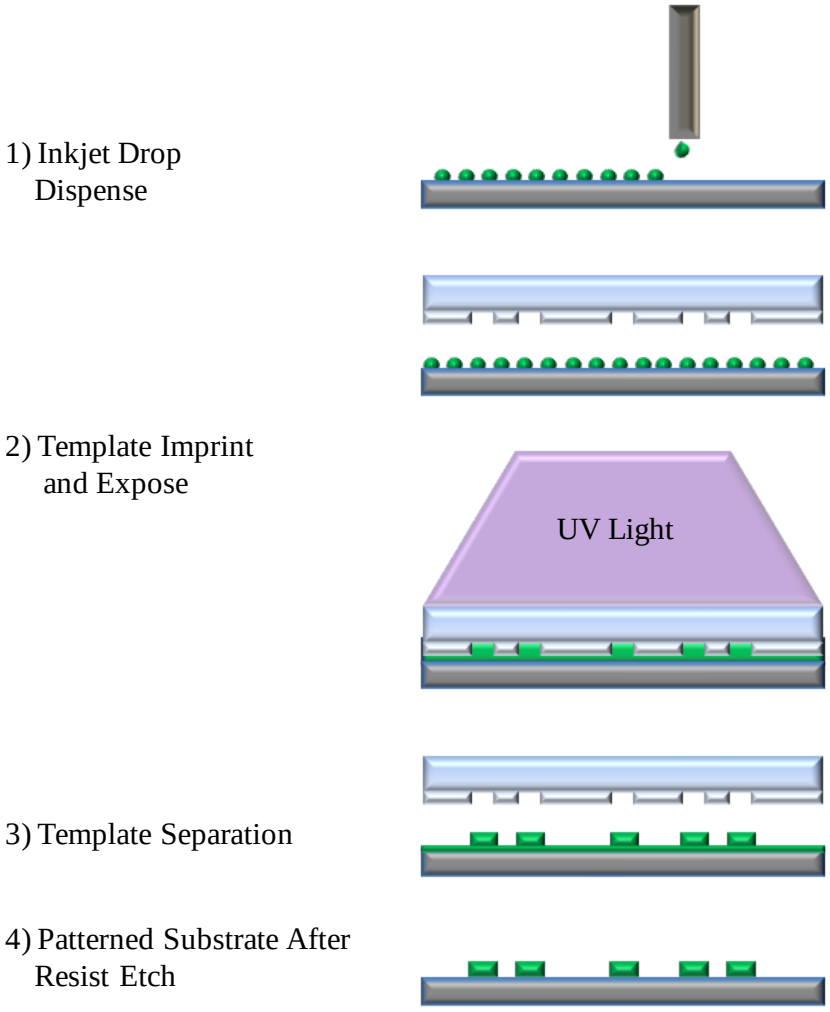

Figure 1-2: Figure of the Jet and FlashTM imprint process

(see Section 1.1.1 for details on process)

1.2 USE OF NANOTECHNOLOGY IN DRUG DELIVERY IN MEDICINE

One of the most widely researched areas in modern medicine involves the search for effective treatment of cancerous growths. The term cancer refers to the uncontrollable growth of abnormal cells that are detrimental to the healthy tissues of the body.

Environmental conditions and hereditary genetics can increase the chances of an individual developing cancer. Cancer is generally treated using the following methods:

- Surgery to physically remove the cancerous growth.
- Radiation therapy to target and kill the cancerous cells using ionizing radiation.
- Chemotherapy to kill cancer cells using pharmaceutical drugs.

These treatments, while proven to be effective in some cases, have issues that limit their effectiveness. Removal of the cancerous growth via surgery requires that the tumor be in area that is surgically operable and does not address the issue of the cancer spreading throughout the body through metastasis. Radiation therapy also damages nearby healthy cells due to the non-specific targeting of the radiation dose. The damage of healthy tissue can be minimized using proton therapy, but like surgical methods it is most effective for localized tumors. Chemotherapy, like radiation therapy, can kill healthy cells as well due to the imprecise targeting of the drug.

The goal of a successful cancer treatment is to kill or remove cancerous cells with minimal damage to healthy tissue (Brannon-Peppas, 2004). In chemotherapy this is typically done by targeting the fast division that is characteristic of cancer cells. While this method is effective at targeting cancer cells, it also damages fast dividing healthy cells leading to side effects that limit the amount of treatment given and thus the effectiveness of the treatment. Because of this, more specific targeting methods are being researched to limit the effect on healthy cells. This can be accomplished either by developing drugs that target cancer cells more narrowly or by encapsulating the drugs so that the drug is preferentially released at cancer sites. Drug encapsulation is of particular interest since it does not rely on a specific affinity of the encapsulated drug to a particular cancer type, but instead takes advantage of the increased localization of nanoscale

particles at tumor sites, known as enhanced permeability and retention (EPR) effect (Matsumura, 1986).

1.2.1 Desired Nanocarrier Properties

Several factors determine the effectiveness of a nanocarrier for targeted drug delivery, including: size, shape, and material composition (Storm, 1995; Decuzzi, 2008; Miyata, 2002). The size of a particle influences the biodistribution *in vivo*, where with particles greater than 200 nm in size get cleared off in the spleen. The particle size also determines the likelihood that the particle will be internalized by a cell. Because of the way cancerous tissue connects to the body's vasculature, it is highly desirable for nanocarriers to be as near as possible without adhering to the vasculature. Recent studies have shown that high aspect ratio particles exhibit a tumbling behavior that increases their margination within a blood vessel. The material itself should have the following properties:

- It should be biocompatible bio-compatible so it does not damage healthy cells that may ingest the particle.
- It should preferentially release the drug in cancerous cells so it does not damage healthy cells that may ingest the particle.
- It should be capable of loading the active drug in sufficient concentration for effective treatment.

The size and shape of nanocarriers affect both the transport of the particles in the vasculature and the internalization characteristics with respect to cells. Cancer tumors cause leakage of the vasculature allowing particles near the endothelial wall to pass through. Particles that are highly marginating have a greater chance of being at the endothelial wall at the location of a tumor and thus are more likely to be internalized by

the targeted cells due to the EPR effect near the tumor site. A study done by Decuzzi et al. has shown that margination time (the time it takes for a particle to reach the endothelial wall) reaches a maximum, depending on the relative density of the particle to blood, and the margination time can vary sharply for modest changes in radius of spherical particles (Decuzzi, 2005). The shape of a particle also helps determine its margination characteristics. Non-spherical particles exhibit tumbling behavior that tends to increase their margination behavior (Decuzzi, 2008). Studies have also shown that size and shape effect the internalization of nanocarriers by cells (Champion, 2007).

With precise control over the size and shape of the particles, as well as a suitable stimuli responsive bio-compatible material, the nanocarriers can be used to accurately target cancer tissue with minimal effect on healthy tissue within the body.

1.2.2 Top-Down Manufacturing Processes for Nanocarriers

With size and shape potentially playing an important role in the transport to and interaction with cancer cells, nanocarrier manufacturing processes should be capable of producing nanocarriers of uniform size and shape. Several top-down manufacturing methods have demonstrated resolution on the order of nanometers, making them particularly well suited for the manufacturing of nanocarriers of uniform size and shape (Gates, 2005). The next sections will describe a few of these processes reported in literature, which are:

- Particle Replication in Non-wetting Template (PRINT)
- Particle Stretching process
- J-FIL for bio-applications (Bio J-FIL)

1.2.2.1 Particle Replication in Non-wetting Template (PRINT)

The PRINT process, developed by DeSimone and colleagues, was one of the first reported size and shape specific nanocarriers manufacturing methods (Roland, 2005). It is a soft lithography based process that has reported fabrication of sub-300nm monodisperse nanocarriers of polymers such as Poly(ethylene glycol) and Poly(lactic-co-glycolic acid).

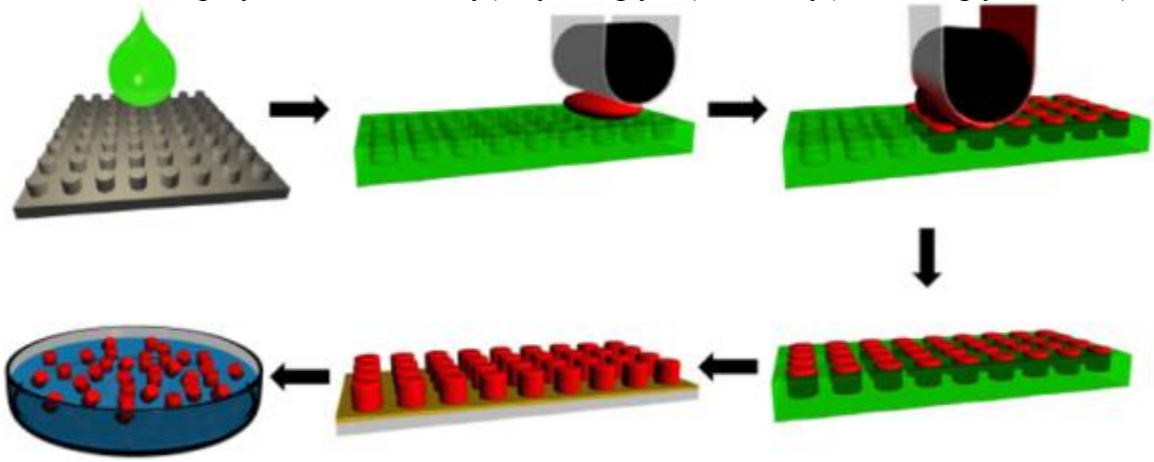

Figure 1-3: Particle Replication In Non-wetting Templates process flow (see Section 1.2.2.1 for details on process) (DeSimone Group)

The first step in the PRINT process involves dispensing a perfluoropolyether dimethacrylate (PFPE-DMA) containing solution on a patterned silicon substrate and UV-cured to form the flexible mold for the nanocarriers, as shown in Fig. 1-3 above. A monomer containing solution is then dispensed on the mold and passed under a roller to distribute the solution throughout the mold. The solution is evenly distributed without an interconnecting residual or flash layer due to the non-wetting nature of the mold. The mold is then processed using different methods (heat treatment, vacuum, UV exposure, etc.) to form the polymer particles. The particles are then removed from the mold using a transfer layer on a second substrate. The particles are then harvested from this film using physical scraping or dissolution of the film. Nanocarriers produced using this method

have been used to study size dependent cellular internalization (Gratton, 2008), as well as the bio-distribution of nanocarriers in laboratory mice (Gratton, 2007). Liquidia Technologies currently employs the PRINT based technique on a roll-to-roll manufacturing platform for the development of therapeutics and vaccines, and is currently conducting FDA Phase 1 clinical trials.

