Microfluidic Bioprinter for Hydrogel Sheet Formation

by

Phoenix Qing Ba

A thesis submitted in conformity with the requirements for the degree of Master of Applied Science
Department of Mechanical and Industrial Engineering
University of Toronto

© Copyright by Phoenix Qing Ba 2014
Microfluidic Bioprinter for Hydrogel Sheet Formation

Qing Ba

Master of Applied Science
Graduate Department of Mechanical and Industrial Engineering
University of Toronto
2014

Abstract

We present a microfluidic bioprinter that is compatible with a wide range of biopolymers and is able to continuously or periodically produce intact homogeneous or heterogeneous hydrogel sheets in Couette flow condition with different throughputs through different cross-linking mechanisms with control over the sheet geometries and mechanical properties. The printer includes a movable platform to achieve the automated production, collection and transportation of the hydrogel sheets, a microfabricated printing device that defines the geometry and composition of the printed hydrogel sheets, pressure controls upstream for introducing biomaterials with different flow rates, and temperature features to fulfill the thermal requirement during the material processing. According features can be enabled for different gelation mechanisms including ionic cross-linking gelation and thermal gelation. Using the concept of Couette flow with controls over printing parameters, homogenous and heterogeneous hydrogel sheets with different geometries and mechanical properties can be produced as predicted using the theoretical model.
Acknowledgments

I would like to thank all the members from the Guenther Research Laboratory, especially my supervisor, Axel Guenther, for guiding and supporting me throughout the project. Special thanks to Ryan Mendell and the university machine shop for helping with the design and fabrication of the bioprinter, to Haotian Chen for programming the LabVIEW interface for all the controls of the printer, to Pak Ki Lam for building and validating the replica of BASX platform, to Lian for teaching me microfluidic device fabrication techniques, and to Lindsey Fiddes for introducing me the imaging facilities. I would also acknowledge the NSERC CREATE MATCH program and Ontario Graduate Scholarship.
# Table of Contents

Acknowledgments ........................................................................................................ iii  
Table of Contents ........................................................................................................ iv  
List of Tables ................................................................................................................ vi  
List of Figures ............................................................................................................... viii  
List of Appendices ....................................................................................................... xiv  
List of Symbols ............................................................................................................ xv  

1. Introduction .............................................................................................................. 1  
   1.1 Tissue Engineering and Biomaterials ............................................................... 1  
   1.2 Different Gelation Mechanisms ........................................................................ 3  
   1.3 Current microfluidic tissue engineering approaches and 3D Printing Technologies .... 4  
      1.3.1 Related Works Using Microfluidic Approaches ............................................. 4  
      1.3.2 3D Bioprinting ............................................................................................. 5  
      1.3.3 Drawbacks and Limitations of Current Approaches ....................................... 6  
   1.4 Thesis Organization .............................................................................................. 7  

2. Microfluidic Bioprinter Design .............................................................................. 9  
   2.1 Microfluidic Bioprinter Design and Fabrication ............................................... 9  
   2.2 Printing Nozzle: Microfluidic Device Design, Fabrication and Characterization .... 12  

3. Materials and Methods .......................................................................................... 17  

4. Printer Specification, Functionality and Characterization .................................... 23  
   4.1 Motion Control .................................................................................................... 23  
   4.2 Pressure Control ................................................................................................. 25  
   4.3 Temperature Control .......................................................................................... 28  

5. Results and Characterization of Hydrogel Sheet Formation .............................. 36  
   5.1 Analytical Model for Biopolymer Flow, Transport and Gelation ...................... 36
5.1.1 Couette Flow ........................................................................................................ 36
5.1.2 Parameters and Theoretical Model ................................................................. 46
5.2 Homogeneous Hydrogel Sheets ........................................................................... 50
5.3 Pressure Driven Printing ..................................................................................... 55
  5.3.1 Pressure versus Flow Rate Control ................................................................. 55
  5.3.2 Pressure Driven Printing with Different Nozzles ............................................ 61
5.4 Finite Sheet Length Printing ............................................................................... 65
  5.4.1 Syringe Pump Based Printing ........................................................................ 65
  5.4.2 Pressure Driven Printing ................................................................................ 67
5.5 Printing Parameter Variation in Sheet Formation ............................................... 69
  5.5.1 Varying Velocity while Fixing Flow Rates ...................................................... 69
  5.5.2 Varying Matrix Flow Rate while Fixing Velocity .......................................... 70
5.6 Measured Sheet Tensile Properties .................................................................... 72
5.7 High-throughput with Extreme Wide Nozzle Printing ....................................... 76
5.8 Heterogeneous Hydrogel Sheets ......................................................................... 78
6. Conclusion ............................................................................................................. 81
  6.1 Conclusion .......................................................................................................... 81
  6.2 Future Work ....................................................................................................... 83
References ............................................................................................................... 84
Appendices ............................................................................................................. 90
List of Tables

Table 1 Advantages and drawbacks of three bioprinting techniques ........................................ 6

Table 2 Summary of microfluidic device geometry ......................................................................... 14

Table 3 Measured thermoelectric element performance under different voltages ....................... 29

Table 4 Experimental condition summary of homogeneous sheet printing ............................... 50

Table 5 $p^*t$ best fit equations for each specific geometry/configuration of device with 2%w.t. alginate solution flowing through and the determined D values as combined fluid physical resistance constants .......................................................................................................................... 60

Table 6 Experimental parameter predictions and actual experimental trails for 10mm device .... 61

Table 7 Experimental parameter predictions and actual experimental trails for 25mm device .... 62

Table 8 Experimental parameter predictions and actual experimental trails for 42mm device .... 62

Table 9 Measured geometries of segmental 25mm wide 2%w.t. alginate sheets printed using syringe pump .................................................................................................................................. 66

Table 10 Measured geometries of segmental 25mm wide 2%w.t. alginate sheets printed using pressure control ........................................................................................................................................... 68

Table 11 Measured 10mm wide 2%w.t. alginate sheet thicknesses with different printing velocities .................................................................................................................................................... 70

Table 12 Measured 10mm wide 2%w.t. alginate sheet thicknesses with different matrix flow rates .................................................................................................................................................. 71

Table 13 Experimental condition summary of pressure and matrix flow rate ............................ 72

Table 14 Shear rate calculation for the printing of varying belt velocity ...................................... 75

Table 15 Shear rate calculation for the printing of varying matrix flow rate ............................... 75
Table 16 Experimental condition for each lane of material being printed onto the belt .......... 80

Table 17 Limitations of the microfluidic bioprinter .................................................................. 82

Table 18 Specifications of Peltier Thermoelectric Element HP-199-1.4-0.8 Potted.............. 108

Table 19 Seidon 120M model RL-S12M-24PK-R1 Specification ............................................. 109

Table 20 Pressure resistance calculation................................................................................. 114

Table 21 P*t average of CaCl₂ solutions through different devices ......................................... 117

Table 22 Combined fluid physical resistance constant for specific geometry/configuration..... 118
List of Figures

Figure 1 Schematic of the working principle.................................................................................................................................................. 8

Figure 2 (a) Schematic of hydrogel sheet formation in Couette flow; (b) Rendered 3D image of design of the microfluidic bioprinter .................................................................................................................................................. 10

Figure 3 Photographs of microfluidic bioprinter ........................................................................................................................................... 11

Figure 4 (a) Schematic of flows going through and exiting the microfluidic device printing nozzle; (b) Section view of a three-layer device; (c) Confinement implementation at the exit of the device .................................................................................................................................................. 13

Figure 5 Illustration of the replica of BAXS platform. Zoom in view of the roller clamp: the set screws push the sandwiched samples into the top groove of the cylinder, scale bar 20mm ....... 20

Figure 6 Detailed procedures of hydrogel clamping........................................................................................................................................ 22

Figure 7 Illustration of a 25mm 2%w.t. alginate hydrogel sheet being printed onto the moving conveyor belt, scale bar 10cm.................................................................................................................................................. 24

Figure 8 Schematic of the pressure setup .............................................................................................................................................. 25

Figure 9 Illustration of the cartridge sealing test (a) liquid sealing (b) gas sealing...................... 26

Figure 10 Illustration of a 10mm 2%w.t. alginate hydrogel sheet being printed onto the moving conveyor belt using pressure control and cartridge ................................................................................................................................... 27

Figure 11 Measured thickness of samples printed under the condition of $Q_s=500\mu l/min$, $p=1\, \text{psi}$, $Q_m=410\mu l/min$, $v=3\, \text{mm/s}$.................................................................................................................................................. 27

Figure 12 Illustration of temperature control feature, the thermoelectric element clamped between the manifold (copper plate extension feature) and the cooler plate................................. 29

Figure 13 Illustration of the thermal performance test, heat pumping direction as marked and thermal sensor attachment........................................................................................................................................... 29
Figure 14 Measured temperature profile for heating (red) and cooling (blue) ......................... 30

Figure 15 COMSOL simulation of heat transfer and distribution on manifold with cartridges and device ........................................................................................................................................................................ 31

Figure 16 Illustration of thermal performance test with all the on-manifold features on .......... 33

Figure 17 Measured temperature profile with all the on-manifold features on for heating (red) and cooling (blue) ........................................................................................................................................................................ 33

Figure 18 Measured temperature profile for cooling below 0°C ............................................ 34

Figure 19 Illustration of 4%w.t. agarose being printed using pressure control and temperature control ........................................................................................................................................................................ 35

Figure 20 Couette flow with a fixed top confinement and bottom moving plate ............... 37

Figure 21 Velocity distribution of viscous flow between parallel plates with the top plate fixed and bottom plate moving ........................................................................................................................................................................ 40

Figure 22 Shear stress profile of viscous flow between parallel plates with the top plate fixed and bottom plate moving ........................................................................................................................................................................ 41

Figure 23 Two fluid layers between top fixed confinement and bottom moving plate .......... 42

Figure 24 Velocity profile of two different viscous flows between parallel plates with the top plate fixed and bottom plate moving ........................................................................................................................................................................ 44

Figure 25 Two fluid layers between top fixed confinement and bottom moving plate with the bottom fluid layer forming a hydrogel as a moving wall ........................................................................................................................................................................ 44

Figure 26 Velocity profile of the hydrogel and the focusing stream when alginate sheet forms with a focusing stream on top with confinement (an extended roof after the exit of the device). 45

Figure 27 Flow condition in the microfluidic bioprinter, with the top confinement fixed and the bottom conveyor belt moving, the streaming fluid layer is on top of the matrix fluid layer .... 47
Figure 28 3D plot of the relationships among four parameters: matrix solution volumetric flow rate, belt moving velocity, thickness of printed sheet and printing device width with the rest parameters fixed................................. 48

Figure 29 3D plot of velocity and sheet thickness when varying matrix flow rate at different streaming flow rates with a device of 10mm exit width and 1mm confinement height............. 49

Figure 30 Sheet thickness vs. matrix volumetric flow rate plot for different Qs.......................... 49

Figure 31 Illustration of (a) 10mm, (b) 25mm and (c) 42mm 2% w.t. alginate sheets being printed .................................................................................................................. 51

Figure 32 Theoretical predictions, measured thicknesses and standard deviations of samples printed using 10mm device under multiple printing conditions as indicated ................. 52

Figure 33 Theoretical predictions, measured thicknesses and standard deviations of samples printed using 25mm device under multiple printing conditions as indicated ..................... 52

Figure 34 Theoretical predictions, measured thicknesses and standard deviations of samples printed using 42mm device under multiple printing conditions as indicated ................ 53

Figure 35 Illustration of the setup for determining flow rate vs. pressure relationship........ 55

Figure 36 Experimental data of 2% w.t. alginate solution flow rate at different pressure levels of 10mm, 25mm, 42mm wide devices and the best fit flow rate vs. pressure curves............. 56

Figure 37 Rheology measurements and the best fit natural logarithm curve of 2% w.t. alginate solution dissolved in DI water ........................................................................................................ 57

Figure 38 Pressure vs. shear rate data and best fit natural logarithm curve of 2% w.t. alginate solution flowing through a 10mm wide device......................................................... 58

Figure 39 Pressure vs. shear rate data and best fit natural logarithm curve of 2% w.t. alginate solution flowing through a 25mm wide device......................................................... 58

Figure 40 Pressure vs. shear rate data and best fit natural logarithm curve of 2% w.t. alginate solution flowing through a 42mm wide device......................................................... 59
Figure 41 Illustration of 42mm 2\%w.t. alginate sheet being printed using pressure control and cartridge. Scale bar: 4cm

Figure 42 Predicted and measured matrix solution volumetric flow rate vs. pressure data

Figure 43 Predicted and measured sheet thickness vs. pressure data

Figure 44 Predicted sheet thickness vs. matrix flow rate plots using the theoretical model and measured thicknesses

Figure 45 Illustration of segmental 25mm wide 2\%w.t. alginate sheets being printed

Figure 46 Measured thickness and length comparison between theoretical and measured values for sheets printed using syringe pump

Figure 47 Measured thickness and length comparison between theoretical and measured values for sheets printed using pressure control

Figure 48 Measured 10mm wide 2\%w.t. alginate sheet thicknesses with different printing velocities

Figure 49 Measured 10mm wide 2\%w.t. alginate sheet thicknesses with different matrix flow rates

Figure 50 Measured thickness and elastic moduli of 2w.t\% alginate sheets printed using 10mm wide device and pressure control when varying the belt moving velocity

Figure 51 Measured thickness and elastic moduli of 2w.t\% alginate sheets printed using 10mm wide device and pressure control when varying the matrix flow rate

Figure 52 Plot of measured elastic modulus vs. shear rate

Figure 53 Illustration of 70mm wide 2\%w.t. alginate sheet being printed

Figure 54 Measured and predicted thicknesses of 70mm 2\%w.t. alginate sheets

Figure 55 Illustration of 8mm multi-layer microfluidic device
Figure 56 Illustration of 2% alginate sheet with three-color alternating strips being printed using the multi-lane cartridge loaded with solutions with different microspheres ........................................ 79

Figure 57 Fluorescent image of the top view of a section of the alginate sheet with three stripes alternating in different colors ........................................................................................................ 80

Figure 58 Design of the assembled reservoir ......................................................................................... 90

Figure 59 Reservoir Piece 1 ...................................................................................................................... 91

Figure 60 Reservoir Piece 3 ...................................................................................................................... 91

Figure 61 Reservoir Piece 5 ...................................................................................................................... 91

Figure 62 Reservoir Piece 7 ...................................................................................................................... 91

Figure 63 Reservoir Piece 10 .................................................................................................................... 92

Figure 64 The eleven polycarbonate pieces form the reservoir frame to support the conveyor and other features of the printer ........................................................................................................ 93

Figure 65 (a) and (b) are the assembled motor feature including (c) L shape mount design and (d) shaft coupler design. (e) The NEMA17 motor with the shaft coupler and the L shape mount attached ........................................................................................................ 94

Figure 66 Mini-Mover low profile series conveyor with a semi-transparent thermoplastic polyurethane belt ................................................................................................................................. 95

Figure 67 Profilometer measurement of the belt material ........................................................................... 96

Figure 68 Confocal image of the belt surface-Slices Snapshot-5μm field of view ................................. 97

Figure 69 AFM contact mode scanning for belt surface smoothness ....................................................... 97

Figure 70 AFM contact mode scanning for belt surface friction ............................................................. 98

Figure 71 One-lane cartridge ................................................................................................................. 100

Figure 72 Eight-lane cartridge ................................................................................................................. 100
Figure 73 Details of the cartridge design ......................................................... 102
Figure 74 Thermal manifold design ............................................................... 104
Figure 75 Different views of the thermal manifold with the thermal elements and the clamping screws on ................................................................. 105
Figure 76 Assembly of the bioprinter cartridges and the thermal manifold .............. 106
Figure 77 (a) Design of the thermal cover; (b) the MEK fused polycarbonate thermal cover ... 107
Figure 78 (a) The complete assembly of all the parts on the cartridge side (b) Side view of the assembly ................................................................. 108
Figure 79 Copper plate design ....................................................................... 110
Figure 80 (a) The copper plate design, (b) Photograph showing cooler attached to thermal manifold .................................................................................. 110
Figure 81 Illustration of the temperature control circuit board ................................. 111
Figure 82 Illustration of the entire pressure setup connections ................................. 112
Figure 83 Shear Rate vs. pressure plots and best fit line \( y = 0.014x - 82.239 \) for 10 mm device ........................................................................................................ 115
Figure 84 Shear Rate vs. pressure plots and best fit line \( y = 0.006x - 27.962 \) for 25 mm device ........................................................................................................ 115
Figure 85 Shear Rate vs. pressure plots and best fit line \( y = 0.0007x - 5.3581 \) for 42 mm device ........................................................................................................ 116
Figure 86 Experimental plot of \( P^t \) against shear rate for flowing 5ml of CaCl\(_2\) solution through different device geometries .......................................................... 117
List of Appendices

Appendix 1 Reservoir ........................................................................................................... 90
Appendix 2 Motor and Associated Mounting Features ......................................................... 94
Appendix 3 Conveyor Belt Specifications .............................................................................. 95
Appendix 4 Printing Cartridges .............................................................................................. 99
Appendix 5 Thermal Manifold ............................................................................................... 103
Appendix 6 Thermal Control Features .................................................................................. 107
Appendix 7 Pressure Control Setup ...................................................................................... 112
Appendix 8 Microfluidic Device Design ................................................................................ 113
Appendix 9 Shear Rate vs. Pressure Plots and Trendlines Based on the Experimental Data.. 115
Appendix 10 Combined Resistance Constant D Determination of CaCl₂ Solution as for Non Viscous Fluids ........................................................................................................... 117
# List of Symbols

## Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{Ba}^{2+}$</td>
<td>barium ions</td>
</tr>
<tr>
<td>BAXS</td>
<td>multi-functional biaxial stretching</td>
</tr>
<tr>
<td>Ca</td>
<td>calcium</td>
</tr>
<tr>
<td>$\text{Ca}^{2+}$</td>
<td>calcium ions</td>
</tr>
<tr>
<td>$\text{CaCl}_2$</td>
<td>calcium chloride</td>
</tr>
<tr>
<td>CAD</td>
<td>computer aided design</td>
</tr>
<tr>
<td>COOH</td>
<td>carboxylic acid</td>
</tr>
<tr>
<td>DI</td>
<td>deionized</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco's modified eagle's medium</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>OH</td>
<td>hydroxide</td>
</tr>
<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
</tr>
<tr>
<td>RGD</td>
<td>arginylglycylaspartic acid</td>
</tr>
<tr>
<td>rpm</td>
<td>revolution per minute</td>
</tr>
<tr>
<td>SEM</td>
<td>scanning electron microscope</td>
</tr>
<tr>
<td>SU-8</td>
<td>epoxy-based negative photoresist</td>
</tr>
<tr>
<td>UHMW</td>
<td>ultra-high-molecular-weight polyethylene</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
</tbody>
</table>
Symbols

$\rho$ fluid density (g ml$^{-1}$)

$\mu$ dynamic viscosity (mPa·s)

$U$ bottom moving plate velocity in Couette flow model

$P$ dimensionless parameter in Couette flow model

$b$ confinement height in Couette flow model

$b_1$ matrix layer thickness in Couette flow model

$b_2$ streaming/focusing layer thickness in Couette flow model

$u$ flow velocity (mm s$^{-1}$)

$Q_{\text{total}}$ total volumetric flow rate (μl min$^{-1}$)

$Q_m$ matrix solution volumetric flow rate (μl min$^{-1}$)

$Q_s$ streaming/focusing solution volumetric flow rate (μl min$^{-1}$)

$w$ width of the microfluidic printing nozzle device exit (mm)

$h$ height of the matrix layer /the thickness of the sheet (mm)

$h_{\text{total}}$ height of the confinement (mm)

$v$ conveyor belt velocity (mm s$^{-1}$)

$l_{\text{confinement}}$ confinement length (mm)

$t_{\text{residence}}$ residence time (s)

$p$ upstream applied pressure (psi)

$\Delta p$ pressure drop (Pa)
R  flow resistance (Pa m$^{-3}$ s$^{-1}$)

$R_h$  hydraulic resistance (Pa m$^{-3}$ s$^{-1}$)

V  volume of fluid (ml)

D  physical resistance constant for a specific geometry of the cartridge combined with a specific configuration of the device

$I_{\text{max}}$  maximum current (amp)

$Q_{\text{max}}$  maximum power (w)

$V_{\text{max}}$  maximum voltage (V)

$DT_{\text{max}}$  maximum temperature difference (°C)

D  rotational shaft diameter (mm)

$\omega_{\text{max}}$  maximum rotational speed (rpm)

$\tau$  shear stress (Pa)

$\gamma$  shear rate (s$^{-1}$)

$\sigma$  tensile stress (kPa)

$\epsilon$  strain

$F$  force (N)

$m$  gram force (g)

$A_0$  cross sectional area of the undeformed sheet (mm$^2$)

$l_0$  original sheet length (mm)

$l$  stretched sheet length with the displacement (mm)
Chapter 1

1. Introduction

1.1 Tissue Engineering and Biomaterials

Millions of patients suffer from organ failure or lost as a result of diseases or accidents. Tissue or organ transplantation, as a currently accepted therapy by general, is highly limited by the numbers of available donors. According to Canadian Institute for Health, in 2011, 2115 organ transplants, including kidney, liver, heart, lung, pancreas and intestine transplants were performed in Canada. Unfortunately, another 4543 patients were still waiting for life-saving organ transplants that 265 patients died while waiting. [1]

Tissue engineering, known as constructing man-made tissues and organs using biomaterials, rises as a revolutionary strategy to overcome the issue of organ donor shortage. Polymer scaffold is the main approach in tissue engineering. Variable biopolymers have been utilized to produce gels or hydrogels as the scaffolds to mimic ECM found in tissues. Preparation methods of hydrogels based on different cross-linking mechanisms have been widely discussed and applied in related biological studies.[2] Patients’ cells can be incorporated into the engineered polymer scaffolds, and these functionalized scaffolds enable cell delivery and interaction within patients’ body to promote new tissue formation. [3, 4] Tissues such as artery, cartilage, bone, ligament, skin and blood vessels have been successfully engineered for in vitro and in vivo studies. [5-12]

Hydrogels from natural polymers have been widely applied as the scaffolds for tissue engineering, since they are biocompatible and structurally similar to the macromolecular-based components in human body. [13] A variety of natural and synthetic biomaterials have been applied for constructing different engineering tissues based on criteria such as biocompatibility, toxicity, biodegradability, material chemistry, mechanism of gelling, and mechanical property. Hydrogels made of biomaterials must not cause any damage to cells, promote interaction between cells, obtain adequate adhesion and have no effect on immune response. [14, 15] The biodegradation rate of a hydrogel relates to the way that the material is originated or synthesised, and it is preferably coordinating to the tissue development rate.[16] For the mechanism of gelling, the material degrades as the ions exchange in aqueous environment in the case of ionic
cross-linking, while none degradable covalent crosslinking might contain toxic molecules. [17] The hydrogels should also be mechanically elastic for being subjected to multi-directional stresses as attaching to the surrounding tissues while allowing the tissue growth. [2] Hydrogels of natural polymers such as collagen, gelatin, elastin, Matrigel™, hyaluronate, alginate, agarose, and chitosan are widely used for different cellular biology studies and tissue scaffolding.

