ABSTRACT

Ngobili, Terrika Adaeze. Characterization of Microprinted Microgels on Functionalized Surfaces. (Under the direction of Dr. Michaele Daniele).

The need to understand and control cell mechanics at a molecular level is ever present. Majority of current regenerative strategies employ the use materials where bulk properties determine tissue interactions. However, in order to precisely control tissue behavior and regenerative properties, materials that allow for interactions on a cellular and molecular level need to be examined. Previous research has also shown the possibility of direct cell patterning however, control of cell proliferation is not possible in these cases. Consequently, bio-inks containing biological polymers and molecules are patterned before cell placement to allow for control of cell adhesion and proliferation. While cell patterning techniques using methods such as stamping or magnetic fluids have been used to control adhesion. The methods fall short in providing either complex features, nano-sized features, or methods conducive to sensitive biomaterials and cells. Contrary to other dispensing methods, ultrasonic dispensing is a simple method to create complex micro-sized patterns with no harmful effects to the bio-inks. Microgels are nano to micro-sized polymer colloidal networks that are currently being examined for their capabilities to be used as a regenerative technique. Microgel nanoparticles have the benefit of being deformable “objects” that have properties that can be modified physically and chemically to tune cellular interactions and respond to external stimuli. When combined with monomers such as acrylic acid and n’n’-methylenbisacrylamide, poly(NIPAM) can be used to create these polymer network microgels. The objective of this study is to characterize ultrasonic printing of poly (NIPAM) microgels inks and their ability to be used in cell patterning techniques.
Ultrasonic printing of poly (NIPAM) microgels was successfully achieved through the incorporation of a viscosity agent. Microgels were successfully patterned on various surfaces that enhanced cell adhesion, increased hydrophobicity, or blocked protein absorption. It was determined that characteristics of pattern features printed using poly (NIPAM) microgels could be specifically controlled using viscosity agents along with surface modifications. Poly (NIPAM) microgel inks have the potential to be used in cellular patterning studies as well as building blocks for larger polymer structures.
© Copyright 2017 by Terrika Adaeze Ngobili

All Rights Reserved
Characterization of Microprinted Microgels on Functionalized Surfaces

by
Terrika Adaeze Ngobili

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Masters of Science

Biomedical Engineering

Raleigh, North Carolina

2017

APPROVED BY:

_______________________________  _______________________________
Dr. Michael A. Daniele            Dr. Ashley C. Brown
Committee Chair

_______________________________
Dr. Matthew B. Fisher
DEDICATION

I would like to dedicate my thesis to mother Terri Elaine Ngobili and my friends who all inspired me to pursue my passions and never give up.
BIOGRAPHY

Terrika Ngobili was born in Atlanta, Georgia on June 7, 1993 to Terri and Alexander Ngobili. At an early age, her desire was to pursue a career in the sciences. After graduating from Chamblee Charter High School, she received her Bachelor of Science in Biological Sciences in 2015. While pursuing her undergraduate degree, she began to research injectable scaffolding materials for breast cancer research. In this environment, she developed a passion for tissue engineering and regenerative medicine, which led her to pursue graduate studies. Under the advisement of Dr. Michael Daniele, she began her graduate studies in the biomedical engineering program at North Carolina State University.
ACKNOWLEDGMENTS

I would acknowledge my advisor Dr. Michael Daniele, who through his mentorship challenged me to be a better researcher and continue to seek knowledge. I also would like to thank my committee member Dr. Ashley Brown for her expertise and guidance in using microgels to pursue regenerative technologies. I would like to express gratitude to my committee member Dr. Matthew Fisher for his patience and willingness to help me understand tissue engineering topics that were very unfamiliar to me. I would also like to thank my teaching advisor Dr. Susan Bernacki for broadening my hands-on experience with tissue engineering techniques. Thank you to my fellow lab students who motivated me to endure through our shared trials and error in research.

I would also like to thank my friends and family for encouraging me thoroughly out this journey. Finally, I would like to specifically thank my mother, who through hard work single handily raised my brother and I. She has inspired and supported me to accomplish any goal I set for myself. She helped me understand that in order to achieve your aspirations in life it requires faith, vision, and determination.
# TABLE OF CONTENTS

LIST OF TABLES....................................................................................................................... vii

LIST OF FIGURES ....................................................................................................................... viii

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

1.1 3-D Printing for Cellular Control....................................................................................... 1

1.2 Cell Patterning & Micro-plotting Techniques................................................................... 3

1.3 Microgel Bio-Inks............................................................................................................... 10

1.4 Objective & Aims............................................................................................................... 12

Chapter 2 Microgel Film Characterization.............................................................................. 14

2.1 Poly(NIPAM) Microgel Ink............................................................................................... 14

2.2 Methods............................................................................................................................ 16

2.2.1 Poly(NIPAM) Microgel Ink Synthesis.......................................................................... 16

2.2.2 Micro-plotting Microgel Inks....................................................................................... 17

2.2.3 Microgel Film Characterization.................................................................................. 19

2.3 Results & Discussion....................................................................................................... 19

Chapter 3 Contact Angle Analysis.......................................................................................... 30

3.1 Variable Surface Chemistries.......................................................................................... 30

3.2 Methods............................................................................................................................ 32

3.3 Results & Discussion....................................................................................................... 33

Chapter 4 Conclusion & Future Directions.......................................................................... 40

REFERENCES.......................................................................................................................... 42
LIST OF TABLES

Table 1: Array of Microgel Inks.................................................................................................................. 18
LIST OF FIGURES

Figure 1: 3-D printed hydrogel osteochondral scaffolds.................................................. 2
Figure 2: Light microscopy images of patterned collagen at 40x magnification............... 4
Figure 3: Light microscopy images of collagen patterns seeded with SMCs............... 4
Figure 4: Schematic of magnetic cell alignment............................................................... 5
Figure 5: Schematic of microcontact stamping................................................................. 6
Figure 6: Human epidermal carcinoma seeded on ligand arrays.................................... 7
Figure 7: Schematic of two inkjet printing methods........................................................... 8
Figure 8: Micropipette used to ultrasonically dispense fluid.......................................... 9
Figure 9: Macrogel vs Microgel polymer structures......................................................... 11
Figure 10: Microgel swelling in response to pH changes............................................... 14
Figure 11: Thermo-responsive changes observed in poly(NIPAM) microgels.............. 15
Figure 12: Illustrating the fabrication of poly-NIPAM microgels...................................... 16
Figure 13: Formation of poly(NIPAM) microgel particles............................................... 17
Figure 14: Printed microgel films on 0.05 monomolar slide ........................................... 20
Figure 15: Printed microgel films on 1% BSA slide......................................................... 21
Figure 16: SEM images of a line array of printed microgels............................................. 22
Figure 17: SEM images of a single dot of printed microgels............................................ 22
Figure 18: Plots of dot radii and line widths for 5% PEG microgels............................... 25
Figure 19: Plots of dot radii and line widths for 10% PEG microgels............................... 26
Figure 20: Plots of dot radii and line widths for 20% PEG microgels............................... 27
Figure 21: AFM image demonstrating microgel with respective film height trace............. 28