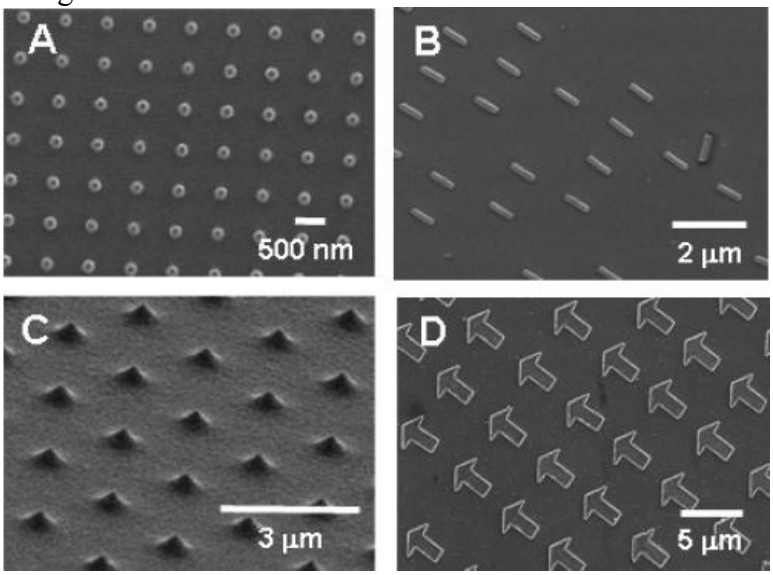

Figure 1-4: Various shapes produced using the PRINT Method (Roland 2005)

1.2.2.2 Particle Stretching

A research group at the University of California, Santa Barbara, led by Samir Mitragotri have used a method for stretching spherical Poly(styrene) (PS) beads into various size and shapes, including ellipsoids, elliptical disk, and barrels(Champion, 2007). The spheres are suspended in a polyvinyl alcohol (PVA) solution and cast into films. These films are then manipulated in two manners in order to form a wide range of particles, as shown in Fig. 1-5. While both processes involve stretching the film, liquefying the PS spheres, and solidifying the final particle, the order in which these steps are done effect the final shape of the particle. The PS spheres are liquefied either by

heating above the glass transition temperature or by use of a solvent. The particles are then solidified by cooling the film or extracting the solvent. After solidification of the stretched particles, the PVA film is dissolved to harvest the particles.

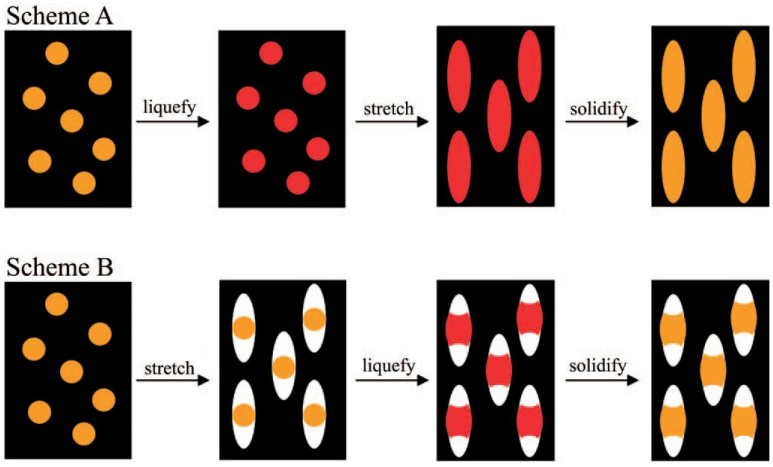

Figure 1-5: Particle stretching process flow (see Section 1.2.2.2 for details on process) (Champion 2007)

The particle stretching method has been used to the shape effect of micron-scale nanoparticles in a synthetic microvascular channel (Doshi, 2010). This method has demonstrated the ability to produce shapes that are difficult or impossible to make with other processes (see Fig. 1-6) which require a physical demolding of the template. The process however, requires exposing the stretched particles to elevated temperatures and this can have a negative impact on the bio-functionality of the loaded drug.

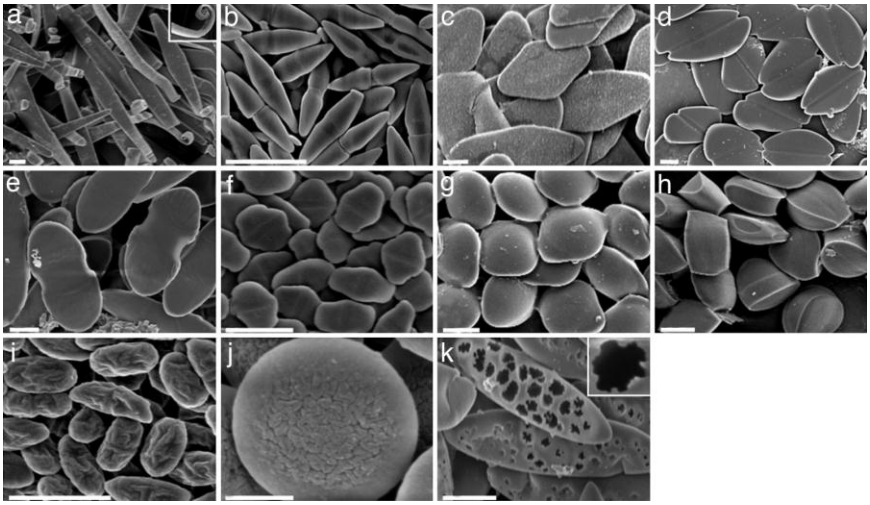

Figure 1-6: SEM of particles formed by particle stretching (Scale Bars: 2 μm) (Champion 2007)

1.2.2.3 J-FIL for Biological Applications (Bio J-FIL)

Drs. Roy and Shi of The University of Texas at Austin have developed a process that takes advantage of J-FIL's ability to accurately pattern sub-50nm features to produce monodispersed, bio-compatible nanocarriers of controllable size and shape. The process differs from the standard J-FIL process in the choice of imprint material and substrate material. The imprint material used is a Poly(ethylene glycol) Diacrylate (PEGDA) based macromer containing a UV reactive photo-initiator and a drug or model drug imaging agent (Glangchai, 2008). To allow for the simple release of the particles after fabrication, a dissolvable sacrificial layer is spun coat on top of a silicon wafer. The macromer is dispensed using an inkjet onto the sacrificial layer. A patterned template is then brought into contact with the solution and UV cured to form cross-linked PEGDA patterned hydrogel imprint. The imprint process leaves a residual layer of PEGDA that interconnects the individual particles. This layer is removed by a brief plasma etch step. The particles are then harvested by dissolving the underlying sacrificial layer with the

appropriate solvent. Figure 1-7 shows a depiction of the Bio J-FIL process and Fig. 1-8 shows nanoscale features imprinted using the J-FIL and a PEGDA based resist

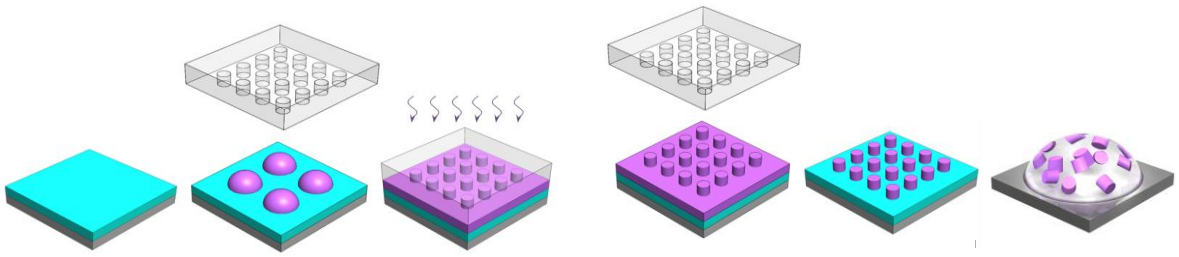

Figure 1-7: Bio J-FIL process flow (see Section 1.2.2.3 for details on process)

The Bio J-FIL process has to manufacture particles for preliminary uptake and bio-distribution studies. One of the key advantages of the process is the ability to use up to 99.9% less bio-material compared to tradition spin or blade coating by selectively dispensing low volume drops using an inkjet dispenser only in the area to be patterned.

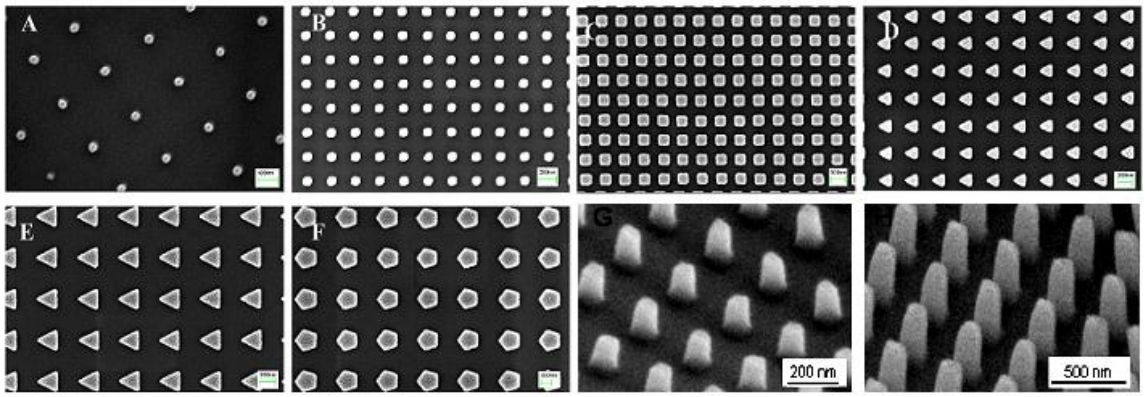

Figure 1-8: Particles produced using the Bio J-FIL process (Glangchai, 2008)

1.3 SCALABLE MANUFACTURING PROCESS FOR NANOCARRIERS

A nano-manufacturing process must possess a few characteristics in order for it to be suitable to be scaled to a mass production level:

- The process must produce repeatable and consistent results.
- The process must be capable of producing nanocarriers at a high volume.
- The process should minimize material consumption and cost.

Because of the effect that size and shape have on a nanocarrier's transport and delivery in the body, it is important that the manufacturing process yield consistent and repeatable results. For mass production, the process should be capable of producing nanocarriers at a high volume. The final characteristic is of particular importance when the cost of cancer drugs is taken into account.

1.3.1 Suitability of Bio J-FIL for Scalability

The scalability of the standard J-FIL process has been studied in depth with respect to semiconductor manufacturing. One of the main advantages of imprint processes is the 1:1 relation between template feature and imprinted feature. Because of this, the mask manufacturing process determines the consistency and repeatability of imprinted features. Template fabrication techniques have demonstrated resolution capabilities for imprint mask as small as 16nm half-pitch (Sasaki, 2009). This resolution capability extends to the Bio J-FIL process and allows for the production of particles that are highly controlled in size and shape.

The newest generation of commercial J-FIL tools are capable of patterning >300 double sided wafers per hour[1]. This throughput is partially dependent on the imprint resist's dispense and filling properties. The imprint material used for Bio J-FIL is non-

[1] http://www.militho.com/products/nutera_hd7000.php

optimized and therefore has sub-optimal dispensed volume of the resist. The volume is much higher (100-400 pL as compared to 1-3 pL for standard J-FIL resists) and it leads to a longer fill time than the standard J-FIL imprint resist. This leads to a drop in wafer throughput. The optimization of drop volume was outside the scope of this research and will be required to achieve high throughput Bio J-FIL.

Perhaps the greatest advantage that Bio J-FIL takes from the standard J-FIL process is the reduced material consumption compared to other methods. By using an inkjet dispense system, imprint material usage can be as little as 0.1% of that used in traditional coating methods (Sreenivasan, 2008). Because of the high cost of drug materials, this translates into significant cost advantages compared to other nanocarrier manufacturing methods.

1.3.2 Challenges of Bio J-FIL

The Bio J-FIL process requires a few key changes to the standard J-FIL process that lead to difficulties in large scale production. Despite the simplicity of the J-FIL process, the process requires materials with a few key properties in order to achieve the level of throughput and consistency required for volume manufacturing. The imprint fluid itself has to be easily and consistently dispensed *via* inkjet, fill the template completely in an acceptably short amount of time, cure under exposure to UV light, and preferentially adhere to the flat substrate surface despite contact over a higher surface area in the patterned template. The adhesion can be further adjusted by the application of a layer that promotes adhesion to the substrate prior to imprinting. While the standard J-FIL process uses an adhesion layer coating on the substrate to promote imprint success, in the Bio J-FIL process the substrate coating's primary purpose is to serve as an easily dissolvable release layer. In standard J-FIL process, the imprint resist is a sacrificial material that is

used to subsequently etch the underlying functional films. The properties of the resist are chosen to ensure high performance during imprinting and demolding, while ensuring that the resist has adequate etch resistance. In Bio J-FIL, the choices of the materials comprising the resist and the underlying release layer are far more restrictive. This is because the material has to satisfy two classes of constraints: (i) The stringent imprinting and demolding requirements for nano-scale patterning; and (ii) The bio-compatibility and the bio-functionality of the resist to ensure that the resist is useful *in-vivo* for therapeutics and/or diagnostics without creating undesirable levels of cytotoxicity.