Collagen, gelatin, hyaluronate and elastin are tissue-derived natural polymers as components of ECM. These materials are favourably applied since they meet the biological design parameters and requirements better with high biocompatibility. Collagen hydrogels with cultured keratinocytes and fibroblasts included have been developed as skin substitutes to prevent contraction and promote epithelial cell differentiation. [18, 19] Collagen was also used in cell and tissue culture scaffold constructions such as in 3D rotary bio vessels and core-shell stem cell delivery system (in combination with alginate) in bone tissue engineering. [20, 21] As a photopolymerizable material, gelatin was electrospun into fibers for different application such as corneal tissue regeneration. [22-24] Similar electrospinning technique was applied to collagen and elastin combination for smooth muscle cell culture scaffold. [25] However, these natural biopolymers obtain low physical strength, and some of them may be expensive to derive. The same drawbacks apply to fibrin as another natural biopolymer produced from blood, which has been applied for skeletal muscle cell and smooth muscle cell proliferation and migration study. [26, 27]

Alginate from brown algae obtains better mechanical property, low toxicity and low cost as oppose to the above materials. The fast gelation with multiple divalent cations enlarges the number of applications that alginate could be applied to, such as cell encapsulation, [28-31] tissue engineering, [32-35] and drug delivery.[36] However, alginate is lack of degradation control and cellular interaction that lectine or RGD-modified alginate was developed to enhance cell adhesion and proliferation. [37, 38] Agarose is another algae derived polysaccharide hydrogel.[39] This non-biodegradable material is widely used for in vitro non adhesive 3D cell culture with higher mechanical strength that is able to maintain the physical structure and shape for a long term.[40] For example, agarose combined with carbon nanotubes was applied in neural tissue engineering, [41] and agarose microgels were created for stem cell encapsulation. [42]

The preparation methods of different biopolymer hydrogels are discussed in the next section.
### 1.2 Different Gelation Mechanisms

The biomaterials undergo the sol-gel transition and form reversible (physical) or permanent (chemical) hydrogels through different types of cross-linking processes such as physical cross-linking, chemical cross-linking, grafting polymerization and radiation crosslinking. [43-47] For various of natural polymers and biological applications that require reversible (physical) hydrogels, physical crosslinking mechanisms including ionic interaction gelation and thermal induced gelation are applied due to the ease of gelation without cross-linking agents. Chemical cross-linking mechanisms, including pH induced gelation, applies to polymers containing functional groups (OH, COOH, etc.) that are able to react with cross-linkers to form permanent gels. Radiation crosslinking such as UV induced gelation is preferable for processes that are required to be free of additives. [43] Gelation takes place when single or multiple biopolymers are exposed to one or additional types of stimuli depending on the physical and chemical properties of the materials.[48]

Alginate gelation is an example of ionic interaction cross-linking process that the guluronic acid groups (G blocks) from sodium alginate exchange with cations (Ca\(^{2+}\), Ba\(^{2+}\), etc.) from an aqueous solution.[49] Solutions containing cations such as Ca\(^{2+}\) ions in calcium chloride solution was commonly used as the source for ionic exchange. The divalent cations bind to the G block of the alginate chains, and the G block form an adjacent polymer chain binds to this junction to form the egg-box-like structure as a gel structure.[50, 51] Alginate fibers, sheets or layered structures were produced based on this material chemistry for different applications. [52-54]

Gelation of natural materials such as agarose, collagen, and gelatin can be induced by a temperature change. All types of agarose go through gelation when being cooled down from a heat-up temperature higher than melting temperature. For example, the SeaPrep\(^{TM}\) Agarose melts at 40ºC-50ºC and gels below 17ºC as a suspension medium for cells, and the gelling and melting temperatures are functions of the agarose solution concentration.[55] Agarose gelling time has been reported experimentally that it varies from 10mins (for droplets) to 1 hour (1.5mm thick gel film) depending on the concentration. [42, 56] Gelatin obtains similar behavior of gelling when being cooled down from above 35ºC (sometimes high up to 60ºC) to 15ºC or even lower temperatures as thermo reversible gels.[57] On the other hand, some thermal sensitive natural materials such as elastin and collagen remain liquid at low temperature at 4 ºC, when being
brought up to physiological temperature of 37 °C (incubator condition), gelation takes place as a slow process that it may take up to hours to mold a collagen scaffold, since the collagen gelation was determined to be a function of not only temperature but also pH, concentration and ionic strength. [58-60]

Photocrosslinkable materials, such as chitosan, go through gelation when exposed to UV light that the process is initiated by the high energy source. Depending on the composition and pairing material, chitosan gel may obtain different physical properties with different exposure time. [61] [62]

1.3 Current microfluidic tissue engineering approaches and 3D Printing Technologies

1.3.1 Related Works Using Microfluidic Approaches

Microfluidic tissue engineering has been a promising strategy for constructing geometries and scaffolds at different dimensional space for different interests of study and applications. Capillary microfluidic devices with confining channels of stable biphasic flows were applied for one-step emulsification of multiple concentric shells.[63] Different diameters of oil/water emulsion droplets were formed through control over the flow rates of the fluids. Moving onto fiber-shaped constructs, microfluidic devices with double-coaxial laminar flow were used for fabricating meter-long core shell Ca-alginate hydrogel microfibers for encapsulating ECM proteins and differentiated cells.[64] Similarly, microfluidic spinning chip and digital flow controller were applied for fabricating alginate microfibers with tunable morphology, structure and chemistry for spatially controlled co-culture of encapsulated cells.[65] Also, microfluidic mimic of the tortuous channels was developed as a modular tissue engineered construct for studying endothelial cell behaviour under shear stress and flow obstruction.[66] Furthermore, microfluidic devices with multi-layer channel networks were designed and applied for one-step alginate sheet and tube formation. Scalable hydrogel sheets and tubes can be extruded continuously with control over the microscale composition for altering the bulk material properties and applied as functional tissues with different cells incorporated in the scaffolds. [67, 68]
1.3.2 3D Bioprinting

3D Bioprinting technology has been commercialized and implemented for producing engineered tissues as 3D structures containing cells. Most available 3D bioprinters adapt the concept of direct writing and can be classified into three techniques: laser-based writing, inkjet printing, and extrusion-based deposition.[69]

Lase based writing involves the usage of laser energy to excite cells suspended in the donor slides and to propel the cells to the collector slides upon the shock wave generation by bubbles created by the laser pulse. Depending on the viscosity of the biomaterial, printing speed, laser energy and pulse frequency, cell patterns in microscale can be achieved, followed by deposition of layers of hydrogel on top of the cells. For example, hepatocytes were patterned between collagen and Matrigel layers using laser-guided 3D cell writing. [70]

Inkjet printing is a technique that prints cells incorporated in droplets through cartridges with biomaterials as oppose to seeding onto layers of bio scaffolds in the case of laser writing. Similar to graphic inkjet printers using printing ink, the bio ink containing biomaterials and cells was printed from the printhead based on the digital data of a tissue’s geometry that there is no direct contact made with the cells as they are embedded in orifices of the biomaterial. For example, alginate microspheres with cells encapsulated were jetted to form a vertical short tubular structure.[71] Adjustable printing parameters include cell concentration, drop volume, printing resolution, nozzle diameter and average diameter of printed cells.[72]

Extrusion based printing is a combination of fluid dispensing system for extrusion and automated three-axis robotic system for printing.[73] The biomaterial containing cells is dispensed by pressure driven systems and extruded in the form of cylindrical filaments to form desired geometry through automatic robotic movements of the nozzle tip. For example, artificial liver was engineered using this method with hepatocytes and adipose-derived stromal cells in gelatin and chitosan hydrogels.[74] Material flow rate, concentration, viscosity, pressure and nozzle geometry are the parameters to be optimized for better printing result and cell viability.

The advantages and drawbacks of each technique are listed in Table 1.
### Table 1 Advantages and drawbacks of three bioprinting techniques

<table>
<thead>
<tr>
<th></th>
<th>Advantages</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Laser-based Writing</strong></td>
<td>-Multiple cell types</td>
<td>-Long fabrication time</td>
</tr>
<tr>
<td></td>
<td>-High resolution (5.6±2.5µm) [75]</td>
<td>-Cell deformation due to laser shock</td>
</tr>
<tr>
<td></td>
<td>-Precise patterning</td>
<td>-Light and gravity effect on cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Lack of third dimension</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Photo-crosslinkable biopolymers only</td>
</tr>
<tr>
<td><strong>Inkjet Writing</strong></td>
<td>-No direct contact with cells</td>
<td>-Cell damage</td>
</tr>
<tr>
<td></td>
<td>-High cell viability</td>
<td>-Sedimentation and aggregation</td>
</tr>
<tr>
<td></td>
<td>-Maintained cell phenotype</td>
<td>-Low density (&lt;5 million cells/ml) [72, 76, 77]</td>
</tr>
<tr>
<td></td>
<td>-Single cell printable</td>
<td>-Poor structural integrity</td>
</tr>
<tr>
<td></td>
<td>-Multiple cell types</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-No flat surface necessary</td>
<td></td>
</tr>
<tr>
<td><strong>Extrusion-based Deposition</strong></td>
<td>-Better structural integrity</td>
<td>-Cell deformation due to shear stress</td>
</tr>
<tr>
<td></td>
<td>-Rapid fabrication</td>
<td>-Limited materials</td>
</tr>
<tr>
<td></td>
<td>-Scale-up approach</td>
<td>-Low resolution</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-High viscosity desired but cause clogging</td>
</tr>
</tbody>
</table>

### 1.3.3 Drawbacks and Limitations of Current Approaches

Different biopolymer processing and scaffolding techniques have been discussed in the previous sections. Although each strategy has its own advantages, there is no perfect approach that certain limitations were discovered about the current technologies, including lack of integrity and precision, long fabrication time with multiple steps, low flexibility of forming different geometries, lack of control over the product properties and targeting only limited numbers of materials due to the cross-linking mechanism. Some of the strategies are non-continuous process that does not allow high throughput. The multi-step process is sequential and time consuming, such as the layer by layer stacking approach, which makes it difficult to scale up the construction to tissue level. Some of the strategies require manual manipulation that process is restricted to either continuous or sequential manner that different macro geometries cannot be achieved, and some strategies cannot produce intact geometries on site initially that long culture time is required at post-production stage. The range of materials each strategy can process is limited that only one type of material chemistry and gelation mechanism can be accommodate since different stimuli (ionic exchange, thermal gradient, UV exposure) are required to enlarge the number of materials that could be processed. In addition, some of the approaches are limited in the fine control over the material composition and heterogeneity; they are lack of dynamic control and organization.
1.4 Thesis Organization

A microfluidic bioprinter that is compatible with a wide range of biopolymers and is able to continuously or periodically produce hydrogel sheets through different types of cross-linking mechanisms with different throughputs is presented in this document. The hydrogel formation is achieved through a process illustrated in Figure 1. The biomaterial is pressurized and transferred from the cartridges to the microfluidic device through pressure control, followed by landing onto the conveyor belt and forming hydrogel sheets that are transferred and processed later by the conveyor belt. Depending on the material being processed, thermal control may be activated for producing desired temperature upstream. Upon exiting the microfluidic device, the flow on the moving belt with a fixed height confinement on top is a well-defined Couette flow. Theoretical analysis and prediction of the produced sheet geometry were generated involving the following experimental parameters: exit width of the microfluidic device, confinement height, matrix biopolymer flow rate, focusing fluid flow rate, and conveyor belt velocity. For a fixed device exit width and a fixed confinement height, varying the flow rates and the conveyor moving speed alters the produced sheet thickness and the mechanical property.

The coming chapter presents the design and the fabrication of the bioprinter and the microfluidic printing nozzles, followed by materials and methods applied for the hydrogel printing process. The next chapter presents the specification, functionality and validation of the features included in the printer. In the fifth chapter, experimental results and characterization of the hydrogel printing are presented, including the Couette flow model for printing parameter and produced sheet geometry prediction, homogenous and heterogeneous sheets printing using the pressure control setup, measured geometry and mechanical properties of sheets printed under various printing parameter conditions.
Figure 1 Schematic of the working principle
Chapter 2

2. Microfluidic Bioprinter Design

2.1 Microfluidic Bioprinter Design and Fabrication

In this section we describe the design, fabrication and validation of the microfluidic bioprinter consisting of: cartridges that contain different biomaterials and streaming fluids, microfluidic devices that define the geometries of the extruded hydrogel sheets, a conveyor system that enables the production and transportation of the hydrogel sheets, the temperature control features, the pressure control that provides adjustable volumetric flow rates and a reservoir as the frame of the setup. Figure 2 illustrates the schematic of the printing and a rendered image of the microfluidic bioprinter design.

The individual parts are described in detail in Appendices 1-7, including the reservoir as a chassis for all the other features (Appendix 1), the motor and associated mounting features (Appendix 2), the conveyor belt specifications (Appendix 3), the printing cartridges (Appendix 4), the thermal manifold (Appendix 5), the temperature control features (Appendix 6) and the pressure control setup (Appendix 7). Custom designed parts were manufactured at the university Machine Shop (Department of Mechanical Engineering, University of Toronto).

The microfluidic bioprinter design is potentially compatible with different hydrogels with or without cells incorporated into the biomaterials as the ink for printing. Therefore biocompatible and sterilizable materials are used. Interchangeable and removable modules are preferred for the flexibility of usage and the ease of assembly. Controls over temperature, pressure, position and movement are integrated into the design.
Figure 2 (a) Schematic of hydrogel sheet formation in Couette flow; (b) Rendered 3D image of design of the microfluidic bioprinter
The reservoir acts as a chassis that secures the positions of the upstream printing cartridges, microfluidic printing devices and the conveyor belt. Figure 3 b-e show the key features of the reservoir assembly. The reservoir is accessible from the side for cleaning and maintaining purposes (Figure 3c), and it allows the rotation of the conveyor belt to have an adjustable contacting angle with the microfluidic devices (Figure 3d-e). A NEMA 17 bipolar stepper motor (Phidgets Inc., Calgary, AB, Canada) was mounted to the conveyor to drive the motion of the belt (Figure 3f). Temperature features include an aluminum manifold for holding the cartridges and device (Figure 3g) and a cooling assembly on the bottom (Figure 3h). Different sizes of printing cartridges (Figure 3i-j) were designed to load variable materials in different volumes. Such assembly allows the temperature control and pressure control over the biomaterials, which will be discussed in the printer characterization section.
2.2 Printing Nozzle: Microfluidic Device Design, Fabrication and Characterization

Microfluidic devices with multiple layers of microfluidic channel networks are used as the printing nozzles. The continuous flows through the microfluidic channels enable the formation of soft hydrogel sheets and the control over the microstructures and properties of the sheets produced. The biomaterials exit the device and land onto the conveyor belt to form sheets in Couette flow condition through solidification process. (Figure 4a) In order to achieve this process, confinement on the left, the right and the top of the exit cross-section of a device is required. (Figure 4c) There are two fluid layers in the gelation process: a focusing or crosslinking fluid on the top right under the confinement on the top, a matrix fluid layer on the bottom in contact with the surface of the conveyor belt. (Figure 4b) The footprint of the focusing fluid channels matches the one of the matrix fluid channels so that the entire bottom matrix stream is fully in contact with the top focusing stream. To avoid the gap between the exit of the device and the moving belt so that the biomaterials are able to be transferred right upon leaving the channels, the bottom layer of the device needs to be as thin as possible while maintaining the sealing property. The channels are designed in a way that the pressure ratio in the transferring tubings to the device is low so that bubbles can be easily pushed out at the beginning stage of the printing to shorten the initialization and stabilization time.

8mm, 10mm, 25mm, 42mm and 70 mm wide devices were designed, fabricated and applied as the printing cartridges for the experiments that will be discussed in the next chapter. All the rectangular channels are 150 µm high (Figure 4b), and there are three sets of channel versus spacing dimensions assigned to the designs within the devices depending on the resistance and resolution requirements, and they are listed in Table2. The resistance calculation of the channel geometry and the device designs are illustrated in Appendix8.
Figure 4 (a) Schematic of flows going through and exiting the microfluidic device printing nozzle; (b) Section view of a three-layer device; (c) Confinement implementation at the exit of the device
<table>
<thead>
<tr>
<th>Device width</th>
<th>Channel width (µm)</th>
<th>Spacing width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mm</td>
<td>300</td>
<td>235</td>
</tr>
<tr>
<td>25mm</td>
<td>420</td>
<td>100</td>
</tr>
<tr>
<td>70mm</td>
<td>1000</td>
<td>800</td>
</tr>
<tr>
<td>8mm Triple Matrix</td>
<td>200</td>
<td>150</td>
</tr>
</tbody>
</table>

**Table 2** Summary of microfluidic device geometry

The microfluidic channel networks were designed using computer aided design software (AutoCAD), and the high precision transparent photomasks containing the designs were printed (CAD/Art services, Brandon, OR, USA) for fabricating the masters.