Figure 22: Film height characterization line plot.................................................................29

Figure 23: Image representing how contact angle image.....................................................33

Figure 24: Contact angle images for water droplets............................................................34

Figure 25: Bar graphs results of contact angle measurements for water..............................36

Figure 26: Bar graphs of contact angle for 0.02 mg/ml microgel concentrations....................37

Figure 27: Bar graphs of contact angle for 0.2 mg/ml microgel concentrations......................38

Figure 28: Bar graphs of contact angle for 2 mg/ml microgel concentrations.......................39
CHAPTER 1: Introduction

In regenerative medicine, new methodologies to produce scaffolds with precisely controlled parameters and characteristics are constantly being examined. More importantly, the capacity of these scaffold products to have specific or concentrated effects on tissues and even individual cells has become the focus of many research studies. Regenerative medicine aims to create systems with the capacity to restore original functions to tissues. To replace or regenerate cells, it is necessary to control behavior and function at a cellular level. While scaffolding strategies exist to alter cellular function and behavior, most of these methods depend on bulk material properties to predict cellular behavior. The complexity of cellular systems and environments make it difficult to predict how bulk material will exactly affect cellular systems. Recently, cell patterning has emerged as a method to control cellular attachment and proliferation onto surfaces. The ability to arrange cells in specific and complex patterns has applications in drug delivery studies, regenerating tissues, and microfluidic research[1].

1.1 3-D Printing for Cellular Control

One quickly evolving system within tissue engineering is 3-D printing. Bioinks are materials capable of being deposited that support cell viability and proliferation[1]. Using bio-inks and bioactive materials, 3-D scaffoldings constructs can be created to cell growth and mechanics. Originally 3-D printing emerged as a method to create 3-D constructs through additive manufacturing techniques. This new concept quickly outweighed the alternative of subtractive
manufacturing processing in which products are created by removing fragments from bulk material. Additive manufacturing techniques are marked by depositing successive layers of materials onto a surface. In addition, 3-D printing allowed for precise control over products characteristics which could not be obtained with subtractive manufacturing techniques. Transitioning from industrial uses to medical techniques, researchers initially created 3-D printing strategies that successfully constructed 3-D scaffolds from biomaterials. Using computer software, researchers developed pre-modeled structures with specific geometrical parameters, tailored porosity and mechanical properties to mimic tissues, shown in Figure 1 [2-4].

![Figure 1: 3-D printed hydrogel osteochondral scaffolds. Reprinted with permission from Springer [3].](image-url)
The first implanted scaffold derived from 3-D printing methods was developed by researchers at the Wake Forest Institute for Regenerative Medicine. Further research and developments have enabled 3-D bioprinting to become a major source for creating 3-D constructs within tissue engineering [5]. These scaffolds mimic the extracellular matrix and structure of tissues. When implanted into a damaged area, the preformed structures enhance the body’s natural regenerative systems by acting as a template for new tissue growth. The effectiveness of scaffolding materials are highly dependent on the application. Scaffolds can be porous and sometimes combined with cultured cells or various proteins and growth factors postproduction [4, 6]. As innovations progressed, bioactive inks containing live cells or other small materials were developed to create biomimetic structures that can be placed directly into the damaged tissue[4, 7]. While the majority of 3-D printing techniques focus on bulk material properties, controlling cell to cell and cell to material interactions needs be explored on molecular level.

1.2 Cell Patterning & Micro-plotting Techniques

Controlling placement and behavior of single cells can improve overall strategies to aid in the regeneration of tissues. Understanding how cell patterning affects cell behavior and proliferation is also essential to constructing methods of precisely altering tissues. For example, cell patterning along with signal expression can be used to determine the fate of differentiating stem cells [8]. Cell patterning can be achieved either through patterning the cell with a physical barrier, dispensing solutions containing living cells, or by patterning materials that permit preferential cellular adhesion. Direct cell patterning can achieve desired cell control
initially, however without secondary measures unguided cell proliferation will still occur. Patterning of a biomaterials creates methodologies that not only control cell adhesion but control cell proliferation. Extracellular proteins, such as collagen, are commonly used in tissue engineering applications because of their ability to enhance the natural regenerative capacity of tissues. Figure 2 shows collagen substrates patterned into circular and oval shapes, while cells seeded onto those shapes are shown in Figure 3. Currently researchers have been able to successfully pattern cells using collagen as the precursor for creating unique cell patterns [9]. Although these organic materials have an innate capacity to facilitate cellular adhesion, there

![Figure 2: Light microscopy images of patterned collagen at 40x magnification. Reprinted with permission from Elsevier [9].](image1)

![Figure 3: Light microscopy images of collagen patterns seeded with smooth muscle cells; a) 4 days at 200x mag, b) 4 days at 40x mag, c) 4 hours after seeding. Reprinted with permissions from Elsevier [9].](image2)
are still limitations with complexity of cellular control possible. Direct cell patterning methods forgo the use of substrate to allow for cellular attachment. Krebs and Erb employed magnetic fluid and electric forces to guide cells into linear patterns determined by magnetic field directions. The methodology of this technique can be seen in Figure 4. Although straightforward, this approach is limited by weak forces and potential of disrupting electrochemical processes within cells [10]. Laser guided cell patterning uses near-infrared optical forces to guide cells from medium onto a surface. When coupled with hollow fibers cells were guided into and along the distance of the fibers, with no negative affects to cell viability [11].

Photolithography and soft lithography methods are commonly used in the fabrication of microscale objects to create precise patterns. Exposing photo sensitive substrates to light reacts
to create specific patterns within the materials. Photolithography techniques allow for the creation of nanoscale patterns as well as the deposition of variable layers [12] [13]. Subsequent to protein deposition, further sample manipulation is usually required to remove protein attachment to specific surfaces [14]. Some research however has developed techniques which don’t require modifications following the protein absorption. Bhatia et al. used photolithography etching to produce substrates with differential affinities to protein adhesion [15]. Dip-pen nanolithography creates nano-sized patterns through molecular ink
deposition from an atomic force microscope tip. Producing nano arrays of proteins on substrates creates a composite for cell attachment[16]. Nanolithography methods are commonly used to prints dots arrays line features, however not much evidence of printing complex patterns using the method exists.