The fact that the material properties of the imprint solution and substrate coating were determined primarily by bio-functional factors that do not necessarily promote success in the scalability of the J-FIL process, this has led to inconsistent results in the Bio J-FIL process. Even small scale imprint runs of <20 imprints of 100nm features are not possible using the method initially proposed in Figure 1.7 (Glangchai, 2008).

1.3.3 Proposed Research in This Thesis

For the Bio J-FIL process to be scalable, the inconsistent results caused by the use of non-optimized materials must be eliminated. This must be done while still allowing for imprint material flexibility, such as the variation of composition and solvents. The particles should also still remain easily harvestable from the sacrificial substrate. In this thesis, an understanding of the limitations of Bio J-FIL for scalability is established followed by the development of a modified Bio J-FIL process (Bio JFIL-*I*). This process is capable of consistently imprinting >100 imprints. By implementing wafer automation, the process has the potential to be scalable for production level manufacturing.

1.4 THESIS OUTLINE

This rest of the thesis is divided further into three chapters. In Chapter 2 of this thesis, a detailed failure analysis of the current Bio J-FIL process is presented in order to determine the source of inconsistency in imprinting bio-functional resist as earlier reported. Chapter 2 also details experimental tests carried out on four different polymer substrate coatings which demonstrate various levels of imprint success. Fluid properties including contact angle and dissolution behavior have been tested and discussed if failure is occurring in the resist's liquid phase. Qualitative imaging data such as optical microscopy, fluorescence imaging, and scanning electron microscopy, of completed imprint fields have also been collected and presented. Chapter 3 covers the experiments conducted to examine a novel approach of using a thin masking layer, selected from Chapter 2, to allow for the use of water and organic solvents in the imprint solution. This approach, Bio JFIL-*I* increases the process versatility of the Bio J-FIL process in its ability to use various types of resists. Chapter 4 provides conclusions and a discussion on future work which can increase particle shape retention of Bio JFIL-*I* during the etch process using a modified substrate described experimentally in Chapter 3.

Chapter 2: Failure Analysis Using Aqueous Resist in Bio J-FIL

2.1 Evidence of Substrate Effect on Imprint Failure

Three of the main components of the Bio J-FIL process are the imprint material, the substrate coating, and the template. The imprint materials used are made up of PEGDA macromers, UV photoinitiator, drug/model-drug agent, and solvent. Example solvents used in the Bio J-FIL process are de-ionized (DI) water and Dimethyl Sulfoxide (DMSO). For the bulk of studies, the imprint material composition remained the same with only the choice of solvent changing. The templates used are patterned quartz templates that vary only in pattern dimension and density from template to template. The substrate materials used have ranged from Molecular Imprints proprietary Transpin™ adhesion layer to various polymers that are soluble in different solvents for particle harvesting.. To enable scalability, test to determine the most common cause of failure were conducted over various polymer coated substrates with varying resist solvents.

2.1.1 Imprint Test

In order to gauge the scalability of the Bio J-FIL process using various substrates, extended imprint runs were carried out using silicon wafers coated with Poly(vinyl alcohol) (PVA), Poly(acrylic acid) (PAA), and Poly(methyl methacrylate) (PMMA) and benchmarked against wafers coated with Transpin™. Transpin™ was chosen as a bench mark substrate due its strong adhesive properties but is insoluble in solvents and cannot be used as the sacrificial layer. Each coated wafer was then used in an imprint run of performing fifty consecutive imprints with a 5mmx 5mm imprint field using two different imprint solutions, one with DI water as the solvent and the other with DMSO. The same template pattern was used for all imprint trials. Following the completion of the imprint

run, the wafers were examined to determine if the run was successful (all fifty fields imprinted successfully) or unsuccessful (fewer than fifty fields imprinted successfully). The results are summarized in Table 2-1. It was found that the imprints runs were either completely successful or failed in less than 1~5 imprints depending on the substrate coating.

Table 2-1: Imprint run results with varying substrate and solvent

	Solvent	
Substrate Coating	**DI Water**	**DMSO**
Transpin	Successful	Successful
PVA	Unsuccessful	Unsuccessful
PMMA	Successful	Successful
PAA	Unsuccessful	Successful

2.1.2 Discussion of Imprint Test Results

The results of the extended imprint runs on the different substrates seem to indicate that the main cause of failure occurs at the substrate/imprint material interface. Upon examining the template, it was found that the imprint material was sticking to the template after unsuccessful imprint runs. Visual inspection of the imprint fields indicated that the failure is initially localized in one area of the imprint region and expands in subsequent imprints. The successful imprint runs showed no sign of the imprint material adhering to the template. In order to ensure process repeatability, data was collected in order to determine main contributing factor to the preferential adhesion of the imprint material to the template.

2.2 Root Cause Analysis of Bio J-FIL Failure Modes

Failures at the substrate/imprint interface can be caused by a number of factors. The adhesion of the imprint material to the substrate may be low causing the imprint to

preferentially stick to the template. Localized defects may also cause an imprint to fail and "peel-off" in the region surrounding the defect. Higher viscosity and lower wetting behavior of the imprint fluid may also cause incomplete curing in certain areas of the imprint causing, imprints to be unsuccessful. These different failure modes can be classified as either solid phase or liquid phase. A PEGDA water-based solution is used for testing purposes and is presented in later sections.

2.2.1 Liquid Phase Failure and Testing Methods

The imprint material is in the liquid phase for a significant amount of time during the imprint process. Because of this, the characteristics and interactions of uncured imprint material have a large influence on the overall success of the imprint process. Of particular interest are the imprint materials wetting characteristics to the substrate and template, the homogeneity of the imprint material dispensed and subsequent resist drop merging.

The wetting characteristics of the imprint material determine if complete filling of the patterned template region is possible. Filling of the template depends on both the contact angle of the imprint fluid with respect to the substrate as well as with respect to the template. A study conducted by Ki-Don Kim et al. found that using a template with feature width of 1 um, feature aspect ratio of 1, and a resist with a contact angle of 10º with respect to the substrate, the template pattern was completely filled with a resist contact angle of 30º with respect to the template (Kim, 2008). Under the same conditions but with a resist contact angle with respect to the template of 45º, the template was not completely filled. Thus the imprint resist cannot be highly non-wetting to the template, but has to sufficiently wet the template material in order to fill in the template nanoscale

recesses. The imprint resist should also have a low contact angle with respect to the substrate to ensure merging of the dispensed drops.

A Goniometer was used to measure the contact angle of different imprint solutions on the various substrate materials and the template material. Drops of uniform volume were dispensed onto a substrate and the contact angle was measured after 10 seconds, see Table 2-2. Variations of contact angle beyond the acceptable range on a given substrate will indicate that non-merging of the drops may be the cause of imprint failure for a particular imprint solution solvent.

Table 2-2: Contact angle measurement (in degrees) over various substrates compared to fused silica with release monolayer.

Resist	**Transpin**	**PVA**	**PAA**	**PMMA**	**Fused Silica w/ release monolayer**
50% w/v PEGDA400 mw in Water	7.6 ± 0.5	8.5 ± 0.3	10.4 ± 1.2	29.6 ± 2.4	16.6 ± 0.6

UV-curing of the imprint requires the addition of a photo-initiator to the imprint solution. Sufficient distribution of the photo-initiator throughout the imprint fluid pre-exposure is necessary for complete curing of the imprint region. Incomplete curing of the imprint region can lead to local failure during template de-molding and lead to contamination of the template. Subsequent imprints can cause these contamination sites to grow leading to complete imprint failure. However, homogeneity of the imprint fluid should be independent of the substrate material and would not account for substrate dependent failure.

2.2.2 Solid Phase Failure and Testing Methods

Following the UV exposure to cure the imprint resist, the success of the imprint depends on the preferential adhesion of the imprint to the substrate versus the template. Localized defects may cause the adhesion ratio to favor the template and cause the imprint to peel-off of the substrate and stick to the template in that area causing template contamination. These contamination sites can grow in subsequent imprints and can eventually lead to total imprint failure over the entire imprint field.

Optical microscopy images of imprints are collected in order to examine for evidence of localized imprint peel-off that may lead to template contamination. Additionally, Scanning Electron Microscopy (SEM) will be used to look for imprint peel-off regions which are not visible with optical microscopy, as these regions demonstrate poor adhesion of the imprinted material to the underlying substrate.

2.3 Results of Bio J-FIL on Transpin Using Water Based Resist

Transpin^{TM} is Molecular Imprint's proprietary adhesion layer. It has been optimized to increase the selective adhesion ratio of the imprinted material to the substrate versus the template. All Bio J-FIL imprint solutions are benchmarked on Transpin prior to being used on the sacrificial substrates. Using Transpin, Bio J-FIL has been demonstrated to be capable of imprinting all fields over 8" Si wafers. Transpin is insoluble in solvents such as water, DMSO, IPA, etc. and so it is not a viable release layer for the Bio J-FIL process. Transpin contains C=C sites that allow for the chemical bonding of the PEGDA in the Bio J-FIL imprint solution to the substrate. Studying the properties of the imprint solutions with respect to Transpin coated Si substrates gave insight as to what the important parameters are for successful imprinting.

2.3.1 Contact Angle and Dissolution Behavior

The contact angle of the imprint solutions on Transpin is relatively low, < 20º. This low contact angle has been shown to allow for complete filling of the template (Kim, 2008). Complete filling of the template has been confirmed using an overhead camera inside the Imprio® 100D tool at Molecular Imprint, Inc. Transpin was found to be insoluble in the solvents used in the Bio J-FIL imprint solution. This removes the scenario that dissolution of the Transpin layer due to the solvent lead to imprint failures.

2.3.2 Optical Microscopy of Aqueous Resist

Optical microscopy images of water-based Bio J-FIL over Transpin were taken to examine localized imprint peel-off, as shown in Fig. 2-1. Analysis of the images does not show evidence of imprint peel-off. The varying color of the imprint field is due to insufficient volume dispense and can be easily corrected by adjusting the dispense volume.

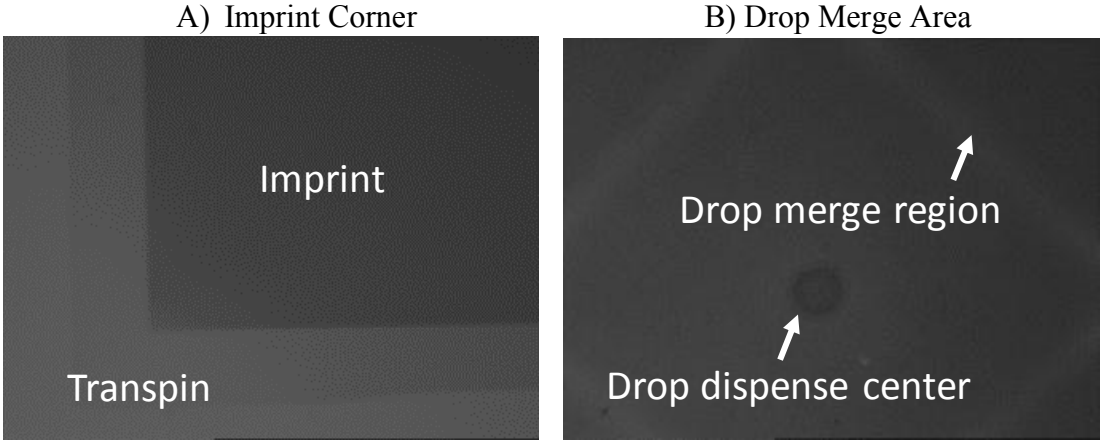

Figure 2-1: Optical microscopy of imprint region (containing cylinders with diameter of 150nm not seen in the micrograph) over Transpin using PEGDA-water based resist.