Standard soft lithography was used to transfer the channel network designs printed on the transparency masks onto masters that are required for device molding.[78]. Glass slides with sizes 75mm×51mm×1mm or 102mm×75mm×1mm were cleaned with acetone and isopropanol, followed by dehydration on hot plates (HP30A, Torrey Pines Scientific, San Marcos, CA, USA) at 180°C for 1 hour. The glass slides were then placed in oxygen plasma (PDC-32G, Harrick Plasma, Ithaca, NY, USA) and treated for 1min. After plasma treatment, a “seed” layer of the negative photoresist SU-8 25 (Microchem, Newton, MA, USA) was spun onto the slides at 2000rpm for 30s with an initial acceleration rate of 300rpm/s for 5s (startup stage at 500rpm for 10s with an initial acceleration rate of 100rpm for 5s), and slides were exposed to UV light with the intensity of 192mW/cm² and the wavelength of 365nm (Model 200, OAI, San Jose, CA, USA). After being baked at 95°C for 3mins, the cooled slides with hardened “seed” layer were spun with SU-8 2050 twice at 1900rpm for 30s with an acceleration of 280rpm/s for 5s (startup stage at 500rpm for 5s with an initial acceleration rate of 100rpm for 5s), which gives 150µm thick feature layers. The glass slides were then baked at 95°C for 1 hour and exposed to the UV light at the exposure energy of 220mJ/cm² and the wavelength of 365nm. After 15mins of baking and cooling, the glass slides were developed using SU-8 developer for 10mins. The channels remained as the features on the slides, while the rest of the SU-8 2050 coated on the slides were developed and washed away. The slides can then be used as the masters for fabricating layers of features for the microfluidic devices.
Multilayers of microfluidic channel networks were patterned in polydimethylsiloxane (PDMS) and bonded into a sealed microfluidic device using a partial curing and bonding technique.[79] This technique allowed the accurate molding of 150μm high channels in multiple layers across the thickness of the device. The elastomer was mixed with 10 % w.t. of curing agent (Sylgard 184 silicone elastomer base and curing agent kit, Dow Corning Corporation, Midland, MI, USA) and degassed in a vacuum chamber at the gauge pressure of -25mmHg and the temperature of 25°C for 1 hour. The uncured PDMS mixture was then poured over the glass masters followed by degassing for 15mins to remove any air bubble between the glass mater surface and the viscous elastomer on top. For the top layer focusing fluid channel design molding, more PDMS mixture was applied for having a thick layer that is later fully cured in the oven at 80°C for 20mins. The thick top layer allows the capillary tubing (1.6mm diameter) insertion easier to be secured with conical through-holes punched through the molded piece that was peeled off from the glass mater. For the lower matrix layers, the mixed elastomer was poured on the masters and spun at 400rpm for 30s with an acceleration of 40rpm/s for 5s (startup stage at 200rpm for 5s with an initial acceleration rate of 40rpm for 5s) to obtain a uniform thickness of 200μm, and the curing time was shortened to 8mins under the same baking condition to have them partially cured and sticky. Fully cured top layer was placed with care on top of the partially cured layer according to the alignment features in the design. The combined layers were then further cured for 15mins at 80°C. The combination of completely cured layer and partially cured layer guarantees reliable bonding between layers with desired sealing property. Such sequence was repeated for each lower layer with a step of peeling and punching internal through-holes for inlet tubings in between the bonding operations.

The device was finally sealed with a bottom plain PDMS layer without pattern. As discussed above, the bottom layer needs to be as thin as possible while it seals the channels. In order to produce such a layer, a glass slide master was fabricated by coating it with only a “seed” layer as described in the master fabrication session. This allows the peeling of cured PDMS on top of the slides. PDMS was spun to 200μm on top of the “seed” layer and partially cured for 8 minutes at 80 °C. The bonded layers of features were then gently placed on top of the partially cured bottom layer for final curing and bonding of the device.

Capillary tubings (IDEX Health & Science) were inserted into the punched holes and secured with epoxy (Lepage Professional speed set, Henkel, Mississauga, ON, Canada) applied around
the insertion holes. The entire device was then covered with extra PDMS and baked overnight. The fully baked device was then peeled off from the glass master and ready to be used.

To implement the confinement at the exit of the printing nozzle, the device was cut using a scalpel at the channel exit that only the end of the channels were exposed to the atmosphere where individual streams from each channel were merged into one planar flow stream and form a sheet upon gelation. The regions on the left and the right of the exit were remained so that the confinement, a 0.51mm thick transparent polycarbonate sheet with the width same as the channel exit, was inserted into the cut-out 1mm to 2mm from the bottom of the device.

The multilayer devices fabricated using the above protocol were tested to be reliable and able to maintain the sealing property when introducing fluids using both syringe pumps and gas pressurization method up to 15psi.

Device fabrication was carried out at the Centre for Microfluidic Systems at the University of Toronto.
Chapter 3

3. Materials and Methods

For characterization of the bioprinter performance, a biopolymer with a fast gelation process that instantaneously forms hydrogel sheets upon exiting the printing nozzle, i.e. contacting the crosslinking fluid, is chosen as the working fluid. Sodium alginate is a biopolymer that gels at room temperature under mild conditions without generating toxic components; therefore it is frequently used in tissue engineering and cell encapsulation.[80] As discussed in the first chapter, alginate acid is an unbranched binary copolymer with guluronic acid monomers (G blocks) and mannuronic acid monomers (M blocks) that the G blocks bind with the divalent cations to form the “egg-box” structures. [50, 51] The percentage of G blocks within the alginate and the degree of binding define the porosity, the mechanical strength and the swelling of the gel. To understand the gelation dynamic between the alginate and the cations such as Ca$^{2+}$, magnetic resonance imaging and numerical simulation of reaction-diffusion of calcium into alginate were generated in previous studies. [81, 82] It was estimated that for a 100µm thick 2%w.t. alginate gel, complete gelation takes approximately 15mins. Alginate is a suitable candidate material for this study since the ionic exchange process happens rapidly with Ca$^{2+}$ cations to induce the gelation without any additional stimulus. Without introducing thermal system or UV light source as for thermally crosslinkable and photopolymerizable materials, alginate simply gels in a fast manner when contacting the Ca$^{2+}$ cations at the interface to form the overall geometry before the complete crosslinking is finished.

For thermally cross-linking materials such as collagen and agarose, the gelation is relatively less rapid that forming hydrogel in moving condition instead of molding scaffolds in static condition is more challenging. For agarose, the gelling and melting temperatures vary depending on the type of agarose used.[55] The gelling time has been reported experimentally that it varies from 10mins (for droplets) to 1 hour (1.5mm thick gel film) depending on the concentration.[42, 56] Collagen remains liquid at low temperature at 4 ºC, when being brought up to physiological temperature of 37 ºC (incubator condition), gelation takes place as a slow process that it may take up to hours to mold a collagen scaffold, since the collagen gelation was determined to be a function of not only temperature but also pH, concentration and ionic strength. According to previous studies, the turbidity measurements have shown that higher initial collagen
concentration, higher temperature, and weaker ionic strength give shorter precipitation time of collagen down to 10mins when the parameters are at the extreme conditions. However the effect of pH level on the precipitation time has not reached agreement that some study indicated that lower pH value (when 6≤pH≤12) gives shorter gelation time, while some reported the reserve trend. In general, the expected gelation time for 1.5ml collagen at different concentrations may vary from 10mins to 4hours. [58-60]

Therefore aqueous alginate solution and calcium chloride solution were chosen as the matrix solution and the cross-linking solution respectively for all the validation and characterization experiments, unless otherwise stated. For temperature induced gelation experiments, SeaPrep agarose (Lonza Group Ltd, Basel, Switzerland) was chosen for related testing, as 4%w.t. SeaPrep agarose solution melts at 70 ºC and gels at 25 ºC approximately.

For solution preparation, 2% w.t. of alginic acid sodium salt (Sigma-Aldrich) and 100mM CaCl2 (Sigma-Aldrich) were dissolved in deionized (DI) water at room temperature prior to the experiments. For visualization purpose, different colours of food dyes (ClubHouse, La Cie McCormick, London, ON, Canada) were added to the solutions. For fluorescent imagining of heterogeneous sheets using fluorescent microscope (Nikon Eclipse Ti), 1% (v/v) of 0.1 µm carboxylate-modified microspheres in yellow-green, nile red and blue (FluoSpheres, Life Technologies, Burlington, ON, Canada) were added to the alginate solutions before the experiments. For thermally sensitive solution, 4% w.t. SeaPrep was dissolved in DI water that was previously heated up to 80 ºC. The solution was quickly transfer using syringe to the previously heated cartridge on the manifold at 80 ºC to maintain at aqueous state without gelation.

The matrix and cross-linking solutions, as the inks for the printing process, were introduced into the microfluidic device that was clamped onto the aluminium manifold through two methods: using the syringe pumps (PHD 22/2000, Harvard Apparatus, Holliston, MA, USA; neMESYS, centoni GmbH, Korbussen, Germany) to directly flow the solutions through the channels from glass syringes (SGE Analytical Science) , or using the pressure control setup to pressurize the printing cartridges that were pre-loaded with the biopolymer solutions to flow the solutions from the cartridges to the device. The pressure control setup will be discussed later in the coming section. The alginate solution on the bottom was then in contact with the CaCl2 solution on top
that at the interface, ionic exchange happened right away and penetrated through the thickness of the alginate solution layer when two fluids left the printing nozzle. The solutions landed onto the conveyor belt that was set to be horizontal and contacting the exit of the printing nozzle with a minimal gap, and formed hydrogels continuously under the confinement which established the Couette flow condition (discussed in the next chapter). The flow rate of the matrix solution, $Q_m$, and the flow rate of the streaming solution, $Q_s$, can be varied by adjusting the syringe pump diffusing rate or the gas pressure level, and the conveyor belt moving velocity, $v$, was adjustable as well. All the parameter assignments can be achieved through LabVIEW control interface, and the input values for all the parameters can be predicted using Couette flow theoretical model that will be presented in the next chapter. By using microfluidic devices with different channel network designs as the printing nozzle and tuning the multiple parameters that the users could control, homogenous and heterogeneous hydrogel sheets with different geometries can be produced.

For the experiments discussed in this document, the alginate samples were collected manually downstream and stored in Petri dishes filled with 100mM CaCl$_2$ solutions and sealed with plastic paraffin film (Parafilm, Pechiney Plastic Packaging Company, Chicago, IL, USA).

For sample geometry characterization, the samples were wrapped around a glass slide and placed onto a microscope (Nikon Eclipse E600) for bright filed imaging using 1x microscope objective (Plan Achromat UW, Nikon, Melville, NY, USA) and high resolution camera (PCO. Imaging Kelheim, Germany). The dimensions of the sheets were determined based on the bright field images using image processing and analysis software (ImageJ). At least five samples of each kind were imaged and analysed with at least ten measurements for statistical significance, and the average values and standard deviations were reported. For heterogeneous samples, since the solutions were loaded with microspheres prior to the experiments, the printed patterns were imaged using florescent microscope.
For elastic modulus study of the produced samples, a replica of the multi-functional biaxial stretching (BAXS) platform designed by Dominique Tremblay and Andrew Pelling from the Centre for Interdisciplinary NanoPhysics at the University of Ottawa was applied for the tensile tests of the hydrogel samples.[83] (Figure 5) A 2%w.t. alginate sheet with a width smaller than 10mm and a length longer than 15mm was taken out from the 100mM CaCl₂ storage solution, clamped onto one axis of the stretcher and pulled at a displacement rate of 0.05mm/s until breakage with a 0-50g load cell attached to the axis of movement. Simultaneous data acquisition of position and load was done via LabVIEW program. The displacement-force data were then converted into stress-strain curves based on the following equations:

\[
\sigma = \frac{F}{A_0} = \frac{mg}{wh} \tag{3.1}
\]

\[
\varepsilon = \frac{l - l_0}{l_0} \tag{3.2}
\]
Where $\sigma$ is the stress, $F$ is the force that is calculated based on the gram force $m$, $A_0$ is the cross-sectional area of the undeformed sheet with width of $W$ and thickness of $h$, $\varepsilon$ is the strain, $l_0$ is the original sheet length, $l$ is the stretched sheet length with the displacement. Therefore the elastic modulus can be calculated as

$$E = \frac{\sigma}{\varepsilon}$$

(3.3)

At least five samples of each kind were tested for statistical significance. The average values and standard deviations were reported. The replicated BAXS platform is illustrated in Figure 5, and the detailed sample clamping procedures are described in Figure 6.
Figure 6  Detailed procedures of hydrogel clamping. Step 1-12 illustrate the novel method of hydrogel sample clamping. 1: A Petri dish containing a piece of sponge inside was wrapped with rubber latex as a mini sample loading stage. This surface allows the sample to be able to hold the liquid content without getting dry while being prepared for the test, and the softness provided by the sponge makes the handling easier without destroying the sample. 2: A sample was transferred from the liquid onto the stage and flattened using a pair of tweezers with rubber tips. 3-4: 100% polypropylene white wiper (KIMTECH) was cut into 1cm by 1cm squares as the material to sandwich the samples in between from the ends. The wiper is absorptive that the square pieces can attach to the samples well. 5-6: The sandwiched sample was then transferred from the stage to the stretcher using two pairs of tweezes holding from both ends. 7: One sandwiched part of the sample was fed onto cylindrical part of the clamp. 8: The other sandwiched end was fed onto the other cylindrical roller. 9: Adjust the vertical position of the two clamps so that the sample is held horizontally. 10: Tighten the two screws on each clamp to lock the sandwiched samples in place against the grooves on the cylinders. The polypropylene wiper distributed the clamping force from the two screws and prevented the samples from being pinched broken. 11. Lock the motor after the sample was distanced just under stretching. 12. Turn on the motor and data acquisition system to start stretching. It is also a side view of how the sample was clamped onto the platform.
Chapter 4

4. Printer Specification, Functionality and Characterization

The hydrogel formation is achieved through a simple process. The biomaterial is pressurized and transferred from the cartridges to the microfluidic device, followed by landing onto the conveyor belt and forming hydrogel sheets that are transferred and processed later by the conveyor. In order to achieve this process, related functions are defined and validated.

Motion control of the conveyor belt guarantees the automatic collection and transportation of the hydrogel sheet, as the belt is the main platform where the gelation takes place. Motor and controller were selected accordingly to mobilize the belt with different speeds.

Pressure control is the key to the transportation of the liquid materials. Knowing the pressure applied upstream will give the liquid exit velocity and flow rate. Therefore the flow rate can be altered by adjusting the pressure level. A pressure regulator, solenoid valves, multi-channel gas control manifold, and related controllers were selected to build the pressure control loop.

Temperature control is required for thermal gelation. Adding related features and controls enables the different gelation mechanism and enlarge the range of materials that can be applied for gelation using this bioprinter.

4.1 Motion Control

The NEMA-17 motor (Phidgets Inc., Calgary, AB, Canada) with an integrated planetary gearbox (ratio 99\( \frac{1044}{2057} \):1) was attached to the conveyor to drive the motion of the belt. At the maximum current of 1.6 amps, the gear box produces 48 kg-cm of continuous torque, and the maximum axial load and the maximum radial load of the shaft are 49.1 N and 98.1 N respectively. The motor was mounted 13 cm away from the conveyor with a driving shaft of 3.6 cm in diameter, so the distance the conveyor belt travels within one rotation of the shaft is:

\[
d = \pi D = \pi \times 35.56 \text{mm} \approx 110 \text{mm}
\]

The maximum rotational speed (\( \omega_{ma} \)) is 44 rpm with 1067 motor controller (Phidgets Inc., Calgary, AB, Canada). Therefore the maximum speed of the belt is:

\[
\nu_{max} = \omega_{max} \times d = (44 \text{rev/min}) \times (111.715 \text{mm/rev}) \times (1 \text{min/60s}) = 81.924333 \text{mm/s} \approx 8.2 \text{cm/s}
\]
At the output of the gearbox, the step angle is approximately 0.018°, so the resolution of the movement is calculated to be:

\[
\text{Step size} = \left(\frac{\text{step angle}}{360°}\right) \times D = \left(\frac{0.018°}{360°}\right) \times 35.56\text{mm} = 0.0056\text{mm} = 5.6\mu m \text{ per step}
\]

Validation experiments were generated using the materials and methods described in the previous section, and a 25mm wide microfluidic device with the confinement of 2.2mm gap size was used as the printing nozzle. The conveyor belt movement was set to be 2mm/s away from the nozzle. The flow rates of 500µl/min for both the alginate solution and the CaCl\textsubscript{2} solution were generated using the neMESYS syringe pump. The printed sheets were measured to be 0.34mm thick with a standard deviation of 0.053. When the flow rate of the alginate solution was lowered to 400µl/min while that for CaCl\textsubscript{2} solution was adjusted to 600µl/min for maintaining the same total flow rate, the sheet thickness was reduce to 0.28mm with a standard deviation of 0.078.

It was observed that when the nozzle exit was not perfectly contacting the moving belt, there is leaking and back flow of less viscous fluids. For example the CaCl\textsubscript{2} solution obtains similar viscosity as water, and a bad contact between the exit with the side confinements and the belt causes disturbed flow and leakage. Therefore extra weight on top of the printing nozzle device will improve the flow control. Further permanent solutions such as hard plastic device with a longer and more stable confinement on the side and the top shall be investigated for producing better contact and more stable flows.

![Image](image.png)

**Figure 7** Illustration of a 25mm 2%w.t. alginate hydrogel sheet being printed onto the moving conveyor belt, scale bar 10cm
4.2 Pressure Control

In order to achieve the pressure control over solutions loaded in the cartridges, 0-15psi pressure regulator (Type3410, Marsh Bellofram, Newell, WV, USA), solenoid valves (The Lee Company, Westbrook, CT ,USA), single or multiple outlet manifolds (McMaster-Carr, Aurora, OH, USA) and pressure controller (National Instrument, Austin, TX, USA) were connected using polyethylene pipes (The Lee Company, Westbrook, CT ,USA) of 6.4mm in outer diameter. The pressure level can be adjusted by assigning different levels of voltage to the pressure regulator, and the solenoid valves allow the opening and closing of each gas line individually. Both controls were programmed in the measurement and control system software (LabVIEW) with the help from Haotian Chen. A pressure gauge (MG1-5-A-9V-R, SSI Technologies, Inc, Janesville, WI, USA) was connected right before the gas outlet for measuring the end-of-system pressure, and a sealed glass tank was connected to the regulator to eliminate the instability built within the control system piping when pressure changes took place. Figure 8 illustrates the sequence of the connections of the pressure control system. In this document, all the pressure levels are presented in psi to agree with the actual experimental setting conditions for accuracy purpose.

Figure 8 Schematic of the pressure setup
Using such pressure control system, the accuracy of the regulated pressure was experimentally determined to be acceptable as having errors ranging from 0.13% to 5.6% for pressure levels between 1psi to 15 psi. For pressure levels lower than 1psi, the error may go up to 10%.

Even though the stabilization time for pressure change is shorter when using gas pressurization method comparing to using Harvard apparatus syringe pump and neMESYS syringe pump, individual pressure regulators are required for each different level of pressure that is regulated, so that different flow rates can be assigned to different solutions.

The cartridges were tested in terms of liquid sealing performance and gas sealing performance. The cartridges filled with deionized water were connected to the pressure line, and the pressure level was adjusted from 5psi to 15 psi. There was no leakage observed. For gas sealing test, the empty cartridges were pressurized while being submerged in water. No bubble appeared up to pressure level of 13psi, which indicates acceptable gas sealing performance. For pressure higher than 15psi, the hex head plugs and the outlet vacuum tight fittings at the outlet experienced high stresses that minor gas leakage appeared at the threads.

![Figure 9](image.png)  
*Figure 9* Illustration of the cartridge sealing test (a) liquid sealing (b) gas sealing
To validate the pressure control system and to determine if it is compatible with the printing process, experiments were generated using the materials and methods described in the previous section and a 10mm wide device with 1mm confinement height. The crosslinking stream of CaCl$_2$ solution was introduced using syringe pump at a flow rate of 500µl/min, and the alginate solution was loaded into the single lane cartridge and pressurized under a pressure level of 1psi. The $Q_m$ was later determined to be 410 µl/min based on the time it took to run out the fixed volume of solution loaded into the cartridge. The conveyor belt was moving at a speed of 3.0mm/s. The continuously produced sheet was cut into multiple pieces for thickness measurement and consistency evaluation. The average thickness of the ten samples was 0.26mm, and the error was within 40µm as illustrated in Figure11.