Contrary to photolithography methods, soft lithography methods do not require the use of a light-sensitive substrate or chemicals that have the potential to harm cells or modify
biomaterials. Furthermore, soft lithography allows for the manipulation of soft polymers frequently used as biomaterials to control cell function, while producing patterns with feature sizes that are smaller than photolithography techniques [17]. Microcontact printing is a form of soft lithography that uses similar stamping techniques to transfer proteins and materials patterns onto a surface. This method is widely used to modify surfaces with the goal of enhancing cell adhesion. In Figure 5, microcontact printing methods have been used in combination with extracellular matrix proteins to create patterns where self-assembling peptides present functional groups that favor cell attachment [18]. Cellular attachment to these ligands are seen in Figure 6. Microprinting techniques have demonstrated how control of cell shape affected cell function and growth [19, 20]. Microcontact printing applies material patterns to a PDMS to be stamped on a surface. Conversely, microfluidic patterning creates channels throughout a PDMS mold that allows biomaterials to flow and be deposited in the channels[21].

Figure 7: Schematic of two inkjet printing methods. Reprinted with permissions from Nature Publishing Group [4].
The development of bio-inks composed of cells, protein, and biomaterials allow for ink jet printing techniques to be used in creating cellular, scaffolding and biosensor constructs. Contrary to previously discussed techniques, this method enables the user to create highly complex structures without the need of a molds or secondary chemicals. Furthermore, inkjet printing has the capacity to print 3-D in layer by layer fashion with inks composed of nanomaterials. There are currently two main types of inkjet printing methods used to deposit fluid visualized in Figure 7. Thermal ink-jet printing uses heat to produce pressures pulses to push inks drops from the nozzle. However, inks containing biological molecules or can are sensitive to temperatures changes, which limits the applications of this techniques[4]. While inkjet printing can be used to print nanomaterials, they are limited in producing nano-sized features smaller than 50 µm. Also by design, inkjet printers are not able to print continuous
lines. Instead, continuous features are produced by creating dots arrays in close proximity to one another [22]. Micro-plotting, a similar technique to ink-jet printing, allows for printing of bio-inks with variable nano-sized features. The Sonoplot GIX microplotter is an instrument that uses ultrasonic methods to dispense fluids from a micropipette needle. Ultrasonic dispensing techniques drive a small ac current along a piezoelectric element. This causes a light vibration throughout an attached micropipette, allowing biological solutions to be wicked onto the surface[23]. Demonstrated in Figure 8, this approach produces a system that allows for continuous printing of bio-inks into complex features at least 1µm in size, while using lesser amounts of fluids. Moreover, this printing methods involved the use of a precision positioning system, that allows fluid deposition on the x, y, and z axes.

1.3 Microgel Bio-inks

In majority of injury cases, the tissues are not uniformly damaged. While 3-D printing does allow for custom constructs to be created, majority of cell to material interactions within these materials are controlled by bulk material properties. To fully control cell interactions to at a microscopic level, microscopic materials must be used. Seeding cells onto deposited biomaterials allows for cellular control using a secondary platform. Popular materials such a self-assembling peptides and extra-cellular matrix proteins, provide sufficient support to allow for cellular attachment[24, 25]. The methods however, only function provide support for cell attachment and fall short in the potential to alter cell functions and behavior. In tissue engineering, nanoscale particles are being using to deliver enzymes, antibodies, and interact
with tissues in regenerative capacity. Nanoscale particles have been used in medicine to target specific tissues and deliver proteins or drugs to these tissues. In many cases, the composition alone of the nanoparticle can affect cellular growth and function[26, 27].

![Figure 9: Macrogel vs Microgel polymer structures. Reprinted with permissions from Springer [28].](image)

Traditionally hydrogel structures have been used to enhance regenerative tissues because of their mechanical similarity to biological tissues[28, 29]. Like hydrogels, microgels are made of polymer networks with mechanical properties that can be tuned by chemical composition and network structures. Contrary to macroscopic hydrogels, microgels dimensions range from nano to micro meters and have the added benefit of being able to interact with tissues on a particle to cellular level[30]. Tuning the mechanically properties of these objects can control cellular interactions, as well as microgel environmental responses [31]. Microgels will swell
in a solvent and respond to various environmental factors such as pH or temperature. For example, temperature responsive properties of microgel suspensions composed of gellan gum have been employed to prevent aggregations and settling within cell laden bio-inks[32].

Poly (N-isopropylacrylamide), or poly(NIPAM) microgels are a class of biodegradable microgel nanoparticles that have the capacity to provide relief in a tissue engineering aspect through delivery of regenerative factors or drugs [33]. Combining poly(NIPAM) with other co-polymers such as n’n’-methylenebisacrylamide and acrylic acid, produces crosslink microgels with varying function. Tissue engineering scaffolding techniques use films and coating to directly affect cell attachment, proliferation and signaling in some cases. 3-D printing of poly(NIPAM) microgels inks will create microgel thin bioactive films capable of being patterned to affect cell proliferation or adhesion [34, 35]. These thin films will serve as methods to precisely control surface behavior of scaffold as well as building blocks to construct whole products.

1.4 Objective & Aims

Controlling scaffolding parameters at a microscopic scale will create new opportunities to produce biomimetic constructs. In this study, we characterized ultrasonic printing printing of poly(NIPAM) microgel particle inks on functionalized surfaces by evaluating surface wetting chemistries, printing resolution, and film height characteristics. We developed bio-inks with microgels ranging in concentrations and viscosity to examine effects on surface contact and
particle clumping. Using ultrasonic printing techniques to pattern bio-inks onto glass substrates with variable surface properties will allow the determination of optimal microgel ink characteristics for cellular control.
CHAPTER 2: Microgel Film Characterization

2.1 Poly(NIPAM) Microgel Ink

Poly(NIPAM) microgels have been reported to have applications in drug delivery, biosensor, and scaffolding areas of tissue engineering. In contrast to hard nanoparticles, microgels possess soft and deformable characteristics that allow for unique particle interaction and adhesion as a bio-ink. Poly(NIPAM) microgels have been extensively examined for their pH-responsive and thermosensitive properties, [36]. Variations in pH and temperature dictate microgel swelling under wetting conditions (observed in Figures 10 and 11), as well as, adhesion properties to proteins and cells[37, 38].

Figure 10: Microgel swelling in response to pH changes. Reprinted with permissions from Springer [33].
Furthermore, these nanoparticles can be mechanically and chemically tuned under specific reaction conditions to possess bioactive properties [26, 39]. When culturing poly(NIPAM) microgels with monocytes and leukocytes, cellular uptake of microgels was dictated by microgel surface chemistries [40]. When modified with antibody, poly(NIPAM) microgels can serve as bio-sensing inks that can be printed to surfaces [41]. Tuning the mechanical properties of the nanoparticles has shown to have differential effects on cellular function and interactions. Poly(NIPAM) microgels have been thoroughly characterized in literature however, using micro-plotting techniques and poly(NIPAM) microgel inks to create complex patterned films for controlling cell adhesion and proliferation has been unreported.