2.3.3 SEM of Aqueous Resist

SEM imaging of the imprint fields following the imprint process was conducted to look for localized failure of the imprint. No evidence of localized failure was seen in the SEM images (Fig. 2-2). The imprints showed good shape retention of template dimensions with minimal loss of critical dimension (Fig. 2-3).

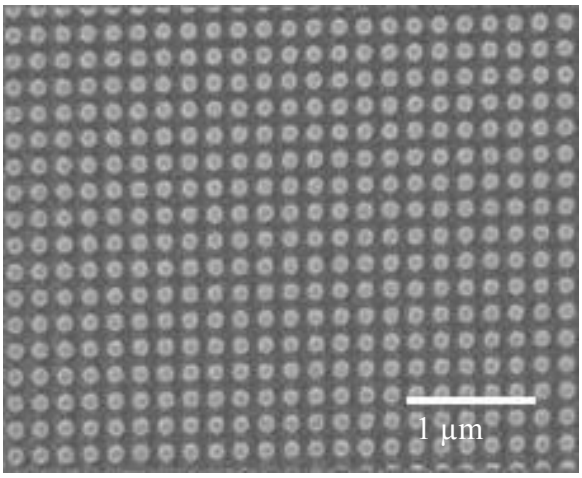

Figure 2-2: Top down SEM image of imprinted cylinders with diameter of 150nm over Transpin using PEGDA-water based resist.

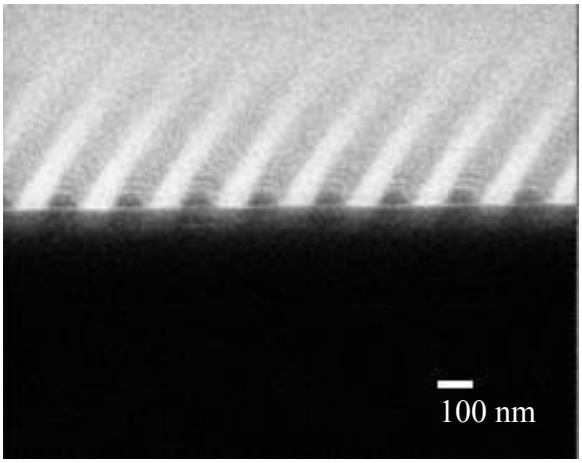

Figure 2-3: Cross-section SEM image of imprinted cylinders with diameter of 150nm over Transpin (2nm) using PEGDA-water based resist.

2.4 Results of Bio J-FIL on Poly(vinyl alcohol) Using Water Based Resist

Poly(vinyl alcohol) (PVA) is a water soluble polymer that was the first reported sacrificial substrate material for the Bio J-FIL process (Glangchai, 2008). PVA does not contain acrylate sites on the polymer chain and thus does not form as strong of a bond with the imprint material as Transpin does. The imprint performance of imprints over PVA was much worse than those done over Tranpsin. The imprint trials over PVA failed with as few as < 5 imprint fields completed. Because of this unreliability, PVA is not a well suited substrate for large scale manufacturing of Bio J-FIL drug particles.

2.4.1 Contact Angle and Dissolution Behavior

The contact angle of the imprint solution (50% PEGDA 400Da in water) on Transpin is very small at 8.5º, see Table 2-2. This low contact angle has been shown to allow for complete filling of the template (Kim, 2008).. PVA is a known water soluble bio-compatible polymer. This solubility may lead to localized defects that can cause complete imprint failure over time. Imaging of the completed imprint fields was completed in order to look for evidence of imprint failure.

2.4.2 Optical Microscopy of Aqueous Resist

Optical microscopy of the imprint fields shows evidence of localized peel-off in the drop merge area of the imprint field. The increased concentration of water in this region appears to lead to increased dissolution of the PVA substrate. Due to the dissolution of the substrate in this area, the imprint material is unable to even form sufficient physical bonds to overcome the adhesion to the template. This localized failure leads to peel-off and contamination of the template, as seen in Figure 2-4. The presence of the imprint material on the template creates bonding sites for the imprint material on the template which leads to material build up on the template and larger occlusions in the

imprint field, as seen in Figure 2-5. Eventually the material build up on the template is sufficient enough that the imprint fails completely.

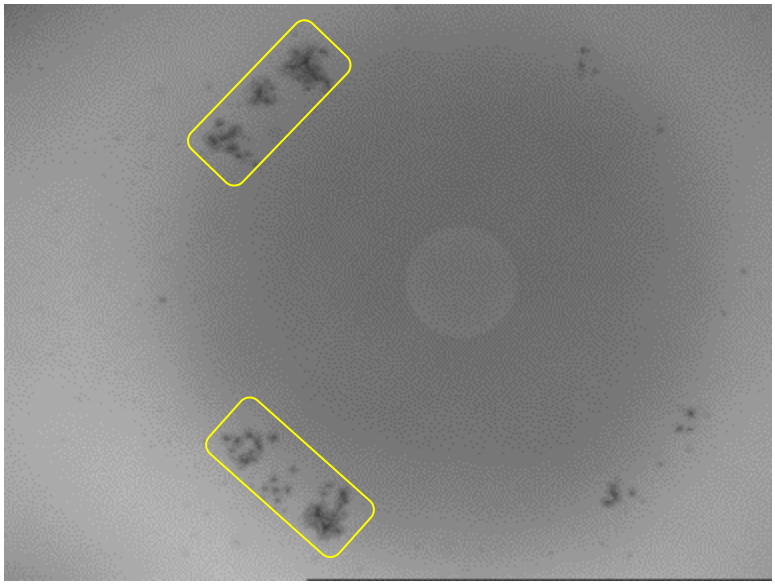

Figure 2-4: Optical microscopy image showing peel-off (yellow boxes in image) at original drop dispensed area of first PEGDA water based imprint on PVA.

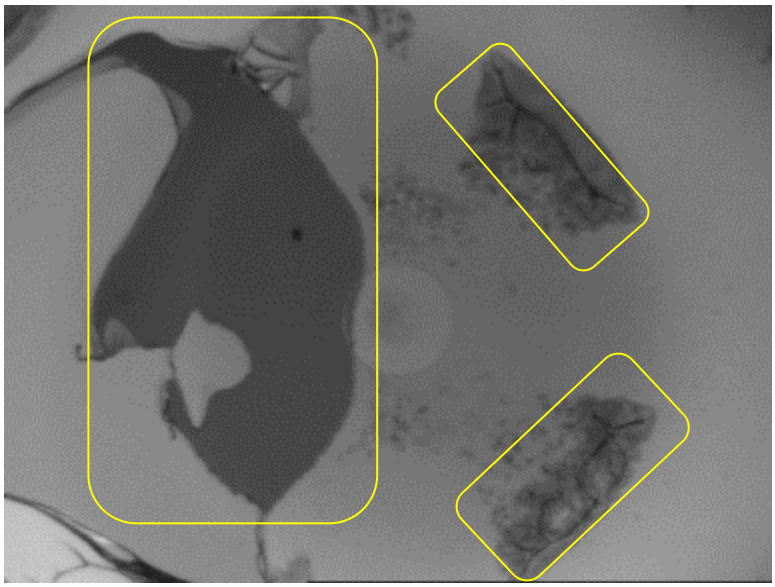

Figure 2-5: Optical microscopy image showing peel-off (yellow boxes in image) at original drop dispensed area of 5th PEGDA water based imprint on PVA.

2.4.3 SEM of Aqueous Resist

SEM images of aqueous based imprints over PVA show peel-off even in regions of the drop merging area inside the imprinted fields. Peel-off in these areas is not significant enough to contaminate the template, but will lead to uneven etching due to the orientation of the particles.

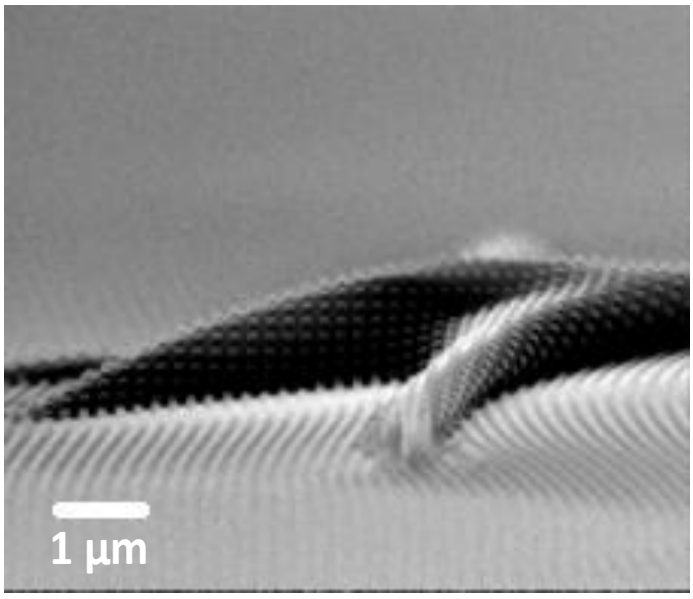

Figure 2-6: SEM image showing stretching of imprint field of cylinders with diameter of 150nm over PVA using PEGDA-water based resist.

2.4.4 Fluorescence Microscopy of Aqueous Resist

Fluorescence Microscopy (FLM) images of aqueous based imprints over PVA was performed using a Zeiss Axiovert microscope with an excitation and emission filter of 480±20nm and 520±20nm, filter set in order to detect the fluorescence agent (Fluorescein-o-Acrylate, FITC). The failed regions of the imprint field due to peel off can be seen as well in the areas without fluorescence, as shown in Fig. 2-7 below.

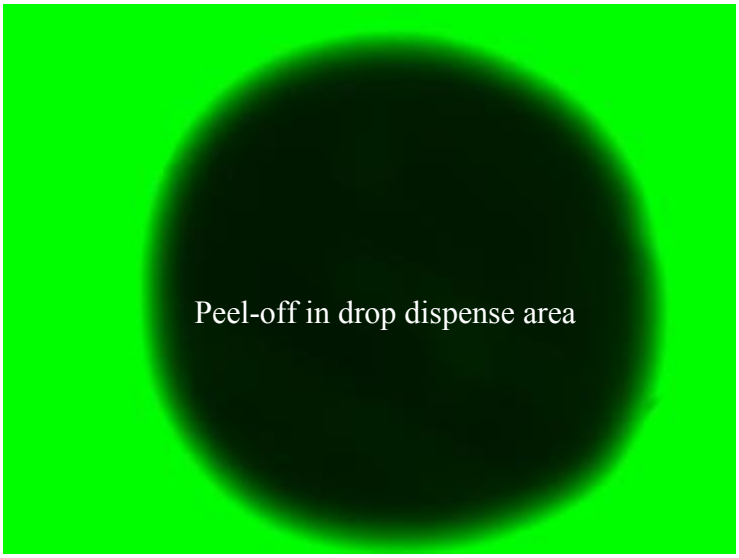

Figure 2-7: FLM of PEGDA water-based imprint over PVA.

