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A Cell Patterning System Based on Thermal Ink-jet Technology

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A Cell Patterning System Based on Thermal Ink-jet Technology

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Abstract

Tissue engineering has a significant impact on biological and medical area. It helps to eventually realize the repair, replace or improve tissue function or even the complete organ. Inkjet cell printing, as a simple and novel way in bio-fabrication, is a promising tool to fabricate cell pattern and scaffolds that directly serve the purpose of tissue engineering.

This thesis introduces a thermal inkjet cell printing device. The mechanical design, software and pertinent test data are described. The specification of the printhead, a customized printing substrate and operating procedure is also presented. The printing device uses a thermal inkjet printhead to create droplet and patterns of cells. This research have found: 1) The lower limit of firing time for cell injection through the applied thermal inkjet printhead is 3 μs; 2) Patterns like circle or band can be produced by this device, with a resolution of 96 DPI; 3) This printing apparatus can provide reasonable cell viability; 4) With a properly prepared printing substrate and procedure, a monolayer of printed cell pattern can be achieved.

1. Introduction

This thesis is primarily focused on the building and testing of a cell printing system.

In this chapter, a brief introduction of the current cell printing technology and objectives of this project will be presented.

1.1 Cell Printing Technology

The idea of printing cells, or biofabrication is brought up with the development of tissue engineering. Tissue engineering, in general, is to seek a feasible approach to fabricate viable body parts including animal, or even human [1]. This research is experiencing fast progress to cope with the shortage of organs for transplantation [2]. In a more practical sense, the technology of cell printing could meet the great need in several neurodegenerative diseases, brain injuries and the spinal cord to find alternative methods to replace unhealthy and injured cells and provide substrates that can promote cell growth in order to achieve functional recovery [3]. In fact the technology of printing also serves other biological purposes other than printing cell, including biosensors [4], DNA arrays [5, 6], DNA synthesis [7], micro-deposition of proteins [8] and the fabrication of polymeric scaffolds [9, 10].

1.2 Methods of Cell printing

Various methods were employed in cell printing. In this part, 2 major ink-jet printing technologies will be reviewed, as well as several other novel or popular cell or microparticle deposition methods.

1.2.1 Thermal inkjet printing

Thermal inkjet printing is a technique commonly used in various brands of inkjet printers including Hewlett-Packard and Canon. In a thermal inkjet printer, a cartridge that with a built-in printhead is utilized to eject the ink out of the nozzle which usually at the bottom of the cartridge. The mechanism is presented in Figure 1.1.

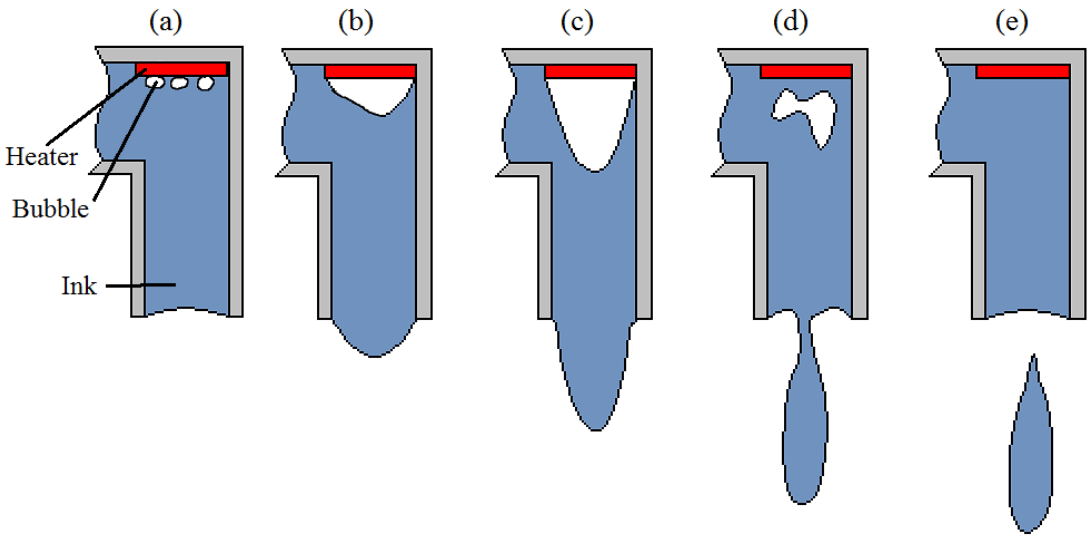

Figure 1.1 The mechanism of thermal inkjet printhead

Fig 1.1 (a) presents the structure of a typical thermal inkjet printhead. Tiny bubbles are left out in the nozzle that filled with ink. An electric heater (red rectangular in the figure) is enclosed in the wall of the nozzle. In Fig 1.1 (b), voltage is applied on the heater to produce heat which makes the tiny bubble in (a) inflates. In Fig 1.1 (c) the bubble inside the nozzle has inflated to an extent that could force a droplet of the ink to come out of the nozzle. In (d), the bubble explodes so that the droplet could come out of the nozzle. Then the surface tension of the ink sucks more ink from the reservoir, which is typically on top of the printhead, to refill the nozzle for the next print procedure. The heater is actually a thin-film resistor (the shape of

which aren't demonstrated in Fig 1.1) [11] that could rise to more than 300 ℃ in few microseconds. The normal heater working time is around 6 microseconds [12].