Figure 11: Thermo-responsive changes observed in poly(NIPAM) microgels. Reprinted with permissions from Springer [33].
2.2 Methods

2.2.1 Poly(NIPAM) Microgel Ink Synthesis

Poly(NIPAM) microgels were synthesized using a precipitation polymerization method, as seen in Figure 13. N-isopropylacrylamide, co-monomer acrylic acid, and crosslinking agent n”n”-methylenebisacrylamide were dissolved in DiH2O at 90.9%, and 5%, and 4%, respectively, with a total solution concentration of 140mM. The solution was filtered and added to a reaction vessel with flowing nitrogen and a condenser attached. The solution was heated to 70 C and incubated for 1 hour while mixing at 450 RPM. Following thermal equilibration,
the immediate addition of a 1mM solution of ammonium persulfate radically initiated the polymer precipitation reaction. The reaction was run for 5 hours and allowed to cool overnight. Using dialysis and filtration methods, all partially formed fragments and large aggregates were removed from the solution. The microgel solution was lyophilized and reconstituted in water at a stock concentration of 5 mg/ml.

![Reaction scheme](image)

**Figure 13**: Formation of poly(NIPAM) microgel particles.

Show in Table 1, parameters for microgel ink synthesis were determined by two criteria; viscosity and microgel ink concentrations. Microgels were diluted in water from 5 mg/ml to obtain three concentrations; 0.02 mg/ml, 0.2 mg/ml, 2mg/ml. To increase ink viscosity and prevent clogging of microgels in micropipette tip a low molecular weight form of polyethylene was used at 5%, 10%, and 20% by volume.

### 2.2.2 Micro-plotting Microgel Inks

Prior to micro-plotting, pattern models were designed using a SonoDraw GIX2 software. The printing template shown in Appendix A was created in the SonoDraw GIX2 software and used...
to print all microgel inks. A 5 µm micropipette tip obtained from Five Photon Biochemicals was secured to a piezoelectric element using super glue. The piezoelectric element was screwed into its compartment in the cartridge unit. The cartridge unit was fit into its holster in the Sonoplot GIX Microplotter II. Detailed instructions for needle and printing setup can be found in Appendix B. Following microplotter setup, the printing proof from the SonoDraw

Table 1: Array of microgel inks printed onto glass slides with various surface modifications.

<table>
<thead>
<tr>
<th></th>
<th>Clean</th>
<th>0.05 mono-molar PEI</th>
<th>1% PEI</th>
<th>BSA</th>
<th>APTMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% PEG</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td></td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
</tr>
<tr>
<td></td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
</tr>
<tr>
<td>5% PEG</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td></td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
</tr>
<tr>
<td></td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
</tr>
<tr>
<td>10% PEG</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td></td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
</tr>
<tr>
<td></td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
</tr>
<tr>
<td>20% PEG</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
<td>0.02 mg/ml</td>
</tr>
<tr>
<td></td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
<td>0.2 mg/ml</td>
</tr>
<tr>
<td></td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
<td>2 mg/ml</td>
</tr>
</tbody>
</table>
GIX2 software was uploaded into Sonoplot program and the printing commenced. Due to needle fragility, fragmenting of needle occurred at various printing points. In this case, the printing was paused and the cartridge was removed for the needle to be replaced.

2.2.3 Microgel Film Characterization

Phase contrast images of each microgel printed film pattern was taken using the Invitrogen EVOS FL Auto Imaging System. 4x objective images of each slide were stitched to display the entire slide image. SEM images were taken of microgel film patterns. Once dried, microgel film proofs were imaged using the Asylum MFP-300 Atomic Force Microscope. Height traces of each film were taken using the Asylum Igor Pro software. Line widths and dot radii were measured using ImageJ software to convert pixel measurements in micrometers. Statistical analyses were used to determine significance of line widths and dot radii data. The following three parameters were compared using a three-way ANOVA; slide type, microgel concentration, and PEG400 percentage. Pairwise t-tests were used to compare output means for line widths and dot radii between each slide types.

2.3 Results & Discussion

For each microgel ink, the Sonoplot microplotter used a 5 µm micropipette tip to print the pattern proof designed in the SonoDraw software, see Appendix A. Figure 13 demonstrates the phase contrast image of dried microgel films patterned with 0.2 mg/ml bio-inks onto a 0.05
monomolar PEI coated glass slide. PEI is a monomer with variable chain lengths and molecular weights. Monomolar measurements account for this and use the "mer" molecular weight instead of the chain as a whole. Increasing PEG percentages allowed for increasing ink viscosity. Increased viscosity enabled the droplet formation at the tip of the needle to have higher contact with the printing surface, resulting in fluid being deposited consistently.

Increasing PEG percentages also allowed for better spreading of the fluid into patterns deposited, as seen in Figure 14.

![Figure 14](image-url)

**Figure 14**: Phase contrast microscopy images stitched at 4x magnification of printed microgel film of 0.2 mg/ml microgel ink with a) 5% PEG, b)10 PEG, c)20% PEG on 0.05 monomolar PEI slide
Figure 15 demonstrates the increase in PEG percentages with microgel inks printed on 1% BSA slides. Again, phase contrast images were taken at 4x and stitched to create the entire slide. Increasing PEG concentrations correspond to large liquid amounts being deposited throughout the pattern. Throughout each microgel concentrations, ink containing 0% PEG resulting in no liquid being deposited onto the slide surface. Using a small micropipette tip could’ve led to drying of microgels on the tip of the needle. Resulting in clogging when all inks containing 0% PEG where printed. Each ink containing microgels and a percentage of PEG successfully deposited films onto each functionalized surface. Increasing PEG percentages resulting in clearer and consistent printing was a trend that was observed through all slide types and microgel concentrations. PEG 400 is a viscous liquid that should increase the hydrophilic nature of the microgels inks. In this sense, we see that the increase in
hydrophilic nature of liquid allows for an increase in the liquid wicked to surface from the needle. Increased liquid spreading onto the surfaces can also be attributed to increasing hydrophilicity by adding PEG 400 [42]. BSA and APTMS functionalized slides observed fluid aggregations once the microgel ink was deposited onto the glass slide. Both surface coatings have been shown to block protein absorption to surfaces [43, 44]. To an extent, this was countered by the increasing hydrophilic nature of PEG 400 that allowed for efficient spreading of the liquids onto the surface. With these two slide types, complete and uniform films were not observed with pattern shapes that covered larger slides areas. No trend was observed between differences in particle concentrations through the inks.

Figure 16: SEM images of a line array of printed microgels at a) 1200x mag. and b) 5000x mag.

Figure 17: SEM images of a single dot of printed microgel ink at a) 350x mag. and b) 250x mag.
SEM images in Figures 16 & 17 demonstrate dried microgel inks of line and dot arrays patterned with the ultrasonic printing method. The features were patterning using a 30 µm needle tip and inks containing 2 mg/ml microgel concentration with 10% PEG onto normal cleaned slides. The following images demonstrate that once dried, the microgel patterns resemble the structure of a dried film. The shrinking nature of dried microgels vs wet microgels can be used to explain the wrinkled appearance of the films.