2.5 Results of Bio J-FIL on Poly(acrylic Acid) Using Water Based Resist

Poly(acrylic acid) (PAA) is a water soluble polymer that was investigated due to its insolubility in DMSO. PAA contains ester sites on the polymer chain which allows strong bonding of the substrate to the imprint. However, the imprint performance of aqueous resist over PAA was as worse, if not more, than imprints performed over PVA potentially to its higher solubility in water. Because of this unreliability, bare PAA is not a well suited substrate for large scale manufacturing of Bio J-FIL drug particles using aqueous resists.

2.5.1 Contact Angle and Dissolution Behavior

The contact angle of the imprint solution (50% PEGDA 400Da in water) on PAA is very small at 10.4º. This low contact angle has been shown to allow for complete filling of the template (Kim, 2008). PAA is highly soluble in water. This solubility may lead to localized defects that can cause complete imprint failure over time. Imaging of the completed imprint fields was completed in order to look for evidence of imprint failure.

2.5.2 Optical Microscopy of Aqueous Resist

Optical microscopy of the imprint fields shows evidence of localized peel-off in the drop merge area of the imprint field. The high concentration of water in this region appears to lead to increased dissolution of the PAA substrate. Similar to the PVA substrate, the imprint material is unable to form a physical bond sufficient enough to overcome the adhesion to the template in case of PAA as well. This localized failure leads to peel-off and contamination of the template, as shown in Fig. 2-8. The presence of the imprint material on the template creates bonding sites for the imprint material on the template which leads to material build up on the template. Eventually the material build up on the template is sufficient enough that the imprint fails over the entire imprint field.

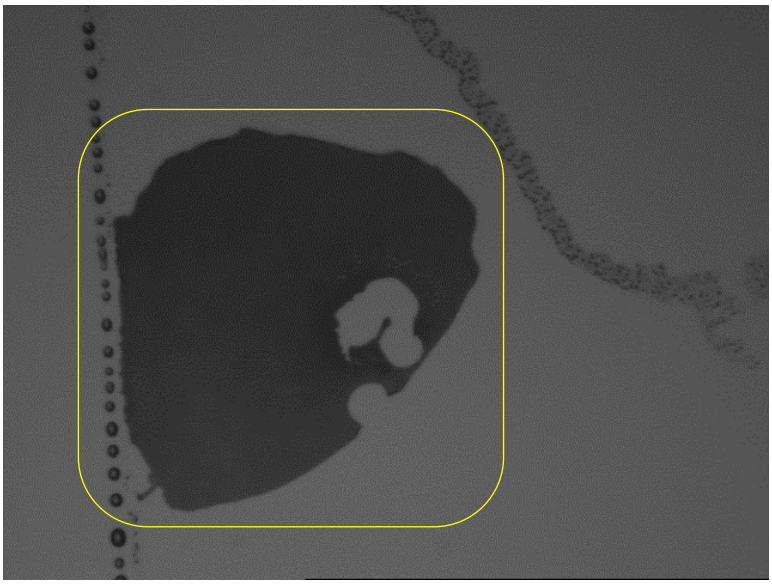

Figure 2-8: Optical microscopy image showing peel-off (yellow boxes in image) at original drop dispensed area of first PEGDA water based imprint on PAA.

2.5.3 SEM of Aqueous Resist

SEM images of aqueous based imprints over PAA show peel-off even in regions in the drop dispensed area of the imprint field. Stretching and cohesive failure of the imprint is also evident in the drop merged area, as shown in Fig. 2-9.

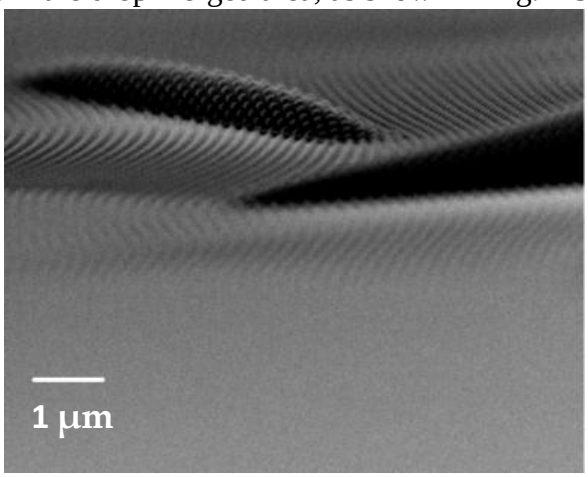

Figure 2-9: SEM image showing stretching due to insufficient adhesion of imprint field of cylinders with diameter of 150nm and height of <80nm over PAA using PEGDA-water based resist.

2.5.4 FLM of Aqueous Resist

FLM Images of aqueous based imprints over PAA show the failed regions of the imprint field due to peel off similar to FLM images of imprint peel off seen over PVA (see Fig. 2-7).

2.6 Results of Bio J-FIL on Poly(methyl methacrylate) Using Water Based Resist

Poly(methyl methacrylate) (PMMA) was investigated as a potential release layer. PMMA contains ester sites which allow for the chemical bonding of the PEGDA in the Bio J-FIL imprint solution to the substrate. Imprints using water-based solutions were done over a PMMA substrate. The imprint performance was comparable to imprints done

over Transpin. PMMA is readily soluble in organic solvents such as Acetone, Toluene, etc. which would allow for harvesting of the particles after etching.

2.6.1 Contact Angle and Dissolution Behavior

The contact angle of the imprint solutions on PMMA is relatively low, 16.6º (see Table 2-2). This low contact angle has been shown to allow for complete filling of the template (Kim, 2008).PMMA has been found to be insoluble in the solvents used in the Bio J-FIL imprint solution, such as water and DMSO. This removes the scenario that reactions of the solvent with substrate are leading to imprint failure or success.

2.6.2 Optical Microscopy of Aqueous Resist

Optical microscopy images of water-based Bio J-FIL over Transpin were taken to examine localized imprint peel-off. Analysis of the images does not show evidence of imprint peel-off.

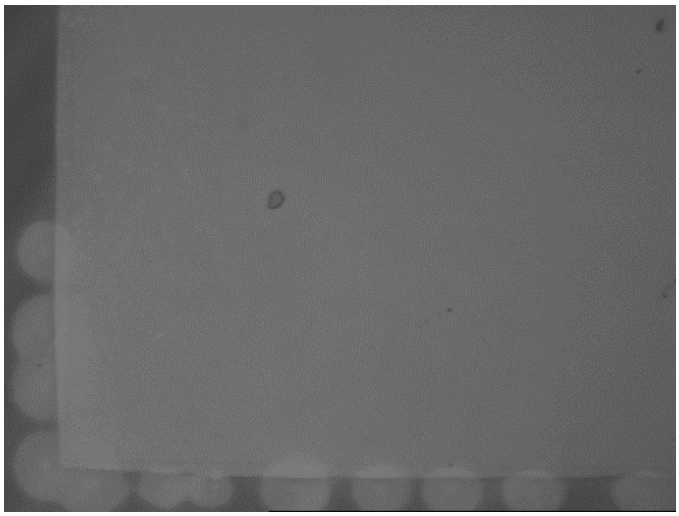

Figure 2-10: Optical microscopy of imprint field over PMMA using PEGDA-water based resist.

2.6.3 SEM of Aqueous Resist

SEM imaging of the imprint fields following the imprint process was conducted to look for localized failure of the imprint. No evidence of localized failure was seen in the SEM images. The imprints showed good shape retention of template dimensions with minimal loss of critical dimension, see Fig. 2-10. Fluorescence imaging showed even distribution of the

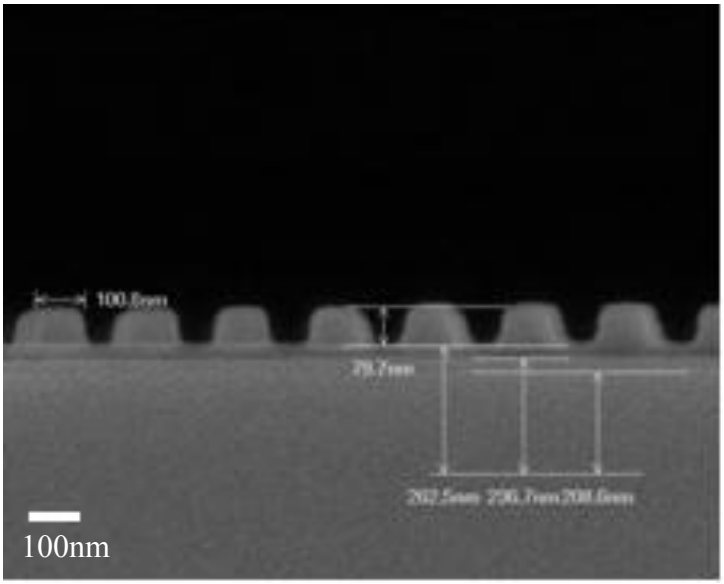

Figure 2-11: Cross-section SEM image of imprinted cylinders with diameter of 100nm and height of <80nm over PMMA using PEGDA-water based resist.

2.7 Discussion of Results

Dissolution of the substrate due to interaction with the imprint solution solvent has been shown to be the primary cause of imprint failure. Transpin and PMMA performed the best in the imprint test using water based solution. Of the two substrate materials, only PMMA is a viable choice for the Bio J-FIL process due to its solubility in organic solvents such as Acetone. However, the use of organic solvents is a concern

when it comes to bio-compatibility due to their recognition as carcinogens, neurotoxins, and reproductive hazards (CDC). Because of their negative effects, the use of organic solvents necessitates additional washing and filtration steps in order to remove residual solvent, which are standard procedures followed in the fabrication of nanoparticles for *in vivo* delivery.

Water based harvesting would eliminate the issue of bio-compatibility and negate the need for additional filtration steps. In trials concurrent with those conducted using water based solutions, imprints using a DMSO based imprint solution were completed over PAA. DMSO was initially chosen for the increased solubility of FITC in the solvent needed for particle imaging in uptake trials. Due to its insolubility in DMSO, PAA provided a viable substrate for the DMSO-based imprints. Imprint runs of over 100 fields have been completed without failure using the DMSO-based solution on PAA. The use of PAA allows for the water harvesting of the imprinted particles and avoids the use of organic solvents.

While PAA provides a solution for DMSO-based imprint solutions, a solution for water-based imprint solutions that avoids the use of organic solvents is still needed. Some drug materials (i.e. siRNA) are insoluble in DMSO and must be dissolved in water. Because of the effect of substrate solubility on the imprint process, a direct conflict exists between the use of water-based imprint solutions and water based harvesting. Altering the solubility of the substrate depending on the particular process step would allow for the use of water in both the imprint solution and particle harvesting. Utilizing materials and equipment already on-hand, a method was developed, referred to as Bio JFIL-*I*, in which an imprint substrate was formed by masking a water soluble sacrificial layer with a thin, water insoluble intermediary PMMA layer. The results of this method are outlined in the following chapter.

Chapter 3: Intermediate Adhesive Masking Layer for Imprinting Solvent Versatility in Bio J-FIL

3.1 Need for Modified Bio J-FIL Process for Water-Based Imprints

As discussed in the previous chapter, compatibility of the substrate material with the imprint fluid solvent is necessary for a reliable process. The use of certain drug and imaging agents requires the use of water as a solvent. PMMA is a compatible substrate for water-based imprints and bio-compatibility of PMMA *in vivo* has been shown (Thomas, 1992). In order to allow for water and DMSO based imprinting and particle harvesting in water, the sacrificial substrate used in the Bio J-FIL process was modified by the addition of a thin water insoluble polymer layer over a water-soluble sacrificial polymer layer. The water insoluble layer acts as a masking layer to prevent the dissolution of the underlying layer. The process, Bio JFIL-*I*, is described and demonstrated in the following sections.