Figure 10 Illustration of a 10mm 2%w.t. alginate hydrogel sheet being printed onto the moving conveyor belt using pressure control and cartridge, scale bar 10cm

Figure 11 Measured thickness of samples printed under the condition of $Q_s$=500µl/min, $p$=1psi, $Q_m$=410µl/min, $v$=3mm/s
4.3 Temperature Control

Thermoelectric element HP-199-1.4-0.8-Potted (TE technology, Traverse City, MI, USA) was used as the thermal source to control the temperature of the materials upstream. The Peltier thermoelectric high performance heater/cooler operates from -40°C to 80°C with internal solder type 58/42 Bi/Sn of solder melting temperature at 138°C. The element is reversible depending on the direction of the current through the module. As a result, the hot side and the cold side can be reversed based on the direction of the heat conduction through the wire.

By applying a voltage difference, heat is pumped towards assigned direction. In the case of heating, heat is continuously pumped towards the hot side to heat up the target. However for cooling, while the heat is pumped away from the cold side to the hot side, an effective heat remover is required to remove heat on the hot side efficiently and constantly so that the cooling can be maintained. In order to use the thermal element for both heating and cooling, a heat sink is required on the side that is not contacting the target objects to maintain the temperature gradient across the element. A cooler (Seidon 120M model RL-S12M-24PK-R1, Cooler Master, Richmond, BC, Canada) that consisted of a fan and liquid cooling system was attached on the bottom of the thermal element. In addition, better surface contact and high pressure compression clamping (500-1200kPa) between the off target side of the thermal element and the cooler are suggested for less heat loss and more effective heat removal. Therefore carbon-based thermal compound (Arctic Cooling AC-MX4, Arctic Switzerland AG, Switzerland) and hex socket cap machine screws (Plastic Nuts and Bolts, Edneyville, NC, USA) were used to assemble all the temperature control related features.

Single thermoelectric element performance was tested using an adjustable power supply (PWI-AC120LE, Prudent Way, Walnut, CA) for different levels of voltage applied. Table3 lists the testing result of the temperature difference between the hot side and cold side under each voltage condition.
**Figure 12** Illustration of temperature control feature, the thermoelectric element clamped between the manifold (copper plate extension feature) and the cooler plate

<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>Hot Side (°C)</th>
<th>Cold Side (°C)</th>
<th>Difference (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>31</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>15</td>
<td>31</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>16</td>
<td>32</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>18</td>
<td>34</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>19</td>
<td>34</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>24</td>
<td>36</td>
<td>12</td>
<td>24</td>
</tr>
</tbody>
</table>

**Table 3** Measured thermoelectric element performance under different voltages

**Figure 13** Illustration of the thermal performance test, heat pumping direction as marked and thermal sensor attachment
The appropriate input voltage was decided based on the hot side temperature of the module. An approximate rule to follow is to use an input voltage that is no more than 75% of $V_{\text{max}}$ when the module is used with a typical fan-cooled heat sink. For this module, it means that the input voltage should be no more than 18 V. In order to take advantage of the full capacity and to increase the performance of the thermal element, two power supplies with 18V of voltage and 4-6A of current each were applied parallelly to power the element that was attached to the bottom of the thermal manifold. A thermal couple (3109_0 - TPK-01G Bead Probe K-type Thermocouple, Phidgets Inc., Calgary, AB, Canada) was attached to one of the device clamping feature in the center region of the aluminum manifold with the plastic cover on for sensing the actual temperature and creating a feedback signal to the control system. The system ran for more than 1 hour for both heating and cooling (with the liquid cooling system and fan on only when cooling) under ambient temperature at 26°C, and the temperature was controlled and recorded through LabVIEW program.

It took approximately 12 minutes to heat the aluminum manifold from 0°C to 80°C (overshoot of 1°C) with the cooling fan off and 15 minutes to cool it down from 80°C to 0°C with the fan on. It reached -1.8°C and stayed at the limit of approximately -1.4°C for 20 minutes until the control was turned off. The heating rate and cooling rate were calculated to be 6.7°C/min and 5.3°C/min respectively under the operating condition specified above.

![Figure 14 Measured temperature profile for heating (red) and cooling (blue)](image-url)
It was observed that thicker side of the aluminum manifold was colder than where the sensor was attached since there was ice formed on the surface, which indicated that there is a non-uniform distribution across the entire manifold. With the polycarbonate cartridges and PDMS device attached on top of the aluminum manifold, it is necessary to understand the temperature difference across the entire assembly to make sure the solutions loaded upstream receive sufficient amount of thermal energy. The heat transfer and distribution were modelled using simulation software (COMSOL Multiphysics).

The model was defined with the following input parameters. The heat source was set to be 180W power boundary heat source. The ambient temperature was set to be 26°C. The boundary conditions were assumed that convection happened to some free surfaces to air at room temperature with heat transfer coefficient of 25W/m²K; while some surfaces were set to be thermally insulated. The materials involved were aluminum, copper, polycarbonate (with thermal conductivity of 0.21W/mK, density of 1210 kg/m³, heat capacity at constant pressure as 1250J/kgK) and PDMS (thermal conductivity of 0.17W/mK, density of 965 kg/m³, heat capacity at constant pressure as 1200J/kgK). The heat resistance between the thermoelectric element and the manifold was omitted.

![Surface: Temperature (degC)](image)

**Figure 15** COMSOL simulation of heat transfer and distribution on manifold with cartridges and device
The result shows that due to the heat capacity difference between different materials, the aluminum manifold could be heated up to a relatively high temperature while the polycarbonate carridges were not so hot as the rest of the assembly. The temperature calculated may not perfectly match the real heating scenario due to the assumptions made to the model, but it illustrated the situation that there is a temperature difference between components made of different materials, and the location closer to the thermal element obtains a better thermal status. This modelled heat distribution suggested that when heat up or cool down the system, the thermal sensor should be attached to the cartridge to sense a more accurate temperature that the solutions actually obtain, so that the system keeps operating until it reaches desired temperature.

Further thermal test was generated with all the cartridges and microfluidic device inserted onto the manifold as illustrated in Figure 16. Two power supplies were applied to power the thermoelectric element with 120W maximum power and 18V of voltage each. The ambient temperature was 25°C, and the thermal sensor was inserted into the cartridge.

It was determined that it took approximately 14 minutes to heat up the cartridges from 0°C to 80°C and 30 minutes to cool them down from 80°C to 0°C. The heating performance was similar to the result determined previously in terms of the heating rate and the fluctuation of overshoot at target temperature, but the cooling curve in this case was not so linear that it showed a faster rate when the temperature was above ambient temperature. It was slower to bring the temperature from ambient temperature to 0°C. For cooling below 0°C, Figure shows that the cooling rate was 1.4°C/hour. It is a relatively much slower process to bring the temperature down to negative.

In conclusion, the overall heating and cooling performance between 0°C to 80°C indicates that the thermal system satisfies the requirements of heating or cooling the upstream solutions in a fast and effective manner.
Figure 16 Illustration of thermal performance test with all the on-manifold features on

Figure 17 Measured temperature profile with all the on-manifold features on for heating (red) and cooling (blue)
Thermal induced gelation experiment was generated using 4% w.t. SeaPrep agarose with the temperature control features and pressure control. The manifold and cartridge were heated from 22°C to 80°C within 7mins and maintained at 80°C throughout the experiment using two power supplies at 16V each. The pressure level was set to 6psi with an actual reading of 6.01psi, and the flow rate of agarose was determined to be 980µl/min. The streaming fluid, DI water in this case, was introduced using syringe pump (Harvard Apparatus) at a flow rate of 400µl/min. The conveyor belt velocity was set to 1.5mm/s. The upstream agarose solution maintained aqueous throughout the experiment that the temperature control is capable for fulfilling the temperature requirement upstream. Even though 4% w.t. agarose was expected to gel at room temperature, the downstream temperature on the belt was not cold enough, in another word, the temperature gradient as the cross-linking stimulus was not introduced to the material rapid enough for the gelation to happen immediately. The reservoir was therefore filled with dry ice to cool down the conveyor environment, and some dry ice was located on the belt directly to give the thermal gradient to the material. (Figure 19) As a result, agarose hydrogel sheets were formed in a slower manner comparing to the gelation of alginate sheets. The geometry was not maintained as desired since the gelation process was slow that the confinement was not long enough to restrict the aqueous non-gelled material before enough gelation happened to form the overall geometry.
Also, the agarose gel was hard to collect with a brittle property that any manipulation resulted in breakage of the sample, which made the post-printing characterization difficult.

The thermal gelation was seen relatively less rapid that forming agarose hydrogel in moving condition instead of molding scaffolds in static condition is more challenging. Some system improvements are required for conducting thermal gelation printing. One approach to consider is to implement thermal control features to the conveyor belt side or the confinement piece that downstream temperature control is available so that the material will experience the thermal gradient in a faster manner. Since thermal gelation is different and less rapid than ionic crosslinking such as alginate with CaCl₂, longer confinement and slower conveyor speed are preferred to expand the residence time that the material can be better restricted under the desire flow condition for a longer period of time.

Figure 19 Illustration of 4%w.t. agarose being printed using pressure control and temperature control
Chapter 5

5. Results and Characterization of Hydrogel Sheet Formation

5.1 Analytical Model for Biopolymer Flow, Transport and Gelation

Upon leaving the microfluidic device, a Couette flow is established between the moving bottom wall (belt surface) and the stagnant top wall. Theoretical analysis and prediction of the produced sheet geometry were generated involving the following parameters: the width of the exit section of the microfluidic device, the height of the confinement, the volumetric flow rate of the biopolymer matrix solution, the volumetric flow rate of the focusing fluid, and the velocity of the conveyor belt. It is shown that, for a fixed device exit width and a fixed confinement height, varying the flow rates and the conveyor moving speed alters the produced sheet thickness and mechanical property.

We demonstrated the formation of homogeneous and heterogeneous hydrogel sheets through ionic crosslinking using 2% w.t. alginate solution as the matrix biopolymer and 100mM CaCl₂ solution as the crosslinking/focusing reagent. The experimental result agrees with the Couette flow condition prediction, and the printer is capable of producing hydrogel sheets with different thickness. The throughput of the printer can be easily scaled up by increasing the volumetric flow rates and the conveyor belt moving speed using a microfluidic device with a wide exit up to 70mm. With the thermal features and controls added to the printer, biopolymers such as agarose and collagen are the candidate materials that can be processed using the microfluidic bioprinter.

5.1.1 Couette Flow

Two miscible fluid streams exit from the microfluidic device into the confined region between the bottom moving belt and the spatially fixed top wall as the confinement roof. The top focusing fluid retains a constant viscosity while the bottom biopolymer solution undergoes a gelation process while being transported on the belt. It is assumed that the flows are steady, laminar, and incompressible as two dimensional flows with no-slip boundary conditions, constant densities (ρ) and constant viscosities (μ).
General Analysis:

Figure 20 Couette flow with a fixed top confinement and bottom moving plate

Navier-Stokes Equations:

\[ \rho \left[ \frac{\partial u}{\partial t} + u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} + w \frac{\partial u}{\partial z} \right] = - \frac{\partial p}{\partial x} + \rho g_x + \mu \left( \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} + \frac{\partial^2 u}{\partial z^2} \right) \]  \hspace{1cm} (5.1)

\[ \rho \left[ \frac{\partial v}{\partial t} + u \frac{\partial v}{\partial x} + v \frac{\partial v}{\partial y} + w \frac{\partial v}{\partial z} \right] = - \frac{\partial p}{\partial y} + \rho g_y + \mu \left( \frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2} + \frac{\partial^2 v}{\partial z^2} \right) \]  \hspace{1cm} (5.2)

\[ \rho \left[ \frac{\partial w}{\partial t} + u \frac{\partial w}{\partial x} + v \frac{\partial w}{\partial y} + w \frac{\partial w}{\partial z} \right] = - \frac{\partial p}{\partial z} + \rho g_z + \mu \left( \frac{\partial^2 w}{\partial x^2} + \frac{\partial^2 w}{\partial y^2} + \frac{\partial^2 w}{\partial z^2} \right) \]  \hspace{1cm} (5.3)

Continuity Equation: \[ \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} = 0 \]  \hspace{1cm} (5.4)

Since the flow is assumed to be 2-D, steady, laminar and parallel, \( \frac{\partial}{\partial t} = 0, v = w = 0 \), Equation (5.4) gives \( \frac{\partial u}{\partial x} = 0 \). Therefore \( u = u(y) \) only.

With \( g_y = -g, g_x = g_z = 0 \), Equation (5.1), (5.2) give:
\[0 = -\frac{\partial p}{\partial x} + \mu \left( \frac{\partial^2 u}{\partial y^2} \right)\]  
\[(5.5)\]

\[0 = -\frac{\partial p}{\partial y} - \rho g\]  
\[(5.6)\]

Integrating (5.6) gives  
\[p = -\rho gy + f(x) + C\]  
\[(5.7)\]

Integrating (5.5) gives  
\[\frac{d^2 u}{dy^2} = \frac{1}{\mu} \left( \frac{\partial p}{\partial x} \right)\]  
\[\frac{du}{dy} = \frac{1}{\mu} \left( \frac{\partial p}{\partial x} \right)y + C_1\]

\[u = \frac{1}{2\mu} \left( \frac{\partial p}{\partial x} \right)y^2 + C_1y + C_2\]  
\[(5.8)\]

The boundary conditions are:  
\[u = U\] at  \(y = 0\) and  \(u = 0\) at  \(y = b\); in combination with (5.8):

\[C_2 = U; \quad C_1 = -\frac{1}{2\mu} \left( \frac{\partial p}{\partial x} \right)b - \frac{U}{b}\]

Therefore the velocity is  
\[u = \frac{1}{2\mu} \left( \frac{\partial p}{\partial x} \right)(y^2 - by) + U \left( 1 - \frac{y}{b} \right)\]  
\[(5.9)\]

Or in a dimensionless form:  
\[\frac{u}{U} = -\frac{y}{b} - \frac{b^2}{2\mu U} \left( \frac{\partial p}{\partial x} \right) \left( \frac{y}{b} \right) \left( 1 - \frac{y}{b} \right) + 1\]  
\[(5.10)\]

Define dimensionless parameter  
\[P = -\frac{b^2}{2\mu U} \left( \frac{\partial p}{\partial x} \right)\]  
\[(5.11)\]

\[\frac{u}{U} = -\frac{y}{b} + P \left( \frac{y}{b} \right) \left( 1 - \frac{y}{b} \right) + 1\]  
\[(5.12)\]
To determine where the maximum velocity occurs, differentiate Equation (5.12) with respect to $y$ and set it to zero (assuming dimensionless parameter $P$ obtains a constant value):

$$
\frac{d}{dy}\left(\frac{u}{U}\right) = -\frac{1}{b} + P\left(\frac{1}{b}\right)\left(1 - \frac{2y}{b}\right) = 0
$$

(5.13)

At $y = 0$, $\frac{u}{U} = 1$; at $y = b$, $\frac{u}{U} = 0$. The maximum velocity occurs at the bottom moving surface with the value of the velocity same as that of the belt, and the minimum velocity occurs at the top confinement wall with a value of zero as the wall on top is stationary.

**Pressure Gradient:**

When the movement of the belt is the only driving force that causes the motion of the fluid, there is no pressure gradient in the $x$ direction, i.e. $\frac{dp}{dx} = 0$. Therefore

$$
\frac{u}{U} = -\frac{y}{b} + 1 \quad \Rightarrow \quad u = U\left(1 - \frac{y}{b}\right)
$$

This indicates a linear velocity profile.

When the speed of the belt is not giving a linear profile within the flow, there is pressure gradient in the $x$ direction that $\frac{dp}{dx}$ is taken into account the calculation.

Back flow happens when the pressure gradient is positive, which means that when the pressure increases in the flow direction, back flow occurs somewhere across the $y$ direction as indicated in the velocity profile where dimensionless parameter $P$ is a negative value. On the other hand, when the pressure gradient is negative, which means that the pressure decreases in the flow direction, accumulation occurs as the dimensionless parameter $P$ is positive.
Figure 21  Velocity distribution of viscous flow between parallel plates with the top plate fixed and bottom plate moving. When \( P=0 \), there is no pressure gradient presented in the confined flow, and the velocity profile is linear (red). When \( P<0 \), back flow occurs (pink, almond, purple). When \( P>0 \), accumulation occurs (blue, turquoise, green).

**Shear Stress:**

Assuming there is no normal stress, and there is no shear stress on \( x-z \) or \( y-z \) plane:

\[
\tau_{yx} = \mu \left( \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) = \mu \frac{du}{dy} \tag{5.14}
\]

Where \( u = \frac{1}{2\mu} \left( \frac{\partial p}{\partial x} \right) \left( y^2 - by \right) + U \left( 1 - \frac{y}{b} \right) \) and \( \frac{du}{dy} = \frac{1}{\mu} \left( \frac{\partial p}{\partial x} \right) \left( y - \frac{b}{2} \right) - \frac{U}{b} \).

Therefore \( \tau_{yx} = \mu \frac{du}{dy} = \left( \frac{\partial p}{\partial x} \right) \left( y - \frac{b}{2} \right) - \frac{U\mu}{b} \tag{5.15} \)

Same as for the velocity profile expression, define dimensionless parameter \( P = -\frac{b^2}{2\mu U} \left( \frac{\partial p}{\partial x} \right) \).
Equation (5.15) becomes: 
\[
\frac{\tau}{\mu U} = 2P\left(\frac{y}{b}\right) - P + 1
\]  \hspace{1cm} (5.16)

At \( y = 0 \): 
\[
\tau_{yy} = \left(\frac{\partial p}{\partial x}\right)\left(\frac{b}{2}\right) - \frac{U\mu}{b}, \quad \frac{\tau}{\mu U} = -P + 1
\]

At \( y = \frac{b}{2} \): 
\[
\tau_{yy} = -\frac{U\mu}{b}, \quad \frac{\tau}{\mu U} = 1 \text{ for all } \frac{\partial p}{\partial x}
\]

At \( y = b \): 
\[
\tau_{yy} = \left(\frac{\partial p}{\partial x}\right)\left(\frac{b}{2}\right) - \frac{U\mu}{b}, \quad \frac{\tau}{\mu U} = P + 1
\]

When \( \frac{\partial p}{\partial x} = 0 \), \( \tau_{yy} = -\frac{U\mu}{b} \). The shear stress is constant across y-axis.

Figure 22 Shear stress profile of viscous flow between parallel plates with the top plate fixed and bottom plate moving. For all P values, the shear stress profile is linear across the channel. When \( P=0 \), there is no pressure gradient presented in the confined flow, and the shear stress is constant.
Two-stream Analysis (before Gelation):

**Figure 23** Two fluid layers between top fixed confinement and bottom moving plate

Initially the top fluid and the bottom fluid remain liquid with different viscosities upon leaving the device.

**Top layer:**

\[ u_2 = \frac{1}{2\mu_2} \left( \frac{\partial p}{\partial x} \right) y^2 + C_{21} y + C_{22} \]  

(5.17)

Boundary conditions: at \( y = b \rightarrow u_2 = 0 \); at \( y = b_1 \rightarrow u_2 = u_1 \)

**Bottom layer:**

\[ u_1 = \frac{1}{2\mu_1} \left( \frac{\partial p}{\partial x} \right) y^2 + C_{11} y + C_{12} \]  

(5.18)

Boundary conditions: at \( y = b_1 \rightarrow u_1 = u_2 \); at \( y = 0 \rightarrow u_1 = U \)

Assume that the motion of the fluid is caused entirely by the movement of the conveyor belt on the bottom, and there is no pressure gradient existed within the confinement.