Various groups have successively applied the thermal inkjet printing techniques to print cells. T. Xu *et al* have used a modified Hewlett Packard (HP) Desktop 550 thermal inkjet printer or similar models to conduct multiple researches, including the printing of viable mammalian cells [13], high-throughput cell patterning [14], the print of neural cell structures [15], human microvasculature fabrication [16] and gene transfection into living cells [17]. Other researches includes printing macromolecules hydrogels to steer neural stem cell differentiation [18], growth factor array fabrication [19], characterization of patterned self-assembled monolayers and protein arrays [20], construction of high-density bacterial colony arrays [21], transfer of nucleic acids to solid supports [22].

For this method, the general cell viability after printing is quite satisfying. For neural cells a viability of 74.2 ±6.3% can be achieved [15], and a viability of 89% of printed Chinese hamster ovary (CHO) cells was obtained and only 3.5% apoptotic cells were observed afterwards [23]. These researches have proved that thermal inkjet printing can achieve a high viability and indicated that this technique has a promising application for cell or microparticles delivery.

1.2.2 Piezoelectric inkjet printing

Piezoelectric inkjet printing is another technology applied to normal office/home printers. Different from thermal ink-jet printing technology, it's protected by patent. Epson Inc. first invented this technology and named it "Micro Piezo" and applied for a patent [24].

Different from thermal inkjet printhead which produces heat to inflate the air bubble, the piezoelectric printhead conducts printing by physical vibration generated by a piezoelectric element. The structure and mechanism of a typical piezoelectric inkjet printer is illustrated in Fig 1-2.

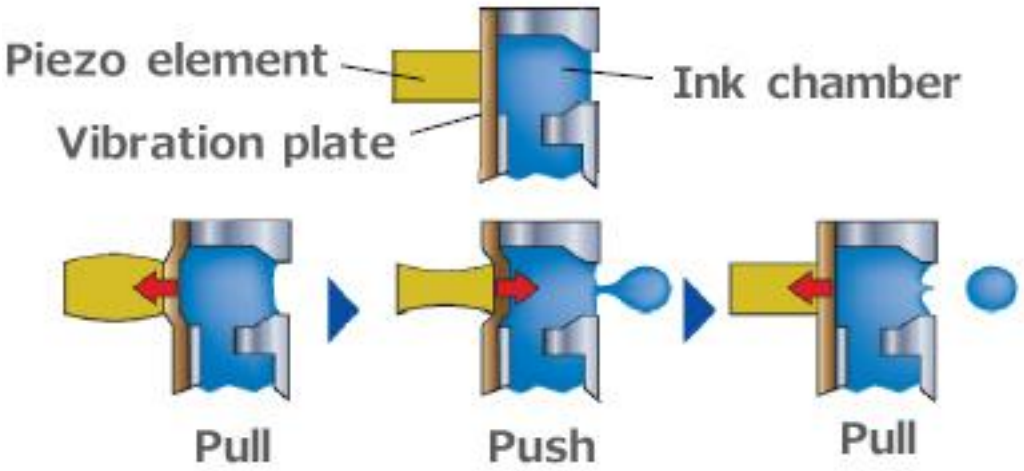

Figure 1.2 The mechanism of piezoelectric inkjet printhead. Source: Epson Inc.

A piezo electric nozzle usually contains a piezo element and a vibration plate to which it is attached. Figure 1.2 presents a 3-step printing procedure for "Pull – Push - Pull". The piezo element will deform when charged with voltage. In the first pull step the element will deform toward the opposite direction of ejecting to "suck" the ink inward. Then it performs a "Pull" action which pushes the ink out of the nozzle. After that the piezo element and vibration plate moves back to its initial situation. The cartridge is kept separate from the printhead and replaceable. The speed of printing can be adjusted by alternating the vibrating frequency of the piezo element, and by changing the voltage that applied to it droplets with different sizes can be acquired.

A number of Bio-printing researches are based on piezoelectric printheads, including: protein printings and hybrid cell-containing materials and structures [25], biocompatible seeding of individual living cell [26], cantilever array sensor preparation [27], delivery of human fibroblast cells [28], pattern printing and microenvironment fabrication [29-31].

Some investigations have shown that the using of piezoelectric based printhead would harm the cells because sometimes the specific frequency of vibration may destroy cell membranes thus cause death [13, 23], but newer researches have indicated that piezoelectric printhead is suitable for various kinds of cells including rat retinal ganglion cells [32], and HeLa cells (ATCC CCL-2) [33]. In those tests a general viability of 70-86% can be achieved [32, 33].

1.2.3 Other methods

Besides thermal and piezoelectric inkjet printing, other methods such as valve-based printing [34, 35], laser guidance [36], soft lithography [37-39], photolithographic techniques [40, 41] and dip-pen nanolithography [42-44] are also developed to conduct micro-scale cell deposition.

Valve-based printing uses solenoid valves to serve as a switch of the nozzle, and the cell printing process is driven by pneumatic pressure. It can achieve better control over the droplet size via adjusting inlet pressure, nozzle diameter and solenoid switch time. However, compared with thermal/piezoelectric inkjet printing, the overall nozzle number of the current valve-based printing device is far less. A typical piezoelectric printhead contains hundreds of individually controlled nozzles while valve-based printhead has only 1 or 2, which will lower the printing speed. The

droplet size it produces is also larger than what's produced by ink-jet printing technology due to the limitation of valve reaction time. The smallest valve on-