3.1.1 Proposed Solution (Bio JFIL-*I*)

The proposed solution is shown graphically in Fig. 3-1 (A) (E), where in Fig. 3-1 (A), the water soluble sacrificial layer is spun coat on top of a silicon wafer. A thin, insoluble polymer layer is then spun coated on top of the soluble layer, as shown in Fig. 3-1(B). This thin layer acts as a masking layer to prevent or slow the dissolution of the underlying layer to allow for successful imprinting on the substrate. A J-FIL imprint process is then carried out as shown in Fig. 3-1(C). During the etch step, the etch time is extended long enough to allow the insoluble layer to be etched away completely, exposing the water soluble layer beneath (Fig. 3-1 (D)). The particles are then harvested using water without the need for non-aqueous solvents (Fig. 3-1 (E)). A 5%wt. solution of PVA in de-ionized water was prepared and spun coated to give a thickness of approximately 200 nm. A 1%wt. solution of PMMA was prepared in Toluene and

spincoated over PVA to form the masking layer, as Toluene does not dissolve PVA readily at room temperature. PMMA is insoluble in water and provided sufficient masking for the underlying PVA layer in imprint tests. The PMMA layer was spin coated to achieve a thickness of 30nm.

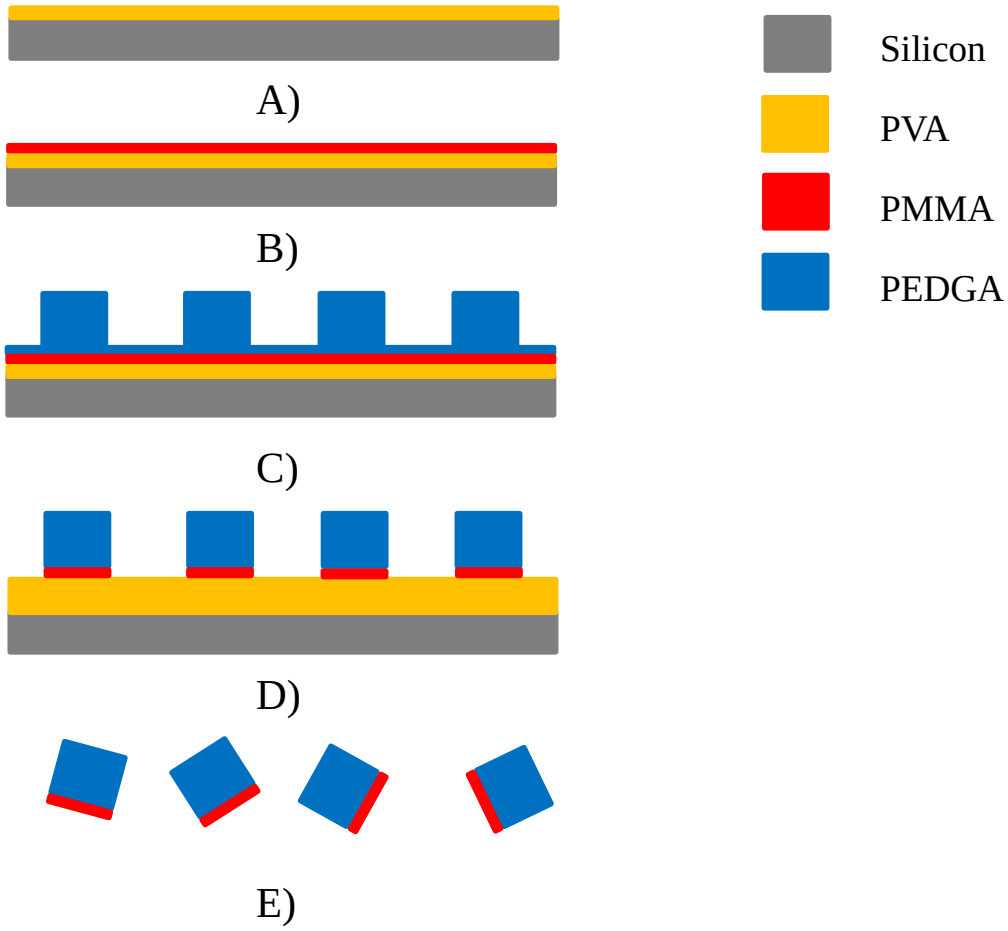

Figure 3-1: Bio JFIL-*I* Process Illustration showing the Intermediate Adhesive Masking Layer (see Section 3.1.1 for details on steps A~E).

In order to harvest isolated nanocarriers, breakthrough etch of the intermediate adhesive masking layer along with the imprint residual layer must be performed. The intermediate adhesive masking layer must be thin enough to where a significant amount

of imprinted material is not etched away. The need to minimize etch time necessitates using as thin of a masking layer as possible, while the need to mask the underlying layer necessitates using as thick of a masking layer as possible. The next section of the chapter will cover the optimization of the layer thickness between the two requirements.

3.2 LAYER THICKNESS OPTIMIZATION

It was first sought to determine the minimum PMMA thickness needed to sufficiently mask the underlying PVA layer. The diffusion rate of water through a PMMA membrane depends on two factors namely i) molecular weight of the PMMA polymer coated, and ii) layer thickness . With respect to PMMA molecular weight, the water diffusion rate through the membrane decreases with increased molecular weight. The diffusion rate of water through the membrane decreases exponentially with decreased layer thickness. In order to confirm this relation, three different molecular weights of PMMA were spun coat on a PVA coated silicon wafer at a thickness of approximately 30nm. Preliminary test were conducted by dispensing 1 μL of de-ionized water on the substrates and allowed to stand for 3 minutes, the average time from dispense to imprint cure in the Bio J-FIL process. At the end of the 3 minutes, the water droplets were blown off using dry compressed air. The substrates were then visually inspected to determine if there was noticeable color change at the drop dispense area which would indicate change in layer thickness. The results are summarized in Table 3-1.

Table 3-1: Preliminary Drop Dispense Results of DI Water Over Substrates Masked with Varying Molecular Weight PMMA

Substrate	Color Change
15kDa PMMA	Severe
495kDa PMMA	Severe
950kDa PMMA	Slight

3.2.1 Optical Microscopy Data of Aqueous Resist Over PMMA

Microscopy images of the drop dispense areas where taken to get a visual indication of substrate dissolution. The microscopy images showed severe discoloration at the drop dispense location on the 15kDa and 495kDa masked PVA, see Fig. 3-2. The discoloration of the 950kDa was imperceptible with microscopy imaging due to lack of contrast between the drop dispense area and the unexposed substrate, as seen in Figure 3-3. In order to confirm dissolution of the substrate, profilometry measurements were taken using a Veeco Dektak 150 stylus profilometer. The profilometry data showed near total dissolution of the PMMA and PVA layers when masked by the thin 495kDa and 15kDa PMMA. However, the profilometry data just indicated substrate swelling in the case of PVA masked with 950kDa PMMA, which was expected and is shown in Fig. 3-5.

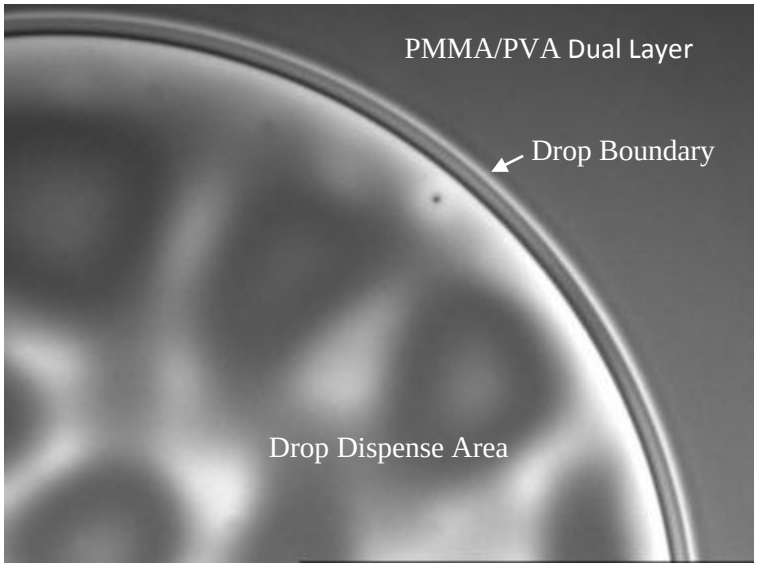

Figure 3-2: Water dispense location on 495kDa PMMA Masked PVA demonstrating partial dissolution of the substrate.

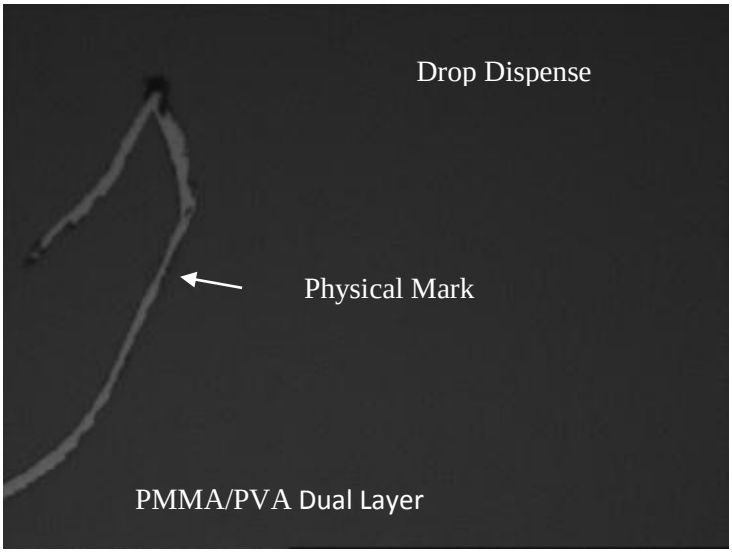

Figure 3-3: Water dispense location on 950kDa PMMA masked PVA. Drop was dispensed to the right of the physical mark and the wafer. No dissolution evident upon physical inspection.