(5.17) \( \rightarrow u_2 = C_{21} y + C_{22} \)

(5.18) \( \rightarrow u_1 = C_{11} y + C_{12} \)

Using the above boundary conditions to solve for the constants:
\[ u_2 = \frac{C_{12} b_1 + U}{b_1 - b} (y - b) \]

\[ u_1 = C_{11} y + U \]

With \( C_{22} = -C_{21} b \); \( C_{12} = U \) and \( C_{21} = \frac{C_{12} b_1 + U}{b_1 - b} \) \hspace{1cm} (5.19)

The velocity distribution is linear in both layers in this case, and shear stress is

\[ \tau_{xy} = \mu \left( \frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) = \mu \frac{du}{dy} \]

The shear stress is constant in each layer and dependent on the viscosity of the fluid:

\[ \tau_2 = \mu_2 C_{21}; \quad \tau_1 = \mu_1 C_{11} \quad \text{and} \quad \tau_1 = \tau_2; \quad \mu_2 C_{21} = \mu_1 C_{11} \Rightarrow \frac{C_{21}}{C_{11}} = \frac{\mu_1}{\mu_2} \] \hspace{1cm} (5.20)

Solve (5.19) and (5.20): \( C_{11} = \frac{\mu_1 U}{\mu_1 (b_1 - b) - \mu_2 b_1} \); \( C_{21} = \frac{\mu_1 U}{\mu_1 (b_1 - b) - \mu_2 b_1} \)

So the velocity profiles are:

\[ \frac{u_2}{U} = \frac{\mu_1}{\mu_1 (b_1 - b) - \mu_2 b_1} (y - b) \] \hspace{1cm} (5.21)

\[ \frac{u_1}{U} = \frac{\mu_2}{\mu_1 (b_1 - b) - \mu_2 b_1} y + 1 \] \hspace{1cm} (5.22)

The velocity at the interface will be at \( y = b_1 \):

\[ u_{\text{interface}} = \frac{\mu_1}{\mu_1 (b_1 - b) - \mu_2 b_1} (b_1 - b) = \frac{\mu_2}{\mu_1 (b_1 - b) - \mu_2 b_1} b_1 + 1 \] \hspace{1cm} (5.23)

The shear stress is constant within the streams:

\[ \tau_1 = \tau_2 = \frac{\mu_1 \mu_2 U}{\mu_1 (b_1 - b) - \mu_2 b_1} \] \hspace{1cm} (5.24)
Figure 24 Velocity profile of two different viscous flows between parallel plates with the top plate fixed and bottom plate moving. Assumptions: each layer of the fluids is half of the thickness of the entire confinement; the bottom matrix fluid is 100x more viscous than the top focusing fluid; no pressure gradient presents in the streams as the motion is driven by only the moving bottom plate (the velocity profiles are linear for both fluids). It is shown that, under the condition that the bottom fluid is more viscous than the top streaming fluid, the bottom fluid obtains less velocity drop as it rises across the y axis; the less viscous fluid on the top obtains a more dramatic velocity change.

Two stream Analysis (after Gelation):

Figure 25 Two fluid layers between top fixed confinement and bottom moving plate with the bottom fluid layer forming a hydrogel as a moving wall
The bottom stream has formed the hydrogel sheet while a layer of streaming fluid flowing on top. The bottom fluid gels into a hydrogel sheet that moves with the conveyor belt, and it is assumed that it is static relative to the surface of the belt without slipping. Therefore the velocity profile is uniform across the thickness of the gel, and it is the same as the velocity of the belt. The top portion of the confinement contains focusing fluid flowing toward x+ direction, which is driven by the movement of the gel on the bottom and possibly the pressure gradient. The boundary conditions are the same as the ones in the general analysis.

Top: \[
\frac{u}{U} = -\frac{y}{b_2} + P \left( \frac{y}{b_2} \left( 1 - \frac{y}{b_2} \right) \right) + 1 \text{ where } P = -\frac{b_2^2}{2\mu U} \left( \frac{\partial p}{\partial x} \right) \text{ and } \frac{u}{U} = -\frac{y}{b_2} + 1 \text{ if there is no pressure gradient, and the shear stress profile is the same as the illustration for shear stress in the general Couette flow with a fixed confinement on the top and a moving plate on the bottom.}
\]

Bottom: \( u = U_{\text{belt}} \), and it is assumed that no shear exists within the formed hydrogel.

**Figure 26** Velocity profile of the hydrogel and the focusing stream when alginate sheet forms with a focusing stream on top with confinement (an extended roof after the exit of the device)
5.1.2 Parameters and Theoretical Model

The flow condition is directly related to the relationship between the flow rate and the conveyor belt moving velocity based on the observation from the early stage experiments of varying experimental parameters. For a fixed volumetric flow rate that the solution exits the device, if the belt is moving with a high speed, there is not enough material to fill the confinement and to catch up with the movement of the belt at the same time that no complete sheet could be formed. This matches with the Couette flow analysis that when $\frac{dp}{dx}$ is positive, which means the pressure increases in the flow direction, “back flow” happens as the lagged, slow streams observed. On the other hand, if the belt is moving with a low speed, some volume of material accumulates within the confinement since there is not enough speed of the belt to drive the movement of the material with blobs formed within the confinement. This again matches with the Couette flow analysis that when $\frac{dp}{dx}$ is negative, which means the pressure decreases as it goes downstream, over amount of material fed into the confinement cannot be dragged out with a slow bottom moving wall that the flow accumulate across the confinement.

There is a flow rate versus belt speed matching condition that the speed assigned to the belt is just adequate to transport the right amount of material as there will be no back flow or accumulation. This relationship gives non disturbed flow and no pressure gradient that may change the geometry of the formed gel. Since the system allows both of the flow rate and the belt speed as adjustable parameters, the matching condition can be applied so that the hydrogel sheet can be formed into certain predictable thickness under control without internal pressure induced shear or flow disturbance.

The theoretical model is generated based on the above theory and the velocity distribution analysis of Couette flow with no pressure gradient presented in the flow direction. This model gives the relationship between the flow rate and the velocity with certain confinement height and all other parameters included. Tuning the parameters of the printer as calculated using this model will produce hydrogel sheets with the predicted thickness.
Figure 27 Flow condition in the microfluidic bioprinter, with the top confinement fixed and the bottom conveyor belt moving, the streaming fluid layer is on top of the matrix fluid layer.

The equations that establish the theoretical prediction model based on the flow condition are:

\[
\begin{align*}
  v_{\text{flow}} &= v_{\text{belt}} (1 - \frac{h}{h_{\text{total}}}) = v (1 - \frac{h}{h_{\text{total}}}) \\
  Q_m &= \int_0^{h_{\text{total}}} w \times v \times (1 - \frac{h}{h_{\text{total}}}) \times dh = \int_{h_{\text{m}}}^{h_{\text{total}}} \left[ w v h - \frac{w v h^2}{2 h_{\text{total}}} \right] dh = w v h_{\text{m}} - \frac{w v h_{\text{m}}^2}{2 h_{\text{total}}} \\
  Q_s &= \int_{h_{\text{m}}}^{h_{\text{total}}} w \times v \times (1 - \frac{h}{h_{\text{total}}}) \times dh = \int_{h_{\text{m}}}^{h_{\text{total}}} \left[ w v h - \frac{w v h^2}{2 h_{\text{total}}} \right] dh = \frac{1}{2} w v h_{\text{total}} - w v h_{\text{m}} + \frac{w v h_{\text{m}}^2}{2 h_{\text{total}}} \\
  Q_{\text{total}} &= Q_m + Q_s \\
  t_{\text{residence}} &= \frac{l_{\text{confinement}}}{v}
\end{align*}
\]

Where \( Q_{\text{total}} \) is the total volumetric flow rate, \( Q_m \) is the matrix solution volumetric flow rate, \( Q_s \) is streaming solution volumetric flow rate, \( w \) is width of the printing nozzle device exit, \( h \) is the height of the matrix layer (the thickness of the sheet), \( h_{\text{total}} \) is height of the confinement, \( v \) is the conveyor belt velocity, \( l_{\text{confinement}} \) is the confinement length, \( t_{\text{residence}} \) is the residence time.
The model was programmed in an equation-solving program (Engineering Equation Solver, EES) that numerically solves the set of equations above with some of the parameters assigned differently based on the purpose of each modelling trial.

Figure 28 3D plot of the relationships among four parameters: matrix solution volumetric flow rate, belt moving velocity, thickness of printed sheet and printing device width with the rest parameters fixed.

Figure 28 illustrates the modelled results of the relationships among the following four parameters: matrix solution volumetric flow rate, belt moving velocity, thickness of the printed sheet and printing device width. The confinement height is 1mm for all conditions, and the total volumetric flow rate is fixed to be 1000µl/min, so the velocity in each device width remains constant with a trend that the wider the device the lower the velocity. By varying the matrix material flow rate (streaming flow rate will change accordingly since the total flow rate is fixed), sheets with different thickness can be produced accordingly. The general matrix flow rate versus sheet thickness relationship is consistent for all cases of different device width.
Figure 29 3D plot of velocity and sheet thickness when varying matrix flow rate at different streaming flow rates with a device of 10mm exit width and 1mm confinement height

Figure 30 Sheet thickness vs. matrix volumetric flow rate plot for different $Q$s
Figure 29 and 30 are the modelled results of varying matrix flow rate at different streaming flow rates with a device of 10mm exit width and 1mm confinement height. It is shown that the sheet thickness will increase with the matrix flow rate in general for all focusing stream volumetric flow rate levels. The velocity increases as either the matrix or the focusing stream flow rate increases. Increases in the focusing stream flow rate at the same matrix flow rate will give a thinner printed sheet at faster belt velocity.

This model can be used to predict the thickness of the sheet printed with certain printing device and confinement height at certain matrix flow rate and focusing flow rate. The same study was generated for 25mm, 42mm and 70mm devices, and the plots will be displaced along with the experimental results in the coming sections.

5.2 Homogeneous Hydrogel Sheets

Homogeneous alginate hydrogel sheets were produced using the materials and methods described in the previous chapter. Figure 31 shows the formation of the hydrogel sheets using different printing device with the width of 10mm, 25mm and 42mm. All the devices were fabricated through partial curing of a 200µm bottom layer, a matrix solution layer in the middle and a focusing solution layer on top with a channel height of 150µm as described in the previous chapter. The alginate solution was coloured as orange and the CaCl$_2$ solution as blue. Both solutions were driven by neMESYS syringe pump with adjustable flow rates. Table 4 lists the experimental parameters that were fixed for each trial. Matrix flow rates and belt velocities were assigned with the values that were generated by the theoretical model.

<table>
<thead>
<tr>
<th>Printing Device Width (mm)</th>
<th>Confinement Height (mm)</th>
<th>Focusing Fluid Volumetric Flow Rate (µl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
<td>250 400 500</td>
</tr>
<tr>
<td>25</td>
<td>1.5</td>
<td>250 500 750</td>
</tr>
<tr>
<td>42</td>
<td>2</td>
<td>500 750 1000</td>
</tr>
</tbody>
</table>

Table 4 Experimental condition summary of homogeneous sheet printing
Figure 31 Illustration of (a) 10mm, (b) 25mm and (c) 42mm 2%w.t. alginate sheets being printed, scale bars 1cm
Figure 32  Theoretical predictions, measured thicknesses and standard deviations of samples printed using 10mm device under multiple printing conditions as indicated. Purple, blue and turquoise lines are predicted \( h \) with \( Qs \) of 250\(\mu\)l/min, 400\(\mu\)l/min and 500\(\mu\)l/min respectively. Purple, blue and turquoise dots are measured \( h \) with \( Qs \) of 250\(\mu\)l/min, 400\(\mu\)l/min and 500\(\mu\)l/min respectively.

Figure 33  Theoretical predictions, measured thicknesses and standard deviations of samples printed using 25mm device under multiple printing conditions as indicated. Purple, blue and turquoise lines are predicted \( h \) with \( Qs \) of 250\(\mu\)l/min, 500\(\mu\)l/min and 750\(\mu\)l/min respectively. Purple, blue and turquoise dots are measured \( h \) with \( Qs \) of 250\(\mu\)l/min, 500\(\mu\)l/min and 750\(\mu\)l/min respectively.
Figure 34  Theoretical predictions, measured thicknesses and standard deviations of samples printed using 42mm device under multiple printing conditions as indicated. Purple, blue and turquoise lines are predicted $h$ with $Q_s$ of 500µl/min, 750µl/min and 1000µl/min respectively. Purple, blue and turquoise dots are measured $h$ with $Q_s$ of 500µl/min, 750µl/min and 1000µl/min respectively.

The measured thicknesses and standard deviations of the samples in each experimental condition are illustrated in Figure 32-34. In general, for the matrix flow rates above 200µl/min, the measured thicknesses are close (within ±50µm) to the predicted values with a trend of increasing thickness as increasing matrix volumetric flow rate. The model well predicts the trend and the thickness values when other operational parameters are assigned as input. When sheets with certain thickness are required, inputting the parameters as the model generates will give sheets that were made under a flow rate versus velocity matching condition without backflow or accumulation.

The thicknesses measured when the matrix flow rate is set to 200µl/min are not agreeing with modelled values that they are much higher (>0.1mm) than the theoretical predicted values, especially for the case of using 42mm wide device. When the matrix flow rate is low, the calculated associated velocity is slow, which agrees with the interpretation based on the model. However there is operational limit of the conveyor system that a too slow velocity creates not enough inertia to initiate the motion that there is clogging and accumulation of material at the
exit as gelation takes place without having the gelled material transported. This model may not predict the sheet thickness well as the higher flow rate operational conditions. However gelation at lower flow rates can happen through other adjustments such as lowering the confinement height, initializing the system with a higher velocity, or varying the downstream velocity experimentally instead of following the modelled values.

The model serves as a valid thickness prediction tool within certain ranges of operational parameters instead of all the conditions modeled. For example, as discussed in the previous paragraph that at low matrix flow rate of 200µl/min with low conveyor belt moving speed in multiple conditions, the predictions are not matching the experimental measurements. The predictable ranges are different and hard to conclude into one statement as conditions vary with different device geometry, confinement height and focusing flow rate. Each case must be discussed separately based on the fixed parameters involved. For example, for 42mm wide device with a 2 mm high confinement, when $Qm$ is 200µl/min, varying $Qs$ below 1000µl/min will always give a belt speed lower than 0.5mm/s, which does not give enough inertia to start the movement of the material and the gel, so the actual experimental conditions cannot be predicted using the modelled values. On the other hand, when $Qm$ to $Qs$ is too high, there is not enough cross-linker to gel the large amount of matrix solution that the gelation will not happen as the model does not count this constraint into calculation.

Within the ranges that the theoretical model is valid, the measured thicknesses may not be exactly matching the predicted values as seen in Figure 32-34. There are several sources that may cause the discrepancy. Since soft PDMS devices were used as the printing nozzles with extra weights placed on top, there is no perfect contact between the bottom of the device and the belt that any gap may cause leakage of the less viscous solution (CaCl$_2$ in this case). The confinement on top was inserted into the cut-out of the device exit that it is not perfectly horizontal, and any micro tilt or bulge may disturb the flow condition within the confinement, which resulted in slightly none uniform sheet with a thickness slightly off from the expected value. In the case of using pressure control to introduce flows, which will be discussed in the next section, the flow rates are not accurate down to single digit of microliter that it is hard to program the parameters to match the condition perfectly.
5.3 Pressure Driven Printing

Pressure control is an important feature of the printer. It allows the on-manifold solution delivery and transportation with faster reaction time. The biopolymers can be stored in sterilized environment along with the other components of the printer as one complete setup, and only with the pressure control validated the solutions can be thermally heated up or cooled down on the manifold. In this section the flow rate determination when using pressure control and the validation experiments will be discussed.

5.3.1 Pressure versus Flow Rate Control

For unknown polymer processing, there are three methods of determining the pressure versus flow rate relationship for a fixed microfluidic printing device with certain geometry. The first method is to experimentally determine the flow rate associated to an applied pressure level by recording the time it takes to run out a fixed volume of solution through a device without making sheets. Figure 35 shows the experimental layout of how 5ml of 2% w.t. alginate solution was loaded in the cartridge and pressurized out through the device to the atmosphere under multiple pressure levels between 1psi to 15psi.

![Figure 35 Illustration of the setup for determining flow rate vs. pressure relationship](image)

Figure 35 Illustration of the setup for determining flow rate vs. pressure relationship
Figure 36 Experimental data of 2% w.t. alginate solution flow rate at different pressure levels of 10mm, 25mm, 42mm wide devices (pink, purple and blue dots) and the best fit flow rate vs. pressure curves (red, purple, blue lines)

The flow rate was calculated by dividing the loaded volume with the time it took to run out the solution. Figure 36 shows the 2% w.t. alginate solution flow rate versus pressure relationships of 10mm, 25mm and 42mm wide devices. Numerical best fit trend lines were also added as for predicting the flow rate at other arbitrary pressure level.

The second method of determining the flow rate of certain fluid at an arbitrary pressure is to apply Ohm’s Law analogy and Poiseuille’s equation:

\[ R = \frac{\Delta p}{Q} = \frac{p}{V/t} = \frac{p \times t}{V} \]  \hspace{1cm} (5.30)

and \( R = \mu \times \text{Constant} \) \hspace{1cm} (5.31)

Where \( R \) is the flow resistance; \( p \) is the upstream applied pressure; \( Q \) is the flow rate; \( V \) is the volume of fluid; \( t \) is the time it takes to run out volume of \( V \) fluid and \( \mu \) is the viscosity of the fluid.
Therefore \( p \times t = \mu \times C \times V \Rightarrow p \times t = \mu \times D \). \hspace{1cm} (5.32)

D is the physical resistance constant for a specific geometry of the cartridge combined with a specific configuration of the device. Knowing D will allow the prediction of how much time it is required to consume a certain amount of fluid under a specific pressure, in another word to be able to predict the flow rate under each pressure condition.

Based on the equation 5.32, D can be determined by finding out the ratio of \( \frac{p \times t}{\mu} \).

The viscosity \( \mu \) values were measured using a rheometer (ARES rheometer, TA Instruments, New Castle, DE, USA) with 1mm apart parallel plate geometry and steady shear rate clockwise one directional sweep mode at temperature of 25°C (Figure 37). The \( p \times t \) valves were experimentally collected and plotted against the shear rates for 150µm high microchannels (Figure 38-40). The curves were compared in order to determine the ratio D.

![Figure 37](image-url) Rheology measurements and the best fit natural logarithm curve of 2% w.t. alginate solution dissolved in DI water
Figure 38 Pressure vs. shear rate data and best fit natural logarithm curve of 2% w.t. alginate solution flowing through a 10mm wide device

Figure 39 Pressure vs. shear rate data and best fit natural logarithm curve of 2% w.t. alginate solution flowing through a 25mm wide device
The same approach was applied to 2% w.t. alginate solution and 100mM CaCl₂ solution (Appendix 10). For alginate aqueous solutions, shear thinning behavior was observed from the rheology data that the viscosity decreases exponentially with increasing shear rate. The natural logarithm curve was fit based on the measurements. The pressure and time products (5ml of solution passing through 10mm, 25mm and 42mm devices under different pressure levels) were also plotted against the shear rates calculated based on the channel heights and widths. By dividing the pressure*time equations by the rheology measurement equation, D values were determined and summarized in Table 5 for each device geometry.

As a result, the equation and constant value D can be used to find out the flow rate of a certain fluid with viscosity at applied pressure $p$:

$$Q = \frac{\Delta p}{R} = \frac{p}{\mu \times C} = \frac{p}{\mu \times \frac{D}{5ml}} = \frac{p}{\mu \times \frac{D}{5ml}}$$  \hspace{1cm} (5.33)
To summarize the approach, three relationships are required for generating the D value: shear rate vs. pressure (Appendix 9), pressure*time vs. shear rate; viscosity of the solution vs. shear rate; as shown above.

Therefore assigning a pressure to a fluid with known viscosity at the shear stress that the pressure creates, it is easy to predict the flow rate using the above equation and relationships.

### Rheology Measurement

<table>
<thead>
<tr>
<th>Device</th>
<th>2% w.t. Alginate Equation</th>
<th>D constant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>10mm device</strong></td>
<td>$y = -1.33 \times 10^6 \ln(x) + 1.14 \times 10^7$ (5.35)</td>
<td>$1.14 \times 10^9$</td>
</tr>
<tr>
<td><strong>25mm device</strong></td>
<td>$y = -4.2 \times 10^5 \ln(x) + 4.9 \times 10^6$ (5.36)</td>
<td>$4.9 \times 10^8$</td>
</tr>
<tr>
<td><strong>42mm device</strong></td>
<td>$y = -2.86 \times 10^6 \ln(x) + 2.63 \times 10^7$ (5.37)</td>
<td>$2.63 \times 10^9$</td>
</tr>
</tbody>
</table>

**Table 5** $p^*t$ best fit equations for each specific geometry/configuration of device with 2% w.t. alginate solution flowing through and the determined D values as combined fluid physical resistance constants

The third approach to have the control over the flow rate using pressure is through indirect sheet thickness measurement. The volumetric flow rate can be determined as $Q = w \times v \times h$ where $w$ is the device exit width; $v$ is the exit velocity and $h$ is the produced sheet thickness. Using imaging technique that the sensed sheet thickness as the sheet being produced can be fed back to the control system to calculate the flow rate, and the value can be then sent to the feedback loop to adjust the pressure until the sensed sheet thickness gives a calculation result of $Q$ value that matches with the desired flow rate. This approach can be explored as an alternative for future study.
5.3.2 Pressure Driven Printing with Different Nozzles

Alginate sheets were printed using the pressure control for alginate solution transportation. The solution was loaded into the single lane 10ml cartridge and was pressurized out under several pressure levels listed in Table 6-8. The flow rates were predicted, followed by generating associated matching velocities using the flow rate prediction method and the theoretical model discussed previously. 10mm, 25mm and 42 mm devices were used with 1mm, 1.5mm and 2mm confinement height respectively. The CaCl$_2$ solution was Harvard Apparatus syringe pump driven with a constant flow rate of 500μl/min.