Printing techniques to examine printing resolution were performed using a 5-µm glass capillary tip. Measuring line widths and dot radii were used to determine printing resolution and accuracy of prints in relation to the diameter of the micropipette used. Three-way ANOVA analysis revealed that there were differences in dot radii and line widths based off interactions between slide type, microgel concentration and PEG400 percentages. It was also determined that individually slide type significantly affected differences in line width (P < 0.0001) and dot radii (P < 0.0001). Interactions between slide type and microgel concentration significantly affected mean dot radii (P < 0.0001). There were no differences in mean line widths based of PEG400 percentage alone. Slide type (P < 0.0001), microgel concentration (P < 0.0387), and all variable interactions (P < 0.0325) resulted in significant differences in mean line widths. In Figures 18-20, dot radii data observed consistently follows the same trend throughout slide types. 0.05 monomolar PEI slides observed a higher average dot radii when compared to other surface modifications (P < 0.0003). Mean dot radii printed onto 1% PEI slides were significantly higher than APTMS, BSA and clean slides (P < 0.0043) and lower than 0.05
monomolar PEI slides (P < 0.0003). APTMS slide types resulted in a significantly lower dot radii than other slide types (P < 0.0001) throughout all PEG concentrations. Similarly, with line width data, APTMS slides displayed significantly (P <0.0001) lower values for line widths.

APTMS coatings have been used in previous research to increase the hydrophobicity of surfaces [45]. Higher printing resolutions for the slide type can be related to a reduction in liquid spreading due to the hydrophobic surface. No slide type however, was observed to have consistently higher line widths. It was expected for changes in viscosity due to varying PEG percentages to affect cell spreading of inks on slides. However, no measurable trend was determined between viscosity percentages on printing resolution. Each microgel film resulted in line widths and dot radii larger than the 5-µm diameter glass capillary used to deposit the bio-ink. This can be attributed to ink spreading once deposited on the glass slides along with a fragile glass tip being damaged during some printed cycles. APTMS coated slides achieved patterns closest to the 5-µm diameter, therefore having the most accurate printing resolutions. Surface response data displayed in Appendix D highlight larger line widths at the highest microgel concentrations and PEG400 percentages. Inks containing high PEG percentages and low microgel concentrations resulted in line widths closest to the 5µm diameter of the micropipette tip used.
Figure 18: Plots with standard deviation bars of average a.) dot radii and b.) line widths for all microgel inks printed with 5% PEG onto glass slide with various surface modifications using a 5-micron micropipette tip (n=5).
Figure 19: Plots with standard deviation bars of average a.) dot radii and b.) line widths for all microgel inks printed with 10% PEG onto glass slide with various surface modifications using a 5-micron micropipette tip (n=5).
Figure 20: Plots with standard deviation bars of average a.) dot radii and b.) line widths for all microgel inks printed with 20% PEG onto glass slide with various surface modifications using a 5-micron micropipette tip (n=5).
Once dried AFM images were taken of lines on each printed film pattern, demonstrated in Figure 21. Height tracing was used to determine heights of each microgel film. Shown in Figure 22, films heights ranged from 200-600 nm though all bio-inks. At 0.02 mg/ml nanoparticle concentrations, clean slides displayed lower film heights than all glass slides with surface modifications. Chemically cleaning a glass slide removed all dirt and particles, creating a smooth uniform surface. The uniform surface would account for an increasing in liquid spreading that would result in the lower film heights. Although it was expected that increases in viscosity due to PEG 400 percentages would results in higher film heights, there is no observed trend with differences in PEG concentrations. Because films were dried before film heights could be traced, surface wetting may have not played an ample role in film heights.

Figure 21: AFM image demonstrating a) microgel with b) respective film height trace.
Figure 22: Film height characterization line plot with a.) film heights in relation to microgel concentrations and b.) film heights in relations to PEG percentages.
CHAPTER 3: Contact Angle Analysis

3.1 Variable Surface Chemistries

Surface modification of materials alters material interactions with environment while maintaining bulk material properties. This is especially beneficial in tissue engineering capacities where surface interactions of biodegradable scaffolding materials need to be controlled or altered. While surface coatings can alter cell interactions with scaffolds, specific coatings have also been used with scaffolds to control drug delivery release systems[46]. Nanosized molecules have been used as coatings to organize the pattern of functionalized groups on surfaces along with reduced platelet aggregations onto those surfaces [47]. The patterning of a biomaterial onto a surface with specific chemical modifications increases the precision of control over cellular adhesion and proliferation. Throughout this particularly study, variable surface coatings were used to establish how differences if surfaces chemistries affected the ability of microgel films to be deposited using the micro-plotting technique.

Chemically cleaning a surface will remove dirt and particulates which may have positive or negative effects on cellular attachment. Printed microgels on chemically cleaned glass serves as a basis for printing characterization. Cleaning a surface before depositing surface modifications with also allow for uniformity in the functionalized surface. Polyethyleneimine (PEI) is an amine conjugate carbon polymer with cationic properties, commonly used in surface modification to increase wetting strength and enhance cellular attachment[48, 49]. PEI coatings of nanoparticles used for drug delivery result in increased attachment of DNA and
RNA molecules to the construct[50]. Bovine serum albumin (BSA) and (3-Aminopropyl) trimethoxy-silane (APTMS) protein coatings have been shown reduce enzyme and cell adherence [43, 44, 51]. In microfluidic channels where nano-sized differences can alter function, APTMS coatings are used to reduce absorption of nonspecific proteins to surfaces[52]. Creating bioactive patterned films on either of these surfaces will create a material surface that allow cells to preferentially adhere to and proliferate on the specific locations the film has been printed.

The piezoelectric printing element uses a vibration technique to dispense liquid from the tip of the micropipette needle. A viscous liquid or a hydrophobic surface have the potential to counter the forces from the vibrations of the piezoelectric element. This results in patterns that are either inconsistent or never dispensed at all. Previous research has shown surface roughness and hydrophobicity can determine the dynamics of protein attachment. When using micropipettes of a specific size, surface wetting characteristics can determine the size and accuracy of the print patterns produced [53]. Contact angle measurements give a quantitative value of the surface wetting of solid by a liquid. By evaluating the array of microgel concentrations and ink viscosities within our inks against varying surface coatings, we can examine surface wetting properties.
3.2 Methods

Prior to all coatings, glass surfaces were chemically cleaned to remove any containments or particles. Glass slides were sonicated in solutions of Alconox® for 20 minutes, deionized water for 15 minutes, acetone for 15 minutes, ethanol for 15 minutes, and isopropyl alcohol for 15 minutes. Following sonication, glass slides were rinsed with water and dried with nitrogen. For APTMS functionalization, glass slides were incubated for 2 hours at room temperature while shaking in a solution containing 2.5 ml of absolute ethanol and 25 ul of APTMS. Following incubation, the slides were rinsed with water and allowed to dry. For 0.05 monomolar PEI coatings, cleaned glass slides were incubated at -4°C in a monomolar solution of PEI with concentration of 3.75 mg/ml. Following an overnight incubation, slides were rinsed with deionized water and air dried. For 1% PEI coating, cleaned glass slides were incubated overnight at 4°C in a 1% by volume solution of PEI, rinsed with deionized water, and left to air dry. For BSA coatings, cleaned glass slides were incubated overnight at 4°C in a 1% by weight solution of BSA, rinsed with deionized water, and left to air dry.