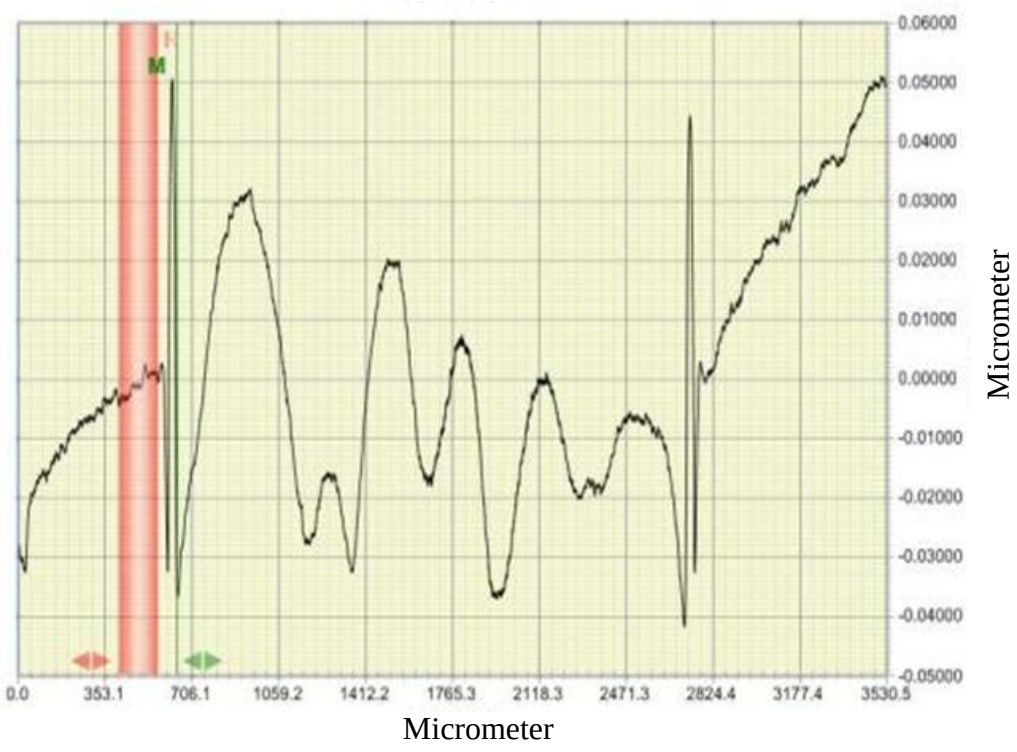

Figure 3-4: Profilometer Measurement of Material Thickness Variation of Drop Dispense Area, 495kDa PMMA Masked PVA

Figure 3-5: Profilometer Measurement of Material Thickness Variation of Drop Dispense Area, 950kDa PMMA Masked PVA

3.3 IMPRINT RESULTS

Following the layer thickness optimization, imprint trial runs were conducted on a substrate layer consisting of approximately 200nm of PVA masked with 30nm of 950kDa PMMA using a water-based imprint solution. As per the procedure outlined in Chapter 2, 50 fields were selected to be imprinted and the success criterion was that all 50 fields imprinted fully. The imprints over the PMMA masked PVA layer performed were comparable to the imprints over regular PMMA with no evidence of peel-off or imprint failure. Metrology of the imprints both before and after etching was conducted in order to confirm imprint performance and show the successful release of particles with encapsulated model imaging agent.

3.3.1 Optical Microscopy Result

Microscopy images of the imprint field showed no evidence of imprint peel off from the substrate. The imprint field was uniform throughout, with no signs of under-or incomplete filling. Imprint peel-off and stretched regions seen earlier in imprints over plain PVA are not seen in the imprints performed over the PMMA masking layer, as shown in Fig. 3-6 and 3-7.

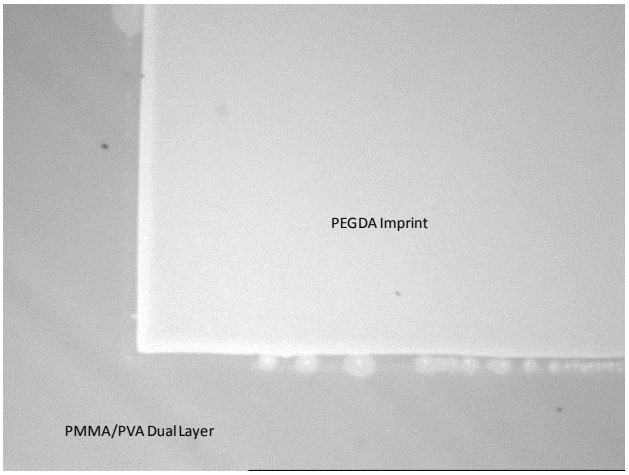

Figure 3-6: Optical Microscopy Image of Corner of Imprint on PMMA/PVA Dual Layer

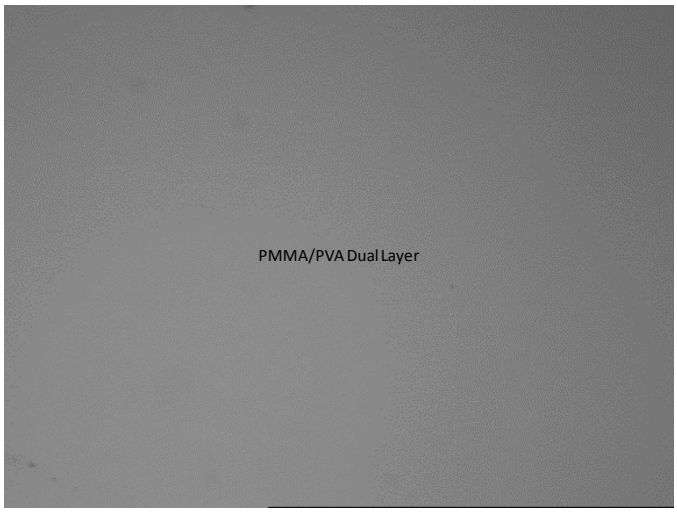

Figure 3-7: Optical Microscopy Image of Drop Merge Area on PMMA/PVA Dual Layer

3.3.2 SEM Result

SEM images confirm the imprint pattern fidelity over the dual layer substrate of PMMA masked PVA. Ellipsometry and SEM data show the PMMA thickness to be approximately 30nm and the PVA thickness to be approximately 400nm.

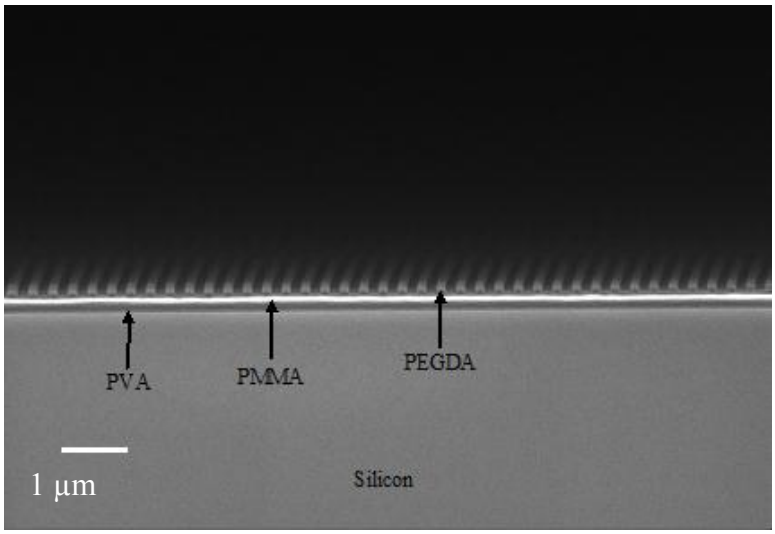

Figure 3-8: Cross-Section SEM of Imprints on PMMA Masked PVA Layer

3.4 ETCH SELECTIVITY

The ability to etch through the PMMA layer while still preserving the imprint material is essential to the Bio JFIL-*I* process feasibility. Additional loss in nanocarrier material can occur after the removal of the residual layer. Because of this, the final dimension of the released nanocarrier depends on the thickness of the PMMA layer and the etch selectivity between the cured PEGDA imprint and the PMMA layer. The minimum thickness of the PMMA layer is determined by the minimum thickness necessary to mask the underlying layer.

The etch selectivity of the imprint material to the masking layer was determined by measuring the etch rate of a flat PEGDA imprint and of a spincoated layer of 950kDa PMMA. Etching was completed using an Oxford Plasmalab 80 plus RIE tool with the

following settings: Argon - 20sccm, Oxygen - 5sccm, Set Pressure - 5 mTorr, RF power - 50W. The etch rate of cross-linked PEGDA was found to be ~24 nm/min. and the etch rate of the spun coated PMMA layer was found to be ~50 nm/min. This gives an etch selectivity rate of the PEGDA imprint to the PMMA layer of about 1:2. With the etch rate in favor of the PMMA layer, the substrate etches at a faster rate than the imprint material and thus more of the nanocarrier geometry and material is preserved. Loss in nanocarrier dimension can also be accounted for in template design.

3.5 WATER-BASED PARTICLE RELEASE DEMONSTRATION

Successful imprinting using a water based imprint resist over the intermediary adhesive masking layer is only the first step in demonstrating Bio JFIL-*I* process feasibility. To complete the process, water-based harvesting of the particles must be demonstrated. The following sections detail the post-imprint processing and present released particle imaging to demonstrate complete Bio JFIL-*I* process feasibility.

3.5.1 Etch of RLT and Underlying PMMA Layer

In order to determine the necessary etch time, cross-sectional SEM was used to determine the thickness of residual and PMMA layers. The pre-etch cross-sectional SEM can be seen in Figure 3-9. Using the etch recipe in Section 3.4 and the pre-determined etch rates and layer thickness, the imprinted wafer was etched to ensure removal of the residual later and the PMMA masking layer. Figure 3-10 shows a cross-sectional SEM of the imprint region following removal of the residual and underlying PMMA layer.

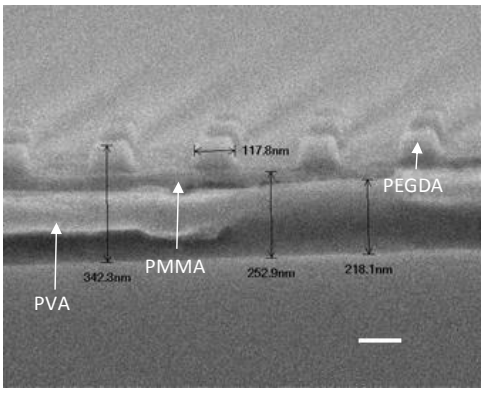

Figure 3-9: Cross-section SEM of 120nm diameter PEGDA pillars on PMMA/PVA dual layer prior to etching.

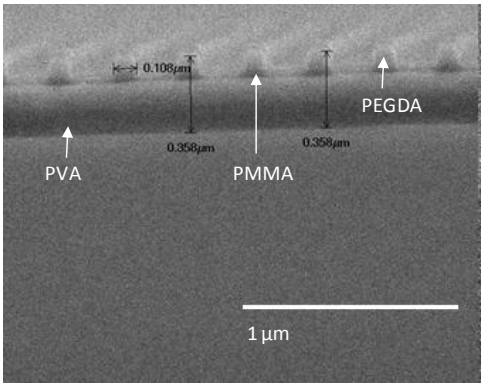

Figure 3-10: Cross-section SEM of 120nm diameter PEGDA pillars on PMMA/PVA dual layer post etching.

3.5.2 SEM of Released PEGDA Nanocarriers

With the removal of the residual and PMMA layers, isolated PEGDA nanocarriers were released using de-ionized water, where 30 μL of filtered de-ionized water was dispensed on the etched imprint field on a silicon wafer. Once completely dry, a 2~3nm gold coating using a sputter coater was performed and top-down SEM images of the released particles was gathered. The irregular spacing of the particles in the SEM images indicates that the particles have been released from the substrate and redistributed across

the wafer upon evaporation of the water. A few of the particles are highlighted in Figure 3-11.

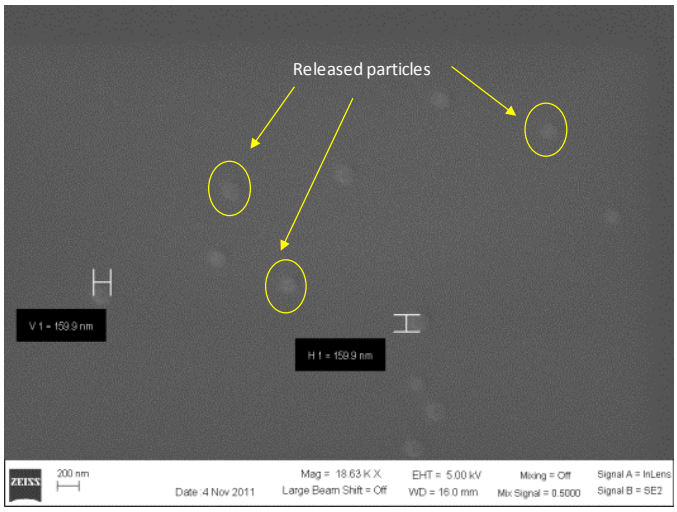

Figure 3-11: SEM of Particles Released From PMMA/PVA Dual Layer

3.5.3 FLM of Released PEGDA Nanocarriers

The SEM image confirms release of PEGDA nanocarriers, but does not necessarily confirm the presence of model imaging agent encapsualted within the nanocarrier. Fluorescence microscopy was used to confirm the presence of the FITC imaging agent in the PEGDA nanocarriers. The particles were released onto a glass slide by dispensing 30 μL of de-ionized water onto the etched imprint and allowed to stand for a brief time to dissolve the underlying PVA. A micro-pipette was then used to gather 4 μL of the water from the wafer and dispensed on a glass slide for imaging. Fluorescence images were captured using a Zeiss Axiovert microscope with an excitation and emission filter of 480±20nm and 520±20nm. Figure 3-12 shows an FLM image indicating encapsulation of model imaging agent, Fluorescein-o-Acrylate, inside released PEGDA nanocarriers..