![Illustration of 42mm 2%w.t. alginate sheet being printed using pressure control and cartridge. Scale bar: 4cm.](image)

**Figure 41** Illustration of 42mm 2%w.t. alginate sheet being printed using pressure control and cartridge. Scale bar: 4cm.

<table>
<thead>
<tr>
<th>Set Pressure (psi)</th>
<th>Expected Pressure (psi)</th>
<th>Expected $Q_m$ (μl/min)</th>
<th>Modelled $v$ belt (mm/s)</th>
<th>Actual Pressure (psi)</th>
<th>Volume (ml)</th>
<th>Run-out Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.55</td>
<td>337</td>
<td>2.79</td>
<td>0.55</td>
<td>5</td>
<td>14min15s</td>
</tr>
<tr>
<td>1</td>
<td>1.04</td>
<td>411</td>
<td>3.037</td>
<td>1.05</td>
<td>10</td>
<td>26min30s</td>
</tr>
<tr>
<td>1.5</td>
<td>1.57</td>
<td>423</td>
<td>3.077</td>
<td>1.57</td>
<td>5</td>
<td>12min40s</td>
</tr>
<tr>
<td>2</td>
<td>2.07</td>
<td>789</td>
<td>4.297</td>
<td>2.07</td>
<td>5</td>
<td>5min54s</td>
</tr>
<tr>
<td>2.5</td>
<td>2.57</td>
<td>1071</td>
<td>5.237</td>
<td>2.58</td>
<td>10</td>
<td>6min20s</td>
</tr>
</tbody>
</table>

*Table 6* Experimental parameter predictions and actual experimental trails for 10mm device
Table 7 Experimental parameter predictions and actual experimental trails for 25mm device

<table>
<thead>
<tr>
<th>Set Pressure (psi)</th>
<th>Expected Pressure (psi)</th>
<th>Expected Qm (μl/min)</th>
<th>Modelled υ belt (mm/s)</th>
<th>Actual Pressure (psi)</th>
<th>Volume (ml)</th>
<th>Run-out Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.06</td>
<td>659</td>
<td>1.03</td>
<td>1.07</td>
<td>5</td>
<td>7min20s</td>
</tr>
<tr>
<td>2</td>
<td>2.08</td>
<td>1190</td>
<td>1.502</td>
<td>2.08</td>
<td>10</td>
<td>8min12s</td>
</tr>
<tr>
<td>3</td>
<td>3.09</td>
<td>1852</td>
<td>2.091</td>
<td>3.08</td>
<td>10</td>
<td>5min30s</td>
</tr>
<tr>
<td>4</td>
<td>4.07</td>
<td>2400</td>
<td>2.578</td>
<td>4.07</td>
<td>10</td>
<td>4min4s</td>
</tr>
<tr>
<td>5</td>
<td>5.08</td>
<td>3529</td>
<td>3.581</td>
<td>5.06</td>
<td>10</td>
<td>3min10s</td>
</tr>
</tbody>
</table>

Table 8 Experimental parameter predictions and actual experimental trails for 42mm device

<table>
<thead>
<tr>
<th>Set Pressure (psi)</th>
<th>Expected Pressure (psi)</th>
<th>Expected Qm (μl/min)</th>
<th>Modelled υ belt (mm/s)</th>
<th>Actual Pressure (psi)</th>
<th>Volume (ml)</th>
<th>Run-out Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.09</td>
<td>311</td>
<td>0.3218</td>
<td>3.1</td>
<td>10</td>
<td>30min</td>
</tr>
<tr>
<td>5</td>
<td>5.07</td>
<td>531</td>
<td>0.4091</td>
<td>5.07</td>
<td>10</td>
<td>15min28s</td>
</tr>
<tr>
<td>7</td>
<td>7.04</td>
<td>857</td>
<td>0.5385</td>
<td>7.06</td>
<td>10</td>
<td>10min19s</td>
</tr>
<tr>
<td>9</td>
<td>9.06</td>
<td>1186</td>
<td>0.669</td>
<td>9.06</td>
<td>10</td>
<td>6min45s</td>
</tr>
<tr>
<td>11</td>
<td>11.03</td>
<td>1478</td>
<td>0.7849</td>
<td>11.04</td>
<td>10</td>
<td>5min10s</td>
</tr>
</tbody>
</table>

Figure 41 illustrates the alginate sheet printing using pressure driven in-cartridge loaded alginate solution with 42mm printing device, and Table 6-8 list all the parameters of each experimental condition. Different pressure levels were set between 0.5psi and 11psi. Since there is certain percentage of error associated with the actual pressure every time running the system, there is expected pressure value for each set pressure, and the actual pressure for each trail of experiment may be slightly different than the expected value. For example, when the pressure was set to be 1psi in the control system, the actual pressured measured by the gauge was 1.04 psi, and during the experimental trail later the pressure could be measured to be 1.05psi. This variation is determined to be less than 0.96% according to the above experimental findings. Therefore the experimental flow rates are closely matching the predicted flow rates as shown in Figure 42.
Figure 42 Predicted and measured matrix solution volumetric flow rate vs. pressure data. Red, purple and blue crosses are predicted $Q_m$ with $w=10\text{mm}$, $25\text{mm}$, $42\text{mm}$ respectively. Pink, purple and blue squares are actual $Q_m$ with $w=10\text{mm}$, $25\text{mm}$, $42\text{mm}$ respectively.

For each trial of experiment listed in the above tables, sheets were collected and the measured thicknesses were presented in Figure 43, 44 with plots against pressure and matrix flow rate. The produced sheets obtained thicknesses that match the predicted values with the same trend. By inputting the parameters as modelled and predicted using the combination of theoretical analysis and experimental interpretation, sheets with different thickness can be produced with full control over the parameters. The pressure control system is validated and proved to be ideally functional for biomaterial upstream transportation.
Figure 43 Predicted and measured sheet thickness vs. pressure data. Red, purple and navy blue crosses are predicted $h$ with $w=10\text{mm}$, $25\text{mm}$, $42\text{mm}$ respectively. Pink, purple and blue squares are measured $h$ with $w=10\text{mm}$, $25\text{mm}$, $42\text{mm}$ respectively.

Figure 44 Predicted sheet thickness vs. matrix flow rate plots using the theoretical model and measured thicknesses. Red, purple and navy blue lines are predicted $h$ with $w=10\text{mm}$, $25\text{mm}$, $42\text{mm}$ respectively. Pink, purple and blue dots are measured $h$ with $w=10\text{mm}$, $25\text{mm}$, $42\text{mm}$ respectively.
5.4 Finite Sheet Length Printing

As discussed in the first chapter, different bioprinting techniques and tissue engineering strategies obtain different approaches to enable the biopolymer hydrogel formation. Some of them were able to produce hydrogels in a continuous manner, while some of the applications require an assembly of multiple layers of features as layer-by-layer stacking fashion was applied during the fabrication. For example, human skin fibroblasts and keratinocytes were embedded in multiple layers of collagen hydrogels as a multi-layer engineered tissue that mimics human skin. [19] Therefore the flexibility of being able to perform continuous printing and step-by-step printing is desired to enlarge the space of related applications without extra manipulation and cutting involved in the process.

The microfluidic bioprinter operates with reliable controls and components under simple Couette flow condition. The system is easy to operate with less manual handling for sheet printing after the channels and the exit are free of bubble. It is flexible to start or stop any time without extra manipulation or procedure. Self-starting segmental sheet printing can be achieved in order to produce finite length of sheets. Formation of alginate laden sheet segments of limited length with control over the size was demonstrated using both the syringe pumps and the pressure driven method.

5.4.1 Syringe Pump Based Printing

A 25mm device with a confinement height of 1.5mm was used, and the parameters were input as the following $Q_s=500\mu l/min$, $Q_m=500\mu l/min$, $v=0.89mm/s$. Both alginate solution and CaCl$_2$ solution were driven by the neMESYS syringe pump. The syringes were turned on for an arbitrary amount of time for the flows to come out and form a limit length of sheet, and the flows were stopped for certain amount of time before they were started through controlling the pumps only to repeat the segmental sheet formation without extra manipulation while the belt was constantly moving with a fixed velocity.
Figure 45 Illustration of segmental 25mm wide 2% w.t. alginate sheets being printed

<table>
<thead>
<tr>
<th>Open Time (s)</th>
<th>Theoretical Length (cm)</th>
<th>Actual Length (cm)</th>
<th>Thickness h (mm)</th>
<th>Standard Deviation of h</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>7.4</td>
<td>6.9</td>
<td>0.44</td>
<td>0.016</td>
</tr>
<tr>
<td>38</td>
<td>3.4</td>
<td>3.1</td>
<td>0.49</td>
<td>0.028</td>
</tr>
<tr>
<td>92</td>
<td>8.2</td>
<td>7.8</td>
<td>0.47</td>
<td>0.014</td>
</tr>
<tr>
<td>59</td>
<td>5.2</td>
<td>4.7</td>
<td>0.46</td>
<td>0.026</td>
</tr>
<tr>
<td>30</td>
<td>2.7</td>
<td>2.7</td>
<td>0.46</td>
<td>0.021</td>
</tr>
<tr>
<td>46</td>
<td>4.1</td>
<td>3.7</td>
<td>0.45</td>
<td>0.032</td>
</tr>
<tr>
<td>36</td>
<td>3.2</td>
<td>3</td>
<td>0.44</td>
<td>0.020</td>
</tr>
<tr>
<td>73</td>
<td>6.5</td>
<td>6.5</td>
<td>0.44</td>
<td>0.017</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>0.46</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Table 9 Measured geometries of segmental 25mm wide 2% w.t. alginate sheets printed using syringe pump
Figure 46 Measured thickness and length comparison between theoretical and measured values for sheets printed using syringe pump, red shaded: measured lengths; blue shaded: theoretical calculated lengths; purple: measured thicknesses

The theoretical lengths were calculated based on the speed the belt traveled at and the amount of time the flows were left open. The actual lengths and thicknesses were measured after the sheets were collected. The results were illustrated in Table 9 and Figure 46. The actual lengths of the sheets are close to the theoretical lengths that the values are matching, or the actual lengths are shorter than the theoretical calculations by maximum 0.5cm. The discrepancy of length is related to the system respond time that there is lagging time when the syringe pump was turned on or off to open or stop the flow, and the end of the segment may not be perfectly straight when cross-linking was not completed through the entire thickness of the gel. The average thickness of all the sheets is 0.46mm with a standard deviation of 0.023. The thicknesses are consistent with the variation less than 50μm.

5.4.2 Pressure Driven Printing

The same experiment (using the same printing device) was conducted using the pressure control system instead of the syringe pump for the alginate solution. The CaCl$_2$ solution was driven by the syringe pump the same way as the previously discussed experiment. The pressure was set to 1psi with an expected system pressure of 1.06psi and flow rate of 660μl/min. The actual system pressure was sensed to be 1.07psi with a flow rate of 680μl/min, and the belt speed was set to 1.0mm/s.
The results are illustrated in Table 10 and Figure 47. The actual lengths of the sheets are close to the theoretical lengths that the values are shorter than the theoretical calculations by maximum 2 cm. The average thickness of all the sheets is 0.55mm with a standard deviation of 0.024. The thicknesses are consistent with the variation less than 40μm. The findings are similar to the ones observed from the previously discussed experiment.

<table>
<thead>
<tr>
<th>Open Time (s)</th>
<th>Theoretical Length (cm)</th>
<th>Actual Length (cm)</th>
<th>Thickness h (mm)</th>
<th>Standard Deviation of h</th>
</tr>
</thead>
<tbody>
<tr>
<td>190</td>
<td>19</td>
<td>17</td>
<td>0.56</td>
<td>0.031</td>
</tr>
<tr>
<td>150</td>
<td>15</td>
<td>14</td>
<td>0.55</td>
<td>0.020</td>
</tr>
<tr>
<td>180</td>
<td>18</td>
<td>16</td>
<td>0.55</td>
<td>0.026</td>
</tr>
<tr>
<td>210</td>
<td>21</td>
<td>18.5</td>
<td>0.56</td>
<td>0.023</td>
</tr>
<tr>
<td>120</td>
<td>12</td>
<td>12.5</td>
<td>0.54</td>
<td>0.017</td>
</tr>
<tr>
<td>90</td>
<td>9.3</td>
<td>8.5</td>
<td>0.55</td>
<td>0.021</td>
</tr>
<tr>
<td>60</td>
<td>6.2</td>
<td>6</td>
<td>0.53</td>
<td>0.024</td>
</tr>
<tr>
<td>45</td>
<td>4.6</td>
<td>4</td>
<td>0.52</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>0.55</td>
<td>0.024</td>
</tr>
</tbody>
</table>

Table 10 Measured geometries of segmental 25mm wide 2%w.t. alginate sheets printed using pressure control

Figure 47 Measured thickness and length comparison between theoretical and measured values for sheets printed using pressure control, red shaded: measured lengths; blue shaded: theoretical calculated lengths; purple: measured thicknesses
5.5 Printing Parameter Variation in Sheet Formation

The theoretical model presented at the beginning of this chapter (Equation 5.25-5.29) can be used as a tool to generate the parameters of a matching condition between the velocity and the matrix flow rate for producing a hydrogel sheet with certain thickness under the ideal condition without back flow or accumulation as a result of pressure gradient. Among the following five parameters: total volumetric flow rate \( Q_{\text{total}} \), matrix solution volumetric flow rate \( Q_m \), streaming solution volumetric flow rate \( Q_s \), thickness of the sheet \( h \), and conveyor belt velocity \( v \), three can be calculated when two chosen parameters are fixed with a device that obtains a fixed exit width and confinement height. When the value of flow rate is fixed, there is a velocity associated with this flow rate that will produce a sheet with a thickness of \( h \). However, such velocity is not the unique operating condition when the flow rate is fixed to a certain value. Slowing down or speeding up the belt relative to the matching velocity within a range will also allow gelation and sheet formation, which result in an increase or a decrease of the produced sheet thickness. This phenomenon also applies to the case of fixing the belt velocity and varying the flow rate.

In this section, two sets of experiments were presented based on the theoretically modelled no pressure gradient operating condition: \( Q_s = 250 \mu l/min, Q_m = 350 \mu l/min, v = 2 mm/s \) when using a device of 10mm wide with a confinement height of 1mm, and the predicted sheet thickness under this operating condition is 0.35mm. The experiments were generated using the materials and methods described in the previous chapter.

5.5.1 Varying Velocity while Fixing Flow Rates

In this set of experiments, the streaming flow rate and the matrix flow rate were set to 250\( \mu l/min \) and 350\( \mu l/min \) respectively using the neMESYS syringe pump, while the velocity was adjusted between 1mm/s to 4mm/s. It is shown that the produced sheet thickness decreases as the velocity increases. For the experimental condition of having the belt moving at 2mm/s, which is the pressure gradient free condition, the measured sheet thickness was 0.34mm which is close to the theoretical prediction of 0.35mm. Faster velocities created more pulling effect on the gelation that the produced sheets were thinner than 0.35mm. Slower velocities could not provide enough
pulling force to match the rate the material was exiting the device, and materials tended to accumulate into thicker gels compare to the 0.35mm gelation condition.

![Graph showing the relationship between sheet thickness and velocity for different matrix flow rates.](image)

**Figure 48** Measured 10mm wide 2%w.t. alginate sheet thicknesses with different printing velocities

<table>
<thead>
<tr>
<th>Velocity (mm/s)</th>
<th>1.0</th>
<th>1.3</th>
<th>1.7</th>
<th>2.0</th>
<th>2.3</th>
<th>2.7</th>
<th>3.0</th>
<th>3.3</th>
<th>3.7</th>
<th>4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (mm)</td>
<td>0.64</td>
<td>0.61</td>
<td>0.52</td>
<td>0.34</td>
<td>0.32</td>
<td>0.28</td>
<td>0.25</td>
<td>0.19</td>
<td>0.16</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Table 11** Measured 10mm wide 2%w.t. alginate sheet thicknesses with different printing velocities

### 5.5.2 Varying Matrix Flow Rate while Fixing Velocity

The same concept can be demonstrated by fixing the velocity while varying the flow rate. In this set of experiments, the streaming flow rate and the velocity were set to 250μl/min and 2mm/s respectively, while the matrix flow rate was adjusted between 50μl/min to 950μl/min. It is shown that the produced sheet thickness increases as the matrix flow rate increases. For the
experimental condition of having the matrix flow rate of 350μl/min, which is the pressure
gradient free condition, the measured sheet thickness was 0.34mm which is close to the
theoretical prediction of 0.35mm. The results agree with the previous experiments that increasing
the matrix flow rate has the same effect on the sheet thickness as lowering the belt velocity, and
decreasing the matrix flow rate gives equivalent effect as speeding up the belt that the sheets
produced will be thinner.

Figure 49 Measured 10mm wide 2%w.t. alginate sheet thicknesses with different matrix flow
rates

<table>
<thead>
<tr>
<th>Matrix Flow Rate (μl/min)</th>
<th>50</th>
<th>150</th>
<th>250</th>
<th>350</th>
<th>450</th>
<th>550</th>
<th>650</th>
<th>750</th>
<th>850</th>
<th>950</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (mm)</td>
<td>0.18</td>
<td>0.27</td>
<td>0.30</td>
<td>0.34</td>
<td>0.36</td>
<td>0.37</td>
<td>0.54</td>
<td>0.38</td>
<td>0.39</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Table 12 Measured 10mm wide 2%w.t. alginate sheet thicknesses with different matrix flow
rates
5.6 Measured Sheet Tensile Properties

As discussed in the last section, different sheet geometries can be achieved by varying one parameter while fixing all the rest ones. The tensile properties of such samples are also important to investigate for studying the effect of different printing conditions on the strength of produced hydrogels. Instead of using the syringe pump for flow control and printing, pressure control system was applied to generate the same sets of experiments of varying printing conditions to produce hydrogels with different thicknesses, followed by uniaxial tensile test using the BAXS platform described in the last chapter.

For all experiments, a 10mm device with a confinement height of 2mm was used. The streaming flow rate was fixed at 250μl/min using the syringe pump. The matrix solution was loaded into the 10ml single lane cartridge and transported using pressure control. For the first sets of experiment, the pressure was fixed at a setpoint of 0.45psi with the actual reading of 0.48psi, so the volumetric flow rate of the alginate matrix solution was constant at 344μl/min. The conveyor belt velocity was adjusted from 1.5mm/s to 3.5mm/s with an increment of 0.5mm/s for each experimental trail. The second set of experiments was generated with a fixed belt velocity of 2mm/s, and the matrix flow rate was varied through different pressure levels as listed in Table 13.

<table>
<thead>
<tr>
<th>Set Pressure (psi)</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual Pressure (psi)</td>
<td>0.22</td>
<td>0.31</td>
<td>0.45</td>
<td>0.54</td>
<td>0.62</td>
</tr>
<tr>
<td>Matrix Flow Rate (μl/min)</td>
<td>135</td>
<td>199</td>
<td>279</td>
<td>373</td>
<td>425</td>
</tr>
</tbody>
</table>

Table 13 Experimental condition summary of pressure and matrix flow rate

The samples were collected and stored in 100mM CaCl₂ solution for 2 days until the tensile tests. The hydrogel geometries and the preliminary data of elastic moduli are illustrated in Figure 50 and 51. The sheet thicknesses vary between 100μm to 500μm, and the elastic moduli vary between 35kPa to 130kPa depending on the printing conditions. The percentage of elongation ranged from 27% to 63% for all samples measured. At a fixed matrix flow rate, lowering the belt moving velocity gives thicker hydrogels, which agrees with the trend determined through the experiment presented in the previous section. Apart from the change of thickness, the elastic modulus of a produced hydrogel decreases as the velocity decreases. Similarly, when the velocity is set to a fixed value, increasing the matrix flow rate will result in an increase of the sheet thickness but a lower elastic modulus. The elastic modulus and operating condition
relationship is concluded as that the elastic modulus increase as the printing velocity increases or the matrix flow rate decreases.