Contact Angle measurements were taken using Rame-Hart Advanced Contact Goniometer – Model 102. DropImage Advanced software was used to obtain images of water droplets on slides. To measure the contact angle, a tangent is drawn at the corner of the liquid droplet. The angle at the boundary where the liquid and solid substrate intersect is measured on each side of the water droplet, seen in Figure 23. The average of the two measurement is determined to be the contact angle. Three-way ANOVA analysis was used to compare to contact angle degree.
outputs for the following parameters; slide type, microgel concentration, and PEG 400 percentage. Oneway and pairwise t-tests analysis was used to compare contact angle mean differences based on slide type.

Figure 23: Image representing how contact angle image measurements are taken; a) water droplet forming on needle tip while, b) the tangent lines used to determine contact angle.

### 3.3 Results & Discussion

Using the contact goniometer, single droplets of water were dropped from a syringe onto functionalize slides and contact angle measurement were taken after the water droplet stabilized. Contact angle images for water droplets onto each slide type can be observe in Figure 24. Figures 24 & 25 demonstrate surface wetting capabilities of various surface modifications. This was repeated for the array of bio-inks containing various PEG percentage and microgel concentrations. Trends that were observed with water contact angle measurements were similarly observed with varying microgel and PEG 400 concentrations. Figures 25-28 shows that with each poly(NIPAM) ink concentration and each PEG percentage, APTMS and BSA functionalized slides consistently have higher contact angles. This trend also
Figure 24: Contact angle images for water droplets on a) chemical cleaned, b) 0.05 monomolar PEI, c) 1% PEI, d) APTMS, and e) BSA coated glass slides.
follows with the water contact angle with each slide type. Comparison of means using paired t-tests for water contact angle revealed that variation in slide type (P < 0.0001) resulted in significantly different contact angles. For water contact angle, clean slides demonstrated lower contact angle (P < 0.0001) than all other slide types. BSA functionalized slides demonstrated the largest contact angle (P < 0.0001) when compared to all other slide types. 0.05 monomolar PEI slides displayed a higher mean contact angle than clean slides (P < 0.001) and lower mean contact angles than all other slide types (P < 0.0219).

Three-way ANOVA analysis indicate that slide type, microgel concentration, PEG400 percentage and all variable interactions produce significant differences in mean contact angle (P < 0.0192). With microprinting wetting chemistries are crucial to printing dynamics. Bovine serum albumin (BSA) is commonly used in scientific applications to block protein absorption to surfaces. BSA having a significantly higher contact angle (P < 0.0001) than all other surface types implies the least surface wetting ability of the various slides types[54]. This corresponds to previous literature that supports BSA as an efficient method of blocking protein absorption to various surfaces. [51, 55]. Surface wetting analysis also demonstrated APTMS coatings maintained a significantly higher contact angle (P < 0.0001) when compared to clean and PEI surfaces. In previous research APTMS coatings produced hydrophobic surfaces were compared to biomaterials coated commonly used in cell attachment [56]. High printing resolutions of APTMS observed in Chapter 2, can be attributed to surface wetting chemistries seen in Figure 7. Higher contact angles can be attributed to low spreading of the bio-ink onto
a surface once its printing. Resulting in line widths and dot radii like the 5-µm tip used to print films. Glass slides with chemically cleaned surfaces were observed to have lower film heights than modified surfaces in Chapter 2. Throughout the surface wetting data, cleaned slides displayed contact angles lower than 60%. Low contact angle resulted in higher spreading area of the bio-ink onto a surface, which can be related to lower film heights observed in Chapter 2.

Figure 25: Bar graphs results of contact angle measurements with respective standard deviation bars for water droplet on glass slides with various surfaces modifications (n=3).
Figure 26: Bar graphs results of contact angle measurements with respective standard deviation bars for 0.02 mg/ml microgel ink droplets on glass slides with various surfaces modifications (n=3).
Figure 27: Bar graphs results of contact angle measurements with respective standard deviation bars for 0.2 mg/ml microgel ink droplets on glass slides with various surfaces modifications (n=3).
Figure 28: Bar graphs results of contact angle measurements with respective standard deviation bars for 2 mg/ml microgel ink droplets on glass slides with various surfaces modifications (n=3).
CHAPTER 4: Conclusion and Future Directions

Throughout the study, we could demonstrate the capabilities of poly(NIPAM) bioactive inks to be used to create patterned nanoparticles biofilms. Microgel bio-inks containing PEG 400 were successfully micro-plotted onto chemically cleaned, 0.05 monomolar PEI, 1% PEI, BSA, and APTMS functionalized slides. Microgel films plotted onto variable functionalized slides types provided unique information on the ability of microgel nanoparticles to be printed on potential scaffolding materials. Phase contrast images displayed microgel printing profiles for each concentration with varying slide types. It was observed that increasing PEG concentrations allowed for films patterns that were clear and consistent. Attempts to print the bio-inks with 0% PEG resulted in films not being deposited from the glass capillary tip. APTMS coatings resulted in the highest printing resolutions, with print features that were closest in size to the needle tip used. Height tracing demonstrated that once dried, PEG percentages had negligible effects on overall film heights. Surface wetting data displayed differences in surface modifications and surface chemistries that coincided. BSA, a common protein used to block protein adhesion, and APTMS, shown to produce surfaces of high hydrophobicity, surface modifications displayed low surface wetting chemistries when compared to PEI coated and chemically cleaned surfaces. Although PEG concentrations played a significant role in overall printing patterns, there was no observed trend with contact angles. Consequently, the ability of PEG 400 to enhance printed features should be associated with printing dynamics involving the ability of fluid to wick to the surface from the micropipette tip. Overall it has been demonstrated that microgel bio-inks in conjunction with modified
surfaces can produce biomimetic films with varying parameters that can have applications in multiple areas of biotechnology.

Future studies of microgel bio-inks should employ the use of highly complex patterns. Sonoplot software has the potential to be used to print single patterns with varying ink combinations, allowing for potential gradients in microgel films. The Sonoplot GIX Microplotter has the capabilities so to produce multilayered features. These microgel solutions can be used to create thicker films and 3-D materials, producing possible new scaffolding avenues that can interact with cells on a molecular level. Nanoparticle and microgel materials are commonly used in tissue engineering to encapsulating proteins, metabolites, and signal molecules to be delivered to cells. Producing and printing microgel bio-inks with added metabolites or other molecules will create films with the potential to control every aspect of cell behavior from proliferation to cell signaling.