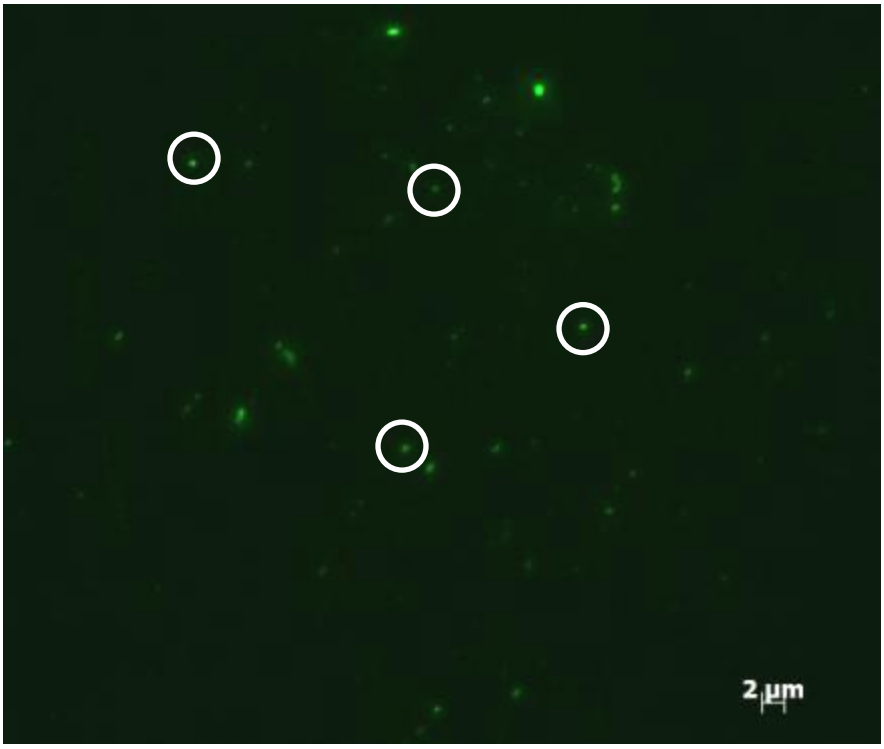

Figure 3-12: FLM of Particles Released From PMMA/PVA Dual Layer (select particles highlighted).

3.5 Results and Conclusion

A new process, Bio JFIL-*I*, for using an intermediate masking layer to allow for both water based imprinting and water based harvesting was investigated. It was found that a substrate layer made up of 30 nm of PMMA over 200 nm of PVA can be successfully imprinted on using a water based solution. The PMMA layer can be removed along with the residual layer during the RIE anisotropic etch step without much geometric or material loss to the PEGDA nanocarrier. This is because of the favorable etch selectivity during anisotropic Ar/O_2 based etching PMMA to cross-linked PEGDA. The isolated PEGDA nanocarriers can then be harvest using water, forgoing the use of

organic solvents during nanocarrier release. The imprint process has been shown to be highly repeatable on the modified PMMA + PVA substrate layer stack.

Chapter 4: Conclusions and Future Work

4.1 CONCLUSION

Prior to this thesis, Bio J-FIL had been proposed as a viable process for the fabrication of size and shape specific polymeric drug carriers. However, when scale-up of this process was attempted in this thesis, it was found that the hydrogel functional materials such as PEGDA patterned on a water soluble release layer such as PVA cause process reliability problems. A detailed analysis of the root cause of these process reliability problems was undertaken here. The findings indicate that the interface between PEGDA and PVA is compromised during the imprint process and this leads to subtle defects in the first few imprints that get aggravated rapidly thereby preventing process scale-up. A refinement of the process materials was next explored leading to the Bio JFIL-*I* process which greatly increased the reliability of the process. It is now possible to scale the process to full wafer imprinting using the process materials described in this thesis. Further potential improvements in the process are described in the following sections.

4.2 FUTURE WORK

In addition to the proposed process in this thesis (use of intermediate PMMA layer), there are other complementary approaches that have been pursued by our research group. This includes creating a switchable water soluble release layer (Agarwal, 2012),

and using a reverse tone process to decouple the imprinting and demolding challenges from the interfacial challenges reported in this work (Singh, 2012). The Bio JFIL-*I* process proposed in this thesis could be further improved as described in sections below.

4.2.1 Backside Etch Process for Bio JFIL-*I*

The etch step is one of the most critical steps in both the Bio J-FIL and Bio JFIL-*I* process. It is required to allow for the release of the particles after fabrication. Incomplete etching leads to large sheets of imprinted material instead of individual particles, substantially decreasing the wafer yield in fabrication of such nanocarriers. Because of this, it is important that the residual layer of the imprint is completely removed during the etch process. The simple solution to this issue is to over-etch the residual layer to ensure that it is completely removed. The over-etching of the residual layer, however, is not an ideal solution to preserve the geometry and bio-functional material composition of the nanocarrier. Due to the chemical isotropic component of the etchant gas, increased exposure of the particle causes the particle to change in dimensions compared to the template shape. Perhaps of more concern is the effect the plasma has on the particle and drug material. Exposure to the highly reactive gas can lead to degradation of the polymer and drug agent (Tao 2011). It is possible to minimize the etch time by minimizing the residual layer of the imprint, but because of the nature of the imprint process, the residual layer is always non-zero.

In an effort to minimize the particles exposure to the etchant gases, the Bio JFIL-*I* process with the adhesive masking layer can be further modified to minimize the exposure of the particle. Figure 4-1 (A)~(I) is a graphical representation of the proposed process. The process follows steps (A)~(C) as depicted in Figure 3-1 of the Bio JFIL-*I* intermediary adhesive masking layer process, as described in Chapter 3. The thickness of

the PVA layer is increased to allow for faster dissolution of the layer. In Fig. 4-1 (A) upon completion of the Bio JFIL-*I* imprint process, a thick layer of polyacrylic acid (PAA) is spun coated on the wafer. The wafer is then adhered to a clean silicon wafer coated with uniform layer of adhesive, as shown in Fig. 4-1 (B) and (C). The wafer "sandwich is then submerged in DMSO to dissolve the PVA layer (Fig. 4-1 (D)) and upon dissolving the PVA layer, the original wafer is allowed to detach such that the imprinted pattern is transferred to the adhesive coated wafer (Fig. 4-1 (E)). The 2nd donor wafer is then put in an RIE tool where the PMMA layer is etched from the planar side along with the non-patterned side of the imprint residual layer, as shown in Figs. 4-1 (F) and (G). After completion of the etch step, the isolated PEGDA nanocarriers embedded in the PAA layer are released by dissolving the PAA layer using water, as shown in Fig. 4-1 (H) and (I).

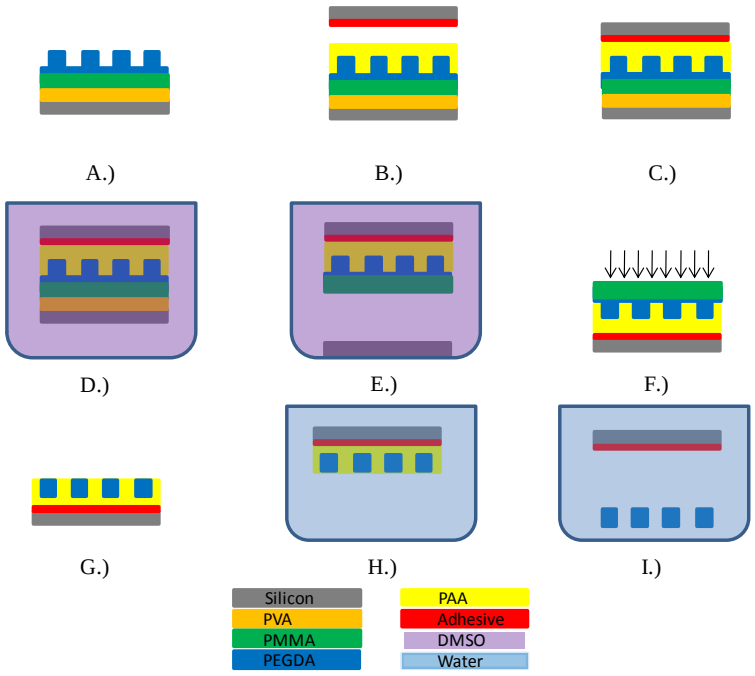

Figure 4-1: Back-Side Etch Process flow to improve the Bio JFIL-*I* process (see Section 4.2.1 for details on steps A ~ I)

4.2.2 Challenges of Back-Side Etch Process

One of the main challenges with the proposed refinement of the Bio JFIL-*I* process is the clean transfer of the imprint from one wafer to a second wafer. Any topographical variation after transferring will lead to uneven etching due to the semi-isotropic nature of the plasma environment. Uneven etching will lead to: i) areas that are over-etched and no longer contain particles, and ii) areas that are under-etched and are a large sheet of interconnected particles as opposed to individual particles. Topographic variations occur because of two main issues: non-planar surfaces at the PAA and adhesive boundary and non-planar adhesion to the secondary wafer due to misalignment of the wafers during adhesion. Spin coating technology can provide an adhesive layer that is sufficiently planar, but the PAA is partially dependent on the imprint topography. A PAA layer that is too thin might be to conformal to the imprint topography and lead to planarization issues. The PAA can be made normally planar by ensuring a thick enough layer is spun over the imprint to ensure that the layer is non-conformal with respect to the imprint topography.

Non-planar adhesion to the secondary wafer is a much more complicated problem because it depends not only on the flatness of the surfaces, but also on planar contact between the layers. Ensuring planar contact in the nanoscale domain can be difficult, however achievable through processes such as chemical mechanical polishing. Another alternative is the use of Molecular Imprints I-1100 and a liquid adhesive to ensure planar contact. The Imprio® 1100 has automatic template and wafer leveling that ensures planar contact at the nanoscale. The Imprio® 1100 uses a thinner fused silica wafer template with a small backing pressure to bow the template at the center which allows for capillary forces to bring the template into planar contact with the substrate. By using a blank quartz

template, the template can be brought into planar contact with the PAA coated imprinted wafer and then release to allow for curing of the adhesive.

4.2.3 Benefits of Back-Side Etch Process

The Bio JFIL-*I* with backside-etch process has a few key benefits over the original Bio J-FIL process. First, utilizing the Bio JFIL-*I* intermediary layer as proposed in Chapter 3, the process is compatible with both DMSO and water-based imprint solutions. Secondly, the PAA layer acts as an isolating layer that prevents the exposure of the PEGDA particles to the plasma environment that might damage the integrity of the particle and drug material. Lastly and perhaps most importantly for current studies, the isolation of the particles in the PAA layer prevents etching of the sidewalls of the particle which leads to greater particle shape retention. Because of these benefits, it is worthwhile to pursue the back-side etch method for its greater reliability in imprint performance, particle shape retention and isolation of particle from the highly reactive etch environment.

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