Figure 50 Measured thickness and elastic moduli of 2w.t% alginate sheets printed using 10mm wide device and pressure control when varying the belt moving velocity, pink shaded: measured elastic moduli, blue squares: measured sheet thicknesses

Figure 51 Measured thickness and elastic moduli of 2w.t% alginate sheets printed using 10mm wide device and pressure control when varying the matrix flow rate, pink shaded: measured elastic moduli, blue squares: measured sheet thicknesses
Similar trend of the elastic modulus of 2% w.t. alginate hydrogel was briefly reported in another work. The homogenous soft material composed of 2% w.t. alginate was produced in conditions of free-extrusion gel formation and pulled-extrusion mode in a reservoir filled with 100mM CaCl₂ solution. The elastic moduli were measured to be 63kPa for the free extruded gels and 150kPa for the pulled-extruded gels. [67] When pulling was introduced into the gelation process, the produced gel obtained higher modulus of elasticity, which essentially agrees with the results presented here that higher printing speed for the same matrix volumetric flow rate or lower matrix volumetric flow rate for the same printing speed gives higher moduli, and they create the same effect of having the sheets being pulling during the extrusion.

Data of other alginate sheets were presented as for comparison of the above data. It was shown that sodium alginate with different percentage of G-block content gives different mechanical properties. 1% w.t. edible sodium alginate (Molecule-R) and 4% w.t. sodium alginate from brown algae (Sigma) showed similar elastic modulus of 52 ± 4.6kPa on the day of production. The material degraded as being cultured in DMEM for 4 days that approximately 63% elastic modulus drop happened to 4% w.t. sodium alginate from brown algae while the 1% w.t. edible sodium alginate maintained the same elastic modulus.

Since hydrogels are widely used for mimicking tissues and cellular environment, it is important to produce such hydrogels that are able to match the elastic modulus of ECM which is often referred as stiffness in a biological context. The stiffness varies between different tissues. [84] Brain tissue is soft that the stiffness is as low as 0.1-1kPa; the striated skeletal muscle’s stiffness is approximately 8-17 kPa; the precalcified bone obtains possibly the highest stiffness of 25-40kPa. [85] Certain collagen gels have been developed to initiate ontogenesis and myogenesis to mimic osteoid and muscle with stiffness of 35kPa and 10kPa respectively.[86] Furthermore, Young’s modulus of skin on volar forearm, dorsal forearm and palm is determined to vary between 20kPa and 100kPa.[87] The sheets printed using the bioprinter under different conditions obtain moduli that match the above tissues.

To further investigate the trend of the elastic modulus change over the operating parameters and the reasons of such phenomenon, the relationship between the modulus of the produced hydrogel and the shear rate that the hydrogel was printed at was studied. The shear rate for a Couette flow between two parallel plates, one moving at a constant velocity and the other one stationary, is
defined as \( \dot{\gamma} = \frac{v}{b} \). Where \( \dot{\gamma} \) is the shear rate in \( \text{s}^{-1} \); \( v \) is the moving velocity of the moving plate in \( \text{m/s} \); \( b \) is the distance between the two parallel plates in \( \text{m} \). In this case, the matrix solution, later the hydrogel, is moving with the conveyor belt at the bottom while the streaming fluid is moving on the top. The bottom matrix solution can be seen as an isolated Couette flow with the top contacting the relatively stationary streaming flow and the bottom contacting a moving plate with a velocity of \( v \) belt minus \( v \) streaming flow. In another word, the velocity difference between the belt and the flow is the absolute velocity that defines the shear rate. The theoretical model that predicts the relationship between the flow rate and the belt moving velocity gives the velocity value that matches with the flow rate with a constant shear stress across the entire thickness of the flow. When the actual belt velocity is different than this calculated value, the difference causes an extra shear that effects the hydrogel formation in terms of the gel thickness and the gel strength. For all the experimental conditions discussed above including varying the velocity or varying the matrix flow rate, the shear rates were calculated as illustrated in Table 14 and 15, where the velocity difference and the hydrogel thickness are applied as the velocity of the moving plate and the distance between the parallel plates in the shear rate definition equation.

<table>
<thead>
<tr>
<th>Actual Velocity (mm/s)</th>
<th>Modeled Velocity (mm/s)</th>
<th>( \Delta v ) (mm/s)</th>
<th>Thickness (mm)</th>
<th>Shear Rate (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>1</td>
<td>2.5</td>
<td>0.12</td>
<td>21.2</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0.17</td>
<td>11.5</td>
</tr>
<tr>
<td>2.5</td>
<td>1</td>
<td>1.5</td>
<td>0.21</td>
<td>7.2</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0.42</td>
<td>2.4</td>
</tr>
<tr>
<td>1.5</td>
<td>1</td>
<td>0.5</td>
<td>0.45</td>
<td>1.1</td>
</tr>
</tbody>
</table>

**Table 14** Shear rate calculation for the printing of varying belt velocity

<table>
<thead>
<tr>
<th>Matrix Flow Rate (µl/min)</th>
<th>Actual Velocity (mm/s)</th>
<th>Modeled Velocity (mm/s)</th>
<th>( \Delta v ) (mm/s)</th>
<th>Thickness (mm)</th>
<th>Shear Rate (s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>135</td>
<td>2</td>
<td>0.64</td>
<td>1.36</td>
<td>0.11</td>
<td>13.0</td>
</tr>
<tr>
<td>199</td>
<td>2</td>
<td>0.75</td>
<td>1.25</td>
<td>0.21</td>
<td>6.0</td>
</tr>
<tr>
<td>279</td>
<td>2</td>
<td>0.88</td>
<td>1.12</td>
<td>0.34</td>
<td>3.3</td>
</tr>
<tr>
<td>373</td>
<td>2</td>
<td>1.04</td>
<td>0.96</td>
<td>0.38</td>
<td>2.5</td>
</tr>
<tr>
<td>425</td>
<td>2</td>
<td>1.13</td>
<td>0.87</td>
<td>0.48</td>
<td>1.8</td>
</tr>
</tbody>
</table>

**Table 15** Shear rate calculation for the printing of varying matrix flow rate
Combining the calculated shear rate for each experimental condition and the measured elastic modulus of according hydrogel produced, Figure 52 illustrates the relationship between the elastic modulus versus the shear rate. The result implies that increasing the shear rate during the printing process gives higher elastic modulus of the printed hydrogel sheet. It was reported in literatures that the mechanical properties of hydrogels mainly depend on the original rigidity of polymer chains, types of crosslinking molecules and the crosslinking density. [88] Introducing higher shear to the gelation process better promotes the alignment of the alginate molecules, the polymer chains and the bound egg box structures between the G blocks and the cations in microscale that the elastic modulus tends to be higher in such condition. To further investigate and validate this hypothesis, approaches such as SEM imaging are recommended for studying the microstructure within different samples.

5.7 High-throughput with Extreme Wide Nozzle Printing

To challenge the physical limitation of the conveyer system, a 70mm wide microfluidic device was used as the largest possible printing nozzle that can be applied, since the width of the conveyor belt is 73mm. The wide printing nozzle enables the extreme high throughout printing with wide hydrogel sheets produced. Theoretical prediction of the operating conditions and produced sheet thicknesses was generated based on the device width, a confinement height of
2mm and the focusing stream flow rate of 2ml/min. The experimental materials and methods were described in the previous chapter. The matrix flow rate was brought from 2.2ml/min to 8.5ml/min with the belt velocities ranging from 1mm/s to 2.5mm/s. The measured thicknesses were compared to the predictions as illustrated in Figure 53, and the produced sheet could be as thick as 1.1mm. Since the width of the sheet was close to the width of the belt that the confinements on the sides were hard to maintain as the flow could not be restricted well in between. Leaving enough margins on the belt is important for the left and the right confinement walls to sit on to avoid leakage and disturbed flow.

![Image](image-url)

**Figure 53** Illustration of 70mm wide 2%w.t. alginate sheet being printed

![Graph](graph-url)

**Figure 54** Measured and predicted thicknesses of 70mm 2%w.t. alginate sheets
5.8 Heterogeneous Hydrogel Sheets

Heterogeneous material composition within one scaffold is preferable for multiple applications such as drug screening or cell coordination and interaction study as discussed in the first chapter. The pressure control and the multi-lane cartridge allow the printing using multiple types of materials or the same material with different concentrations and compositions.

As a case study, a multilayer microfluidic device with alternating channels connecting to three different inlets for different material A, B, C can be used to print sheets with alternating stripe pattern of repeating the sequence of A-B-C. (Figure 55) The inlets will be pressurized individually, and due to the potential resistance difference of the channels each material will go through or the difference of the material viscosities, the same pressure may produce different volumetric flow rate for each material. To control the flow rate of each material separately, multiple pressure regulators and valves are required to establish individual pressure control. In this case, the pressure level was regulated to 0.8psi with only one pressure regulator, and three valves were activated for opening and closing the gas lines separately. Three lanes out of the eight lanes on the cartridge were loaded with 2% w.t. alginate solution with three different colours of microspheres added into the solutions. (Figure 56) The overall device width was 8mm with a confinement 1mm high, and the focusing stream of CaCl₂ solution was regulated using the Harvard Apparatus syringe pump at 400µl/min. The actual experimental condition is listed in Table 16.

As a result, 0.26mm thick alginate sheet with alternating stripes in three different colors was produced as the fluorescent image shows in Figure 57. Since the flow rates of the solutions loaded in different lanes were different, the produced stripe sizes were different accordingly. The solution loaded with nile-red microspheres gave the red stripes with an average width of 140µm; the solution with yellow-green microspheres gave strips with an average width of 190µm; the solution with blue microspheres gave stripes with an average width of 310µm. The higher the flow rate the solution obtains, the wider the printed strips within the sheet. The resolution achieved in this case with a pressure of 0.8 psi was approximately 100µm.
**Figure 55** Illustration of 8mm multi-layer microfluidic device. The yellow channels are in the top layer for streaming fluid on top. The red channels and purple channels are in the second and third layer respectively. The red channels are connected to the blue channels in the bottom layer, and the purple channels are connected to the green ones in the bottom layer. Together with the white channels in the bottom layer, the three different kinds of material alternating strips within a sheet can be produced.

**Figure 56** Illustration of 2% alginate sheet with three-color alternating strips being printed using the multi-lane cartridge loaded with solutions with different microspheres, scale bar 1cm
<table>
<thead>
<tr>
<th>Color</th>
<th>Pressure Reading (psi)</th>
<th>Matrix Flow Rate (µl/min)</th>
<th>Belt Velocity (mm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lane1</td>
<td>Blue</td>
<td></td>
<td>66</td>
</tr>
<tr>
<td>Lane4</td>
<td>Green</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Lane6</td>
<td>Red</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>148</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 16 Experimental condition for each lane of material being printed onto the belt

Figure 57 Fluorescent image of the top view of a section of the alginate sheet with three stripes alternating in different colors, scale bar 100µm
Chapter 6

6. Conclusion

6.1 Conclusion

A microfluidic bioprinter that is compatible with a wide range of biopolymers and is able to continuously or periodically produce intact homogeneous or heterogeneous hydrogel sheets in Couette flow condition with different throughputs through different types of cross-linking mechanisms with control over the sheet geometries and mechanical properties is presented in this work. The printer includes a movable platform to achieve the automated production, collection and transportation of the hydrogel sheets (speed up to 8.2cm/s with resolution of 5.6μm), a microfabricated printing device that defines the geometry and composition of the printed hydrogel sheets (up to 70mm wide), pressure controls upstream for introducing the biomaterials with different flow rates (ranges from 0 to 15 psi), and temperature features (-1°C to 80°C) to fulfill the thermal requirement during the material processing. According features can be enabled for different gelation mechanisms including ionic cross-linking gelation and thermal gelation. The design of the printer components and the specifications were discussed, and functionality of motion control, pressure control and temperature control were validated through multiple experiments.

Using the concept of Couette flow with controls over the printing parameters, homogenous and heterogeneous hydrogel sheets with different geometries and mechanical properties were produced as predicted using the theoretical model. We demonstrated the formation of homogeneous hydrogel sheets 100μm to 1mm thick with different widths through ionic crosslinking using 2% alginate solution as the matrix biopolymer and 100mM CaCl₂ solution as the crosslinking/focusing reagent. The experimental results agreed with the Couette flow condition prediction, and the printer is capable of producing hydrogel sheets with different desired thicknesses. The printer also has spatial control over the composition of the hydrogel that heterogeneous sheets with strip pattern (width of 100μm to 310μm) were printed. The throughput of the printer can be easily scaled up by increasing the volumetric flow rates and the conveyor belt moving speed using a microfluidics device with a wide exit. The geometry and mechanical
properties of the hydrogel can be tuned that by varying the flow rate or the belt moving velocity when the rest parameters were fixed, hydrogel sheets with different geometries obtained different elastic moduli that vary between 40kPa to 130kPa. In addition, the printer can print hydrogel sheets in continuous manner or in finite lengths with self-start or stop flexibility. With the thermal features and controls added to the printer, biopolymers such as agarose and collagen are the candidate materials that can be processed using the microfluidic bioprinter.

Comparing to the previously developed technology in related research conducted in our laboratory, this microfluidic bioprinter produces hydrogel sheets in well-defined Couette flow condition as a new printing mechanism. The printing parameters can be individually and fully controlled with theoretical prediction over the printed sheet geometry instead of tuning the sheet geometry and property without understanding the relationship between the involved operating parameters. The theoretical model gives simple prediction and at the same time provides knowledge about how the sheet geometry and mechanical property relate to the parameters and operating condition. The bioprinter also expands the number of materials that could be applied for printing. The system is more reliable and flexible with less manipulation required for printing sheets in different lengths with the ability of self-starting and stopping, and no large volume of cross-linker is required to be stored in a reservoir as the gelation environment, which reduces the risk of contamination and the complexity of the operation. The current limitations associated with the microfluidic bioprinter, which relate to the future development, are listed in Table 17.

<table>
<thead>
<tr>
<th>Physical limitations</th>
<th>Length and width of the belt (define sheet size)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length of the confinement and belt speed (define residence time for the sheet to form intact geometry)</td>
</tr>
<tr>
<td>Operational limitations</td>
<td>Volume of the on-manifold cartridge 10ml, 3ml</td>
</tr>
<tr>
<td></td>
<td>Maximum belt velocity 25mm/s</td>
</tr>
<tr>
<td></td>
<td>8-valves for eight parallel gas lines</td>
</tr>
<tr>
<td>Prediction limitation</td>
<td>Model only valid for certain ranges of operational parameters</td>
</tr>
<tr>
<td>Ionic cross-linking</td>
<td>The case of not having enough cross-linker (i.e. low Qs/Qm ratio)</td>
</tr>
<tr>
<td>Thermal cross-linking</td>
<td>Thermal gradient not dramatic</td>
</tr>
<tr>
<td></td>
<td>Lack of downstream thermal control</td>
</tr>
<tr>
<td>Sample handling and downstream processing</td>
<td></td>
</tr>
</tbody>
</table>

**Table 17** Limitations of the microfluidic bioprinter
6.2 Future Work

The microfluidic bioprinter should be further developed with the following add-on features: downstream temperature control over the conveyor belt to enable the thermal gelation, features for illumination purpose and enabling UV gelation mechanism. Adding related features and controls enables the different gelation mechanisms and enlarges the range of materials that can be applied for gelation in this bioprinter, therefore printing based on thermal cross-linking mechanism and chemical cross-inking mechanism should be investigated. The microfluidic printing device should be modified, and different fabrication techniques should be explored to have a more stable and longer confinement with a better contact with the belt. Instead of having samples manually collected downstream for the experiments presented here, downstream sheet collecting and transportation features should be added to achieve fully automated printing. Furthermore, different types of cells can be incorporated into the biomaterials as the printer can operate in sterilized environment, such that the microfluidic bioprinter can be applied for biological study and tissue engineering as being able to process multiple types of biopolymers with different cells under various operating conditions.
References


Appendices

Appendix 1 Reservoir

The reservoir acts as the chassis that holds all the components in position while providing the freedom of adjusting the angle between the conveyor belt and the cartridge manifold. (Figure 64a) It consists of eleven parts as indicated in Figure 58. The machined pieces were later fused together using SciGrip cement for plastics (No.3 and No.16, McMaster-Carr, Aurora, OH, USA). All the parts were milled in polycarbonate to allow for machinability, optical transparency and sterilization.

Figure 58 Design of the assembled reservoir
Part 1 is the 1.27mm thick bottom piece that the other pieces sit on. It defines the overall size of the setup and contains two insertion slots on the C shape side for attaching the thermal holder support bars.

Part 2 and 3 are the reservoir side walls. The holes are for the #10-32 shoulder bolts and fixate the universal drive shaft side of the conveyor to the reservoir (Figure 64e). The curved sliding slots are designed to have the other side of the conveyor be able to rotate in order to change the angle between the conveyor belt and the horizontal. The slim slots on the edge allow Piece 9 to slide through (Figure 64d).

Part 4 is the end wall that closes the reservoir. Excess fluid from the hydrogel formation process can be collected and contained within the reservoir.

Part 5 and 6 fill the gap between the frame of the conveyor and the walls of the reservoir; allowing for fixation using #10-32 shoulder bolts (316 grade stainless steel, Accurate Manufactured Products, Indianapolis, IN, USA). These two parts are clamped in between to take the load from the conveyor and to prevent cracking of the walls (Figure 64e).

Part 7 and 8 are the sliding keys that slide along the curved slots for the conveyor to rotate freely with the other end fixed by Part 5 and 6. The smaller cylindrical keys move up and down within the slots while the bigger cylindrical disks
being clamped between the conveyor and wall of the reservoir to take the load (Figure 64d). #10-32 hex socket cap screws with washers exceeding in diameters the width of the sliding slots, the bodies of Part 7 and 8, and the tips screw into the conveyer frame to assemble related parts together. Reservoir Part 7 and 8 allow the rotation of the conveyor for the ease of cleaning between the belt and the manifold and provide a perfect contact between the conveyor and the manifold at the exit of the device.

Part 9 is the end wall of the reservoir that is removable. The slots on Part 2 and 3 allow Part 9 to slide through in order to have the reservoir opened or closed depending on the process (Figure 64c). It gives more flexibly to the handling downstream.

Part 10 and 11 are the support beams as the brackets to clamp and to support the thermal manifold. The position where the manifold is attached to is adjustable along the sliding slots. The manifold is able to be repositioned up and down based on the position of the conveyor. The cut out slots on the top of the inner side of the beams allow the manifold part to be removable from the rest of the setup for flexibility. These two beams are inserted and fused into the slots milled in Part 1 to be permanent bonded to the reservoir structure. Triangular pieces were fused to the side of beams in order to increase the contact area between the beams and the bottom piece of the reservoir for stability.
Figure 64 (a) The eleven polycarbonate pieces form the reservoir frame to support the conveyor and other features of the printer; (b) Side view of the reservoir; (c) The slidable end piece; (d) The sliding key slides along the curved slot while being clamped by the washer and the screw; (e) The shoulder bolt goes through the wall of the reservoir and the block into the conveyor to clamp and fix the end of the conveyor only allowing rotation.
Appendix 2 Motor and Associated Mounting Features

A NEMA 17 bipolar stepper motor with a 99.51:1 planetary gearbox (Phidgets Inc., Calgary, AB, Canada) was applied to drive the motion of the conveyor belt. A steal shaft coupler was designed to connect the 8mm shaft to the universal driving shaft of the conveyor (Figure 65d). An L shape steal mounting bracket was added to the gearbox for fixing one relative to the moving conveyor belt (Figure 65c). Four M2 Screws were used to go through the ring on the L shape mount to match the NEMA 17 mounting plate (Figure 65a), and two screws were used to transfer punch through the piece to attach to the conveyor frame (Figure 65b).