Finally, studies involving cellular adhesion and proliferation can be conducted to examine cell affinity for adhesion to microgels. Microgel inks were patterned onto functionalized surfaces in the above study such BSA and APTMS. Seeding cells onto surfaces such as these which prevent protein absorption, will determine if cells will distinctly adhere to printed microgel patterns. Although PEG400 aided in the printing of inks from the ultrasonic printing system, it would also be beneficial to remove PEG400 following pattern deposition. In this case, cellular adhesion to printed patterns containing purely poly(NIPAM) microgels could be studied.
REFERENCES


APPENDICES
Appendix A

Figure 1: Print schematic for microgel ink characterization study. Designed with SonoDraw GIX II Software. Grid measured in 50 x 50 µm squares. Pattern features are enlarged to visual dots and lines. Actual patterns features are 15 µm.
Appendix B

Sonoplot GIX Microplotter II Instructions

**Disclaimer: The needle tips are very fragile. Touching the tip with your finger or any other surface, as well as dropping it will cause it to break. Broken tips are not nearly as effective in printing and will need to be replaced.**

1. Log into Mac computer

**Drawing a Design**

2. Open SonoDraw software.
   a. In upper right corner of the window click **Inspect** and change dimensions to those desired including the feature width and grid spacing.
   b. To resize the window click **Fit in Window** in the bottom right corner.
   c. In upper right corner click **Solutions** and add all solutions that will be used in design. Name each solution the location on the well plate. For example, carbon ink located in row F column 1 would be named F1.
   d. Leave preset checks.

![Figure 1. Above are the different windows for SonoDraw. 1)The window to the far left is the Properties window. This allows you to change the size of your design in microns as well as the spacing of the grid and the feature width. The spacing of the grid just allows you to make your design more precise and detailed. You want to match the feature width to the needle size you plan on using. 2)The large window in the middle is the drawing space. In this space](image-url)
a variety of designs can be made with different solutions. 3) The window to the right is the Solutions box. In this you can add as many solutions as you need to complete your design. In order to draw in a given solution it must be highlighted in this window. Be sure to name the solution correctly and pick a different color for each solution. **Both the properties window and the solutions window can be opened in the top right corner of the large drawing window.**

3. Use tools on upper left toolbar to draw out the desired pattern with desired solutions.  
   a. Save design. For now most patterns are being saved in the patterns folder.

**Starting Printing**

4. Flip switch on the back upper corner of sonoplot monitor.  
   a. Make sure the desired needle and cartridge is in place on the plotter. If there is not a needle, check the shelf above for already assembled needle. For instructions on setting up a new needle see Needle Setup Instructions below.

5. Open SonoGuide software  
   a. If window pops up asking to home positioner click Home.

6. On CCD Camera window click Solutions and add all the solutions you used in your design  
   a. To do so click the + button in the lower left corner. Name the solution the same as you did on SonoDraw.  
   b. Fill out all the information to the right according to your desired plot. You must change the column, row, and plate fields. If unsure about the other values leave them as the preset.

7. Once all solutions are added click Set A1 Well Position in the upper right corner.  
   a. Using the arrows on the Manual Controls panel maneuver the needle tip to be centered inside of the A1 well so that would be low enough to draw fluid. *Be sure not to hit needle on well plate, as it will break the tip!*  
      i. On the manual controls double arrows on the outside refer to large movements and the single arrows refer to small movements. These values can be changed at the bottom of the window.  
   b. When needle is in desired location click OK.

8. Place the desired printing surface, i.e. glass wafer or slide, on the target location marked off on the metal surface of the microplotter.  
   a. Secure surface using given magnets.

9. Using the arrows on the manual controls, raise the tip out of the well plate and over to the secured printing surface so that it is hovering above the upper left corner of the surface.  
   a. Try to get as close to the surface without touching it. It helps to use movements of 1000 and 100 microns as you approach the surface. *Make sure not to break the needle tip!*
b. Once the needle is in place click **Calibrate Dispenser** on the CCD Camera window and click **Calibrate**.

c. Once calibration is complete go to **Dispenser** in the very top toolbar and click **Show Diagnostics**.

d. Next click **Find Surface** in the top left corner of the Manual Controls window.

   i. The needle will now gradually lower itself to the surface. When the needle has touched the surface the line on the diagnostic will jump above the red line and motion will stop.

10. Using the small upper movement button move the needle so that it is hovering 100 microns above the surface.

   a. On the CCD Camera window click **Surface Calibration**. Change the **distance to calibrate fields** in the lower left corner so that they reflect that of your drawing in SonoDraw.

   b. Click **Calibrate Surface Cant**.

      i. Five points will be taken across the surface in both the X and Y direction. Ensure that the surface you are printing on is large enough.

11. After calibration click **Find Surface** again to find the surface for printing.

   a. Once the needle is on the surface use the small upper movement button again to move the needle about 10 microns above the surface.

12. To begin printing go to File on the upper toolbar and click **Open Pattern**.

   a. Find the pattern you would like to print and open it.

   b. When the window pops up click **Proceed**.

   c. As the pattern prints make sure everything's running smoothly. At any point to stop printing just click **Cancel** on the printing window.

      i. Printing should be stopped if the needle begins to bend or catch on the surface and if the needle stops depositing fluid.
**Figure 2.** 1) In the top left corner is the manual controls panel. This is where you will control the position of the needle and find the surface. 2) The window in the top middle is the surface cant calibration. This is used in step 10 to determine the slant of the surface. 3) At the top right corner is the solutions window. This window allows for solutions and their positions to be added and allows the A1 well position to be set. 4) In the bottom left is the needle calibration window. This is opened by clicking ‘Calibrate Dispenser’ on the camera window. This allows the needle tip to calibrate and should never be used when the needle is touching the surface. 5) In the bottom middle is the diagnostics window. This shows when the needle has touched the surface by jumping above that given red line. 6) The final window is the Camera window. This shows the needle and also has most of the functions for the dispenser on the top bar. **Note: These windows do not all pop up on their own and need to be opened using the directions above. This is just to show you what they should look like when they are opened.**

**Needle Setup Instructions**

1. If the needle you wish to set up is already properly assembled skip to step 9. Otherwise continue with step 2.
2. Obtain desired needle, black casing unit (which includes the part, a cover, and three small screws), and the grey sensor.
3. Using a small screwdriver remove the three small screws from the back of the case and place them in a safe location.
4. Carefully remove the new needle from its box and place on the desk surface.
   a. Using the scoring tool, lightly score the needle so that it is about 2 cm in length (tip to end).
   b. Gently break the needle at the place in which you scored it. If done correctly it should easily snap.
   c. Discard of the back end of the needle.
5. Taking the grey sensor, place a small drop of superglue on the long, flat edge of the piezoelectric. *(See pictures below for clarification.)*
   a. Try to keep superglue only on this edge.
6. Gently place the prepared needle tip on the superglued edge of the sensor. Place the needle so that the piezoelectric metal is just a few millimeters from the flat end. *(The distance between the tip of the needle and the piezoelectric should be no more than 7-8 mm.)*
   a. It may be easier to place the tip on the bench and then use the glued edge of the sensor to pick it up.
   b. Make sure the tip of the needle is facing the opposite direction of the grey sensor lead!
7. Using your fingers or tweezers adjust the needle tip so that it is centered on the sensor. It should be centered in every way so that it lays exactly collinear with the metal edge. *(This is the most important step for ensuring the sensor will work! Any deviation and it might not be picked up properly.)*
   a. After you have centered the tip properly allow the superglue to dry. This should take only a few minutes.
8. After dry, place the whole sensor in the black casing unit.
   a. Adjust the location so that the metal piece lays just over the edge. *(See pictures below for clarification.)*
   b. Gently place the covering on top and secure the three screws with a small screw driver.
   c. Check that the metal piece of the sensor does not hang too far out of the case and that it is not touching any of the case.
9. Place the whole unit on the microplotter. *(See pictures below for clarification.)*
   a. The correct location is on the black piece located to the left of the camera.
   b. When it is placed correctly it will snap into the groove.
   c. Take the grey end of the sensor and connect it to the port above and behind the camera.
Figure 3. A) The figure on the top represents the various parts for the setup of a needle cartridge. You will need the black cartridge unit which includes the top and screws to the right, the grey telephone wire with a piezoelectric and needle attached, and a screwdriver. B) Once the unit pictured in Figure 3A is assembled it is placed on the microplotter as shown above. The hook on the cartridge should be placed in the black groove on the microplotter so it is snug and will not move. The grey wire is then plugged in above the unit as shown. The three grey knobs by the camera on the microplotter adjust the position of the camera so that as you change the needle you can refocus on the tip. In the bottom right corner, you can see the placement of the well plate as well as the glass wafer.
Appendix C

Fabrication of Needle Tips Used in Printing of Bio-inks

This protocol describes how to create micropipette tips used in Sonoplot GIX Microplotter II to dispense bio-ink fluid.

*This protocol uses G-1 Glass Capillaries

**Needle Puller**

The current needle puller protocol is designed to obtain needles with the shortest taper possible. Distance of pull positions and heater values can be adjusted to obtain desired results.

1. Assure that all four weights are fixed to puller and set mode selector of puller to STEP 2 for a two-stage pull.
2. Set distance of first pull to 2 mm by loosening the fixing screw that holds the adjustment plate on the right side of the weight attachments.
   a. Tighten the fixing screw when the top of the adjustment plate is in line with the desired distance.
3. Remove the plastic hood covering the heating element by unscrewing the two knobs on the top of it.
4. Select the heating position for the second pull by loosening the fixing screw for the adjustment plate on the right side of the metal heating element. Set distance at 1 mm.
5. Turn the power switch on.
6. Set the mode selector to NO. 1 HEATER and adjust value of NO. 1 HEATER ADJ. to 55 using knob.
7. Set the mode selector to NO. 2 HEATER and adjust the value of NO. 2 HEATER ADJ. to 50 using knob.
   a. Do not take more than 90 seconds to adjust heating values.
   b. When finished adjusting heating values, return mode selector to Step 2.
8. Set the glass capillary tube by loosening the capillary retaining knobs above the heater element.
   a. Slide the glass capillary down through the spiral heating element until its end reaches the top of capillary retaining knob below the heating element
   b. Tighten the upper capillary retaining knob.
9. Loosen the lower capillary retaining knob
   a. Move the bottom sliding portion containing the weight to its upper limit and tighten the lower capillary retaining knob to the needle.
10. Close the acrylic hood and press the START button.

*Notes for Changing Needle Parameters:
   Increasing heat will make needle tip longer
   Decreasing weights will make needle tip longer
Microforge

1. Turn the microforge power switch on.
2. Maneuver the platinum wire heating element in microscope view using 3 adjustment knobs on the right of microforge.
3. Loosen the pipette holder and remove the metal cleaning rod. Replace this with a pulled micropipette needle.
   a. Use the knobs on the pipette holder to maneuver the micropipette into the microscope view along with the heating element.
   b. Put the micropipette into focus using the adjustment knobs under the eyepiece lens.
4. A spherical glass bead should already be formed on the end of heater. If so, skip to step 6.
   a. Be sure the micropipette is in line with the heater element
5. Turn the heater adjustment to medium-high heat.
   a. Depress the foot peddle and bring the micropipette tip in contact with the heating element.
   b. Continue moving the micropipette tip toward the heating element until a spherical bead is form.
   c. While the foot switch is still depressed move the micropipette tip away from the heating element to leave the glass bead on the heating element.
   d. Set the heater to a medium-high heat and depress the foot switch to make the glass bead spherical.
6. Remove the micropipette tip from the pipette holder and replace with new pulled needle.
7. Turn the graticule on the left eyepiece lens, so that the ruler is vertical.
   a. Using the 5x objective lens, each mark will represent 20 µm distance.
   b. Using the 10x objective lens, each mark should will represent a 10 µm distance.
8. Position needle so that desired needle diameter can be measured with graticule measurements marks.
9. Bring the glass bead in contact with the needle at the desired thickness to be cut. Set the heater adjustment to 0.
   a. Depress the foot peddle and gradually increase heat until the micropipette appears to be slightly melting into the glass.
   b. Release the foot peddle immediately, allowing the micropipette to cut and the tip to adhere to the glass bead.
10. Increase heat until the tip of the micropipette begins to melt and allow tip to completely melt into the glass bead.
Appendix D

Figure 1: Three-way ANOVA results of slide type, microgel concentration, and PEG400 percentage for dot radii.
Figure 2: One-way analysis of mean dot radii by slide type.
Figure 3: Comparison of mean dot radii differences between each slide type using paired t-tests.
Figure 4: Three-way ANOVA results of slide type, microgel concentration, and PEG400 percentage for line widths.
Figure 5: One-way analysis of mean line widths by slide type.
Figure 6: Comparison of mean line width differences between each slide type using paired t-tests.
Figure 7: Three-way ANOVA results of slide type, microgel concentration, and PEG400 percentage for contact angles.
Figure 8: One-way analysis of mean contact angles by slide type.
Figure 9: Comparison of mean contact angle differences between each slide type using paired t-tests.
Figure 10: One-way analysis of mean water contact angles by slide type.
Figure 11: Comparison of mean water contact angle differences between each slide type using paired t-tests.
Figure 12: Response surface on the interaction between microgel concentration and PEG400 percentage on printed line widths.