![Figure 65](image)

Figure 65 (a) and (b) are the assembled motor feature including (c) L shape mount design and (d) shaft coupler design. (e) The NEMA17 motor with the shaft coupler and the L shape mount attached.
Appendix 3 Conveyor Belt Specifications

The conveyor belt is the platform for achieving automatic extrusion and transportation of the hydrogel sheets. A portable, user friendly, sterilizable and adjustable conveyor with a belt that is biocompatible and transparent for light to pass through for imaging purpose is preferred. In this case, a Mini-Mover low profile series small conveyor was purchased from Whipple Enterprises Inc. along with a belt and a bedplate attached to it. The conveyor is 7.4 cm wide and 77.5 cm long with low profile height of 3.81 cm. The bedplate is a 0.95 cm thick aluminum piece with 380 μm think UHMW cover. The belt is made of semi-transparent thermoplastic polyurethane with a smooth non adhesive surface. An alternative belt, which is thinner but with a shorter lifespan, is made of semi-transparent silicone coated PET with a silicone impregnated fabric for a more adhesive surface and more allowance for back light passing through.

Figure 66 Mini-Mover low profile series conveyor with a semi-transparent thermoplastic polyurethane belt

Some modifications will be applied to the conveyor belt and the bedplate to achieve the control over temperature.

Conveyor belt modification: In order to obtain better surface properties for the materials to gel and attach to the belt, certain coating will be applied to the belt.

Bedplate modification: In order to add a thermal source and provide illumination from the bottom of the belt, the aluminum bedplate will be modified. The aluminum plate will be cut out in the center; a ceramic or polymer middle insertion will be added, followed by putting back a piece of aluminum in the cut-out. Then at the first half of the conveyor where the gel first lands there is another cut-out made for the insertion of a piece of sapphire disk (Thorlab, University wafer, or use borosilica glass from McMaster Carr, fuel silicon, or polycarbonate) in the middle.
The ceramic or plastic part is for insulating the heat from transferring outwards, and the sapphire disk will allow the light to transfer through from the LED attached under the sapphire while maintain the thermal effect.

Heating/cooling channel: In the inner aluminum piece, some all-the-way-through channels will be drilled with the end parts of the channels blocked using a string with a series of plugs fed into the channels (The size of the plug has to be twice of the diameter of the pipe as least, so dense coils inside cannot be designed here.). Hot/cold water will pass through the channels to heat up/cool down the conveyor belt.

The surface property of the belt has generated through profilometer measurement, confocal imaging and AFM imaging.

![Figure 67 Profilometer measurement of the belt material](image)
Figure 68 Confocal image of the belt surface-Slices Snapshot-5μm field of view

Figure 69 AFM contact mode scanning for belt surface smoothness
Figure 70 AFM contact mode scanning for belt surface friction
Appendix 4 Printing Cartridges

The cartridges function as the on chip supply features that contain the biomaterials. Two types of cartridges are designed and integrated into the design: one is the single-lane cartridge for the matrix biopolymer or the focusing fluid that obtains a higher consumption rate; the other one is the eight-lane cartridge for up to eight different kinds of biomaterials that may contain different types of cells as the incorporated materials. Liquid biomaterials are loaded into the cartridges at the loading inlets. The gas line is connected to the upstream pressure inlets, which pressurizes the biomaterials out through the downstream exits and the connected tubing to the channels in the microfluidic device.

There are several design criteria that were considered when choosing the material. First of all, visualization of the biomaterial consumption is desired for monitoring the biomaterial usage during the hydrogel formation progress. Secondly, biocompatible and sterilizable material must be used since cells may be contained in the biomaterial. Also, thermal energy needs to be applied to some of the biomaterials through the cartridges as for thermal crosslinking gelation, so thermal conductivity needs to be taken into consideration. Furthermore, depending how the cartridges are machined or molded, the material must allow all the features designed to be produced. In this case, polycarbonate was used by the machine shop for the fabrication of the cartridges. The main body, the inlet feature block and the outlet feature block were machined separately and later boned together using MEK solvent.

Based on the sealing and connection requirement, according fittings, adapters and tubing were selected to complete the cartridges. For the loading inlets on the top of the cartridges, mini high-strength white nylon NDT 1/16 tapered thread pipe fitting hex head plugs were used to have it manually removable, sealed, biocompatible and sterilizable (Figure 71d, 72d). For the pressure inlets on the upstream side of the cartridges, PEEK adapter of 1/4-28 flat-bottom port female to 10-32 flat-bottom port male were purchased from IDEX Health & Science; they connect the 1/4-28 fittings from the gas lines to the cartridges with pressure rating up to 1000 psi as a one piece adapter that is easy to assemble (Figure 71b, 72b). For the fluid exits on the other side of the cartridges, short vacuum tight fittings for 1/16” outer diameter tubing and associated ferrules were purchased from the same supplier (Figure 71c, 72c). These fittings provide airtight
connections under pressure rating of 400-800 psi and obtain small size headless knurls, so eight of them can be placed parallelly at the small exit area.

Figure 71 One-lane cartridge (a) Side view showing the inclined solution loading lane; (b) The pressure inlet side; (c) the solution exit side; (d) Top view of the cartridge with the solution loading inlets; (e) The bottom view

Figure 72 Eight-lane cartridge (a) Side view showing the inclined solution loading lane; (b) The pressure inlet side; (c) the solution exit side; (d) Top view of the cartridge with the solution loading inlets; (e) The bottom view; (f) Overall geometry
In terms of defining the details of the geometry of the cartridges, the following points were addressed during the design process. The overall geometry of the cartridges has to adapt to the thermal manifold which the cartridges sit on to secure the attachment, while maximizing the contact area between the cartridge and the aluminum manifold for the maximum heat conduction. Therefore the bottom of the cartridge has a cut-out as a slot for inserting the cartridge onto the aluminum manifold (Figure 71e, 72e). All the loading lanes that contain the biomaterials are inclined (Figure 71a, 72a), so that the liquids will always accumulate towards the exits downstream due to gravity, and the gas control will pressurize the liquids out and determine how much volume is delivered to the microfluidic device per time. To eliminate the dead volume within the cartridges, the lanes obtain circular cross-section without straight sharp corners inside, and the exit holes with diameter of 1.6mm are located lower than the center of the end of the cylindrical lanes. Different than the concentric condition of the pressure inlet holes and the upper end of the cylindrical lanes (Figure 73c,d), the exit holes are just within the very bottom rim of the lower end of the lanes (Figure 73e,f), so that all the solution can be pumped out without accumulation within the cartridge. In order to adapt the fittings to the inlets and outlets, the inlet feature block and the outlet feature block all have flat ports for the ferrules to be tighten against to achieve desired sealing (Figure 73a,b). The one-lane cartridge is designed for containing matrix material or streaming material for a maximum volume of 10ml; the eight-lane cartridge is for the incorporated materials that may contain cells, and each lane obtains a maximum volume of 3 ml.
Figure 73 Details of the cartridge design (a) One-lane cartridge inlet block with the inlet hole and the flat port; (b) Eight-lane cartridge inlet block with the inlet hole and the flat port; (c) One-lane cartridge pressure inlet concentric with the end of the lane; (d) Eight-lane cartridge pressure inlet concentric with the end of the lane; (e) and (f) show that the solution exit holes are not concentric with the end of the lane, but located lower at the bottom.
Appendix 5 Thermal Manifold

The thermal manifold acts as a support for the cartridges and the microfluidic device. It is also a thermal conduction and distribution media for heating up or cooling down the cartridges and devices. A piece of aluminium was used to fabricate the manifold, since it obtains high heat conductivity and toughness, and the thermal elements were inserted into the slots on the bottom of the manifold plate as the thermal source.

The overall geometry of the thermal manifold is a 5mm thick aluminum plate. One end of the plate is thickened with a trapezoidal feature that matches the design of the bottom side on the cartridges (Figure 74-1). The sharp edges of the feature are filleted so that the cartridges can be easily slidden onto the slot (Figure 74-2). The other end of the plate is tapered with the tip thickness of only 130μm (Figure 74-3); therefore the microfluidic device placed on top of the tapered surface is inclined, so that the gap between the device exit and the conveyor belt that material lands on is eliminated.

On the top of the manifold, some features are added to locate the microfluidic device. Two holes were drilled through from the tapered surface for two sets of ¼-20 screws and nuts to use as for clamping the device in position (Figure 74-4).

On the bottom of the manifold, two 4.1cm by 4.1cm squares with a height of 3.2 mm were cut out along the center line of the manifold. The thermal elements were placed into the slots (Figure 74-5). In order to locate the wires on one side the thermal elements, circular extruded cut slots were made all the way to the side, so that the wires can pass through the manifold and connect to the control board (Figure 74-6). These thermal elements have the wires that are thicker at the connecting joints because of the electrical tapes wrapped around, so the extruded cuts were expanded around those corners (Figure 74-7). Also, on one side of the clamping area of the manifold, the clamping features would block the exit of one of the thermal wire, so an extra slot was cut out from the side to have the wire come out before it hits the clamping feature (Figure 74-8).

On both sides of the manifold, there are extension shoulders on the edges for supporting a plastic thermal cover on top of all the features on the manifold, which will be described in the next section (Figure 74-9). Also, in order to attach the manifold to the reservoir while maintain the
flexibly of adjusting the height of the manifold, two square shape sliding keys are designed to increase the contacting area between the manifold and the reservoir support brackets (Piece 10 and 11) (Figure 74-10). After sliding along the brackets to find the desired height, two #10-32 thumb screws are used to tighten the manifold and the brackets together.

In addition, in order to attach a copper conducting plate and a cooler to the bottom of the thermal elements, four holes of 54mm apart were drilled for M3 plastic screws. The details will be described in the coming sections for the related thermal control features.

Figure 74 Thermal manifold design (The numbered details are discussed in text)
Figure 75 Different views of the thermal manifold with the thermal elements and the clamping screws on
Figure 76 Assembly of the bioprinter cartridges and the thermal manifold
Appendix 6 Thermal Control Features

In order to better insulate the thermal manifold and eliminate heat lost, a transparent polycarbonate cover was machined to be placed on top of the manifold. Piece 1 and 2 (Figure 77a-1, 2) are the side plates that sit on the manifold extension shoulders as indicated in Figure 77-9. Piece 3 is the end piece that allows the exit of the microfluidic device to expose to the conveyor belt surface (Figure 77a-3). Three pieces were fused together using MEK solvent.

Figure 77 (a) Design of the thermal cover; (b) the MEK fused polycarbonate thermal cover
Figure 78 (a) The complete assembly of all the parts on the cartridge side: The cartridges and the microfluidic device are located on top of the manifold, and the thermal cover is applied on the top. (b) Side view of the assembly

Potted thermoelectric elements HP-199-1.4-0.8-Potted (with a perimeter seal around the elements that is recommended for cooling below dew point) were used (TE technology, Traverse City, MI, USA). These Peltier thermoelectric high performance heaters/coolers operate from 40°C to 80°C. The dimensions, the maximum current, maximum power, maximum voltage and maximum temperature differences are listed in Table 17.

<table>
<thead>
<tr>
<th></th>
<th>HP-199-1.4-0.8 Potted</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>40mm</td>
</tr>
<tr>
<td>B</td>
<td>40mm</td>
</tr>
<tr>
<td>H</td>
<td>3.2mm</td>
</tr>
<tr>
<td>Imax (A)</td>
<td>11.3</td>
</tr>
<tr>
<td>Qmax (W)</td>
<td>172</td>
</tr>
<tr>
<td>Vmax (V)</td>
<td>24.6</td>
</tr>
<tr>
<td>DTmax (°C)</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>when T hot side=27°C</td>
</tr>
</tbody>
</table>

Table 18 Specifications of Peltier Thermoelectric Element HP-199-1.4-0.8 Potted (TE Technology)
In order to use the thermal elements for both heating and cooling, a heat sink is required on the side that is not contacting the target objects to maintain the temperature gradient across the element. A cooler (Seidon 120M model RL-S12M-24PK-R1, Cooler Master, Richmond, BC, Canada) that consisted of a fan and liquid cooling system was attached on the bottom of the thermal elements. (Table 18)

<table>
<thead>
<tr>
<th>Model</th>
<th>RL-S12M-24PK-R1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimension</td>
<td>( \phi \text{ 70 x 27 mm} )</td>
</tr>
<tr>
<td>Radiator Dimensions</td>
<td>150.3 x 118 x 27 mm</td>
</tr>
<tr>
<td>Radiator Material</td>
<td>Aluminum</td>
</tr>
<tr>
<td>Fan Dimension</td>
<td>120 x 120 x 25 mm</td>
</tr>
<tr>
<td>Fan Speed</td>
<td>600~2400 RPM (PWM) ± 10%</td>
</tr>
<tr>
<td>Fan Airflow</td>
<td>19.17 ~ 86.15 CFM ± 10%</td>
</tr>
<tr>
<td>Fan Air Pressure</td>
<td>0.31 ~ 4.16 mm H2O ± 10%</td>
</tr>
<tr>
<td>Fan Life Expectancy</td>
<td>40,000 hours</td>
</tr>
<tr>
<td>Fan Noise Level (dB-A)</td>
<td>19 ~ 40 dBA</td>
</tr>
<tr>
<td>Bearing Type</td>
<td>Rifle bearing</td>
</tr>
<tr>
<td>Connector</td>
<td>4-Pin</td>
</tr>
<tr>
<td>Fan Rated Voltage</td>
<td>12 VDC</td>
</tr>
<tr>
<td>Fan Rated Current</td>
<td>0.3A</td>
</tr>
<tr>
<td>Power Consumption</td>
<td>3.6W</td>
</tr>
<tr>
<td>Pump Life Expectancy</td>
<td>70,000 hrs</td>
</tr>
<tr>
<td>Pump Noise</td>
<td>17dBA</td>
</tr>
<tr>
<td>Pump Rated Voltage</td>
<td>12 VDC</td>
</tr>
</tbody>
</table>

Table 19 Seidon 120M model RL-S12M-24PK-R1 Specification (Cooler Master, Richmond, BC, Canada)
In order to guide the heat on the back side of two thermal elements out to the copper plate on the cooler, a copper plate was designed to be clamped in between the thermal elements and the cooler. The top of the copper plate matches the thermal elements, and the bottom will be closely attached to the cooler. Four holes were drilled accordingly for the four plastic #6-32 screws that are used to clamp the cooler, the copper plate and the bottom of the aluminum thermal manifold together for conducting the undesired heat on the other side of the thermal elements out without transferring it back to the manifold.

![Figure 79 Copper plate design](image)

**Figure 79** Copper plate design

**Figure 80** (a) The copper plate design, (b) Photograph showing cooler attached to thermal manifold. Thermal elements were first inserted into manifold. Copper plate matched the layout of TE elements. Cooler was attached using four plastic #6-32 screws.
The original assembly of the thermal control was done by placing copper pieces into the thermal elements slots, followed by putting the copper plate underneath, and then the TE element under the copper plate with the cooler attached the last. The components were screwed together using 6-40 1x hex socket nuts (plastic). All the interfaces had silver thermal paste applied. To have a better head conduction between surfaces and a higher pressure for clamping all the features, a different was applied for the real experimental testing as described in Chapter 4.

Figure 81 Illustration of the temperature control circuit board
Appendix 7 Pressure Control Setup

Solenoid valves and eight-channel manifold (Lee Company), pressure controller (National Instrument), pressure gauge (MG1-5-A-9V-R SSI Technologies, Inc) and 0-15psi pressure regulator (Type3410, Marsh Bellofram) were connected and applied to achieve the pressure control over the materials within the cartridges. The details were described in Chapter 4.

Figure 82 Illustration of the entire pressure setup connections
Appendix 8 Microfluidic Device Design

Low resistance microfluidic channel designs were carried out based on the following calculations. The flow rate $Q$ in a channel is proportional to the applied pressure drop $\Delta P$. Using the analog of electrokinetic law between voltage difference and current, $U = RI$,

$$\Delta p = Q \times R_h$$  \hfill (A1)

with $R_h$ as the hydrodynamic resistance.

The expression for the hydraulic resistance is

$$R_h = \frac{8\mu l}{\pi r^4}$$  \hfill (A2)

for channels with circular cross-section (total length $l$, radius $r$) and

$$R_h = \frac{12\mu l}{wh^3 \left(1 - \frac{0.630h}{w}\right)}$$  \hfill (A3)

for channels with rectangular cross-section (width $w$ and height $h$, expression valid when $h<w$).

In a network of channels, equivalent resistances can be computed (as in electrokinetics) as:

$$R_h = R_{h1} + R_{h2},$$  \hfill (A4)

for two channels in series and

$$\frac{1}{R_h} = \frac{1}{R_{h1}} + \frac{1}{R_{h2}}.$$  \hfill (A5)

for two channels in parallel.

Therefore

$$\frac{\Delta p_1}{\Delta p_2} = \frac{R_{upperchannels}}{R_{lowerchannels}}$$  \hfill (A6)

with the same flow rate $Q$, and the resistance of parallel channels as

$$R = \frac{1}{\frac{1}{R_1} + \frac{1}{R_2} + \ldots + \frac{1}{R_n}} = \frac{n}{\frac{1}{R_1}} = \frac{1}{\frac{wh^3\left(1 - \frac{0.630h}{w}\right)}{n}} = \frac{12\mu l}{nwh^3\left(1 - \frac{0.630h}{w}\right)}$$  \hfill (A7)
For a 25mm wide device with exit channel width 300um, exit channel height 150um (same for all the features in the design) and exit channel length 6mm, to have the same pressure drop upstream at the inlet, i.e. the same resistance, the inlet channel (single branch) is calculated to be 9.96mm wide with a length of 6mm; and the intermediate channels (six branches) are calculated to be 1.74mm wide with a length of 6mm.

<table>
<thead>
<tr>
<th>Device (mm)</th>
<th>25.4</th>
<th>10.5</th>
<th>42.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feature height (mm)</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Exit branch number</td>
<td>48</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>Exit channel length (mm)</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Exit channel width (mm)</td>
<td>0.3</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>Inlet channel number</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Inlet channel length (mm)</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Inlet channel width (mm)</td>
<td>9.96</td>
<td>4.2</td>
<td>21.8</td>
</tr>
<tr>
<td>Intermediate branch number</td>
<td>6</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Intermediate channel length (mm)</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Intermediate channel width (mm)</td>
<td>1.74</td>
<td>1.46</td>
<td>2.81</td>
</tr>
</tbody>
</table>

**Table 20** Pressure resistance calculation (Assume the same fluid flow rate, viscosity and the same pressure drop across inlet channel, intermediate channels and exit channels)
Appendix 9 Shear Rate vs. Pressure Plots and Trendlines Based on the Experimental Data

Figure 83 Shear Rate vs. pressure plots and best fit line $y = 0.014x - 82.239$ for 10 mm device

Figure 84 Shear Rate vs. pressure plots and best fit line $y = 0.006x - 27.962$ for 25 mm device
Figure 85 Shear Rate vs. pressure plots and best fit line $y = 0.0007x - 5.3581$ for 42 mm device
Appendix 10 Combined Resistance Constant D Determination of CaCl\textsubscript{2} Solution as for Non Viscous Fluids

Figure 86 Experimental plot of P*\(t\) against shear rate for flowing 5ml of CaCl\textsubscript{2} solution through different device geometries

For CaCl\textsubscript{2} solutions, since the viscosity of solution is similar to that of DI water, 0.00089Pa·s at 25 °C was used as the reference value. The ratio D values were determined the same way of dividing the average pressure and time products by the reference viscosity as described in Chapter 5.

<table>
<thead>
<tr>
<th>Rheology Measurement</th>
<th>100mM CaCl\textsubscript{2} in DI water in Pa s</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mm device</td>
<td>389988</td>
</tr>
<tr>
<td>25mm device</td>
<td>234166</td>
</tr>
<tr>
<td>42mm device</td>
<td>2438468</td>
</tr>
</tbody>
</table>

Table 21 P*\(t\) average of CaCl\textsubscript{2} solutions through different devices
<table>
<thead>
<tr>
<th></th>
<th>100mM CaCl$_2$ in DI water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>10mm device</strong></td>
<td>0.44*10$^9$</td>
</tr>
<tr>
<td><strong>25mm device</strong></td>
<td>2.62*10$^8$</td>
</tr>
<tr>
<td><strong>42mm device</strong></td>
<td>2.73*10$^9$</td>
</tr>
</tbody>
</table>

*Table 22* Combined fluid physical resistance constant for specific geometry/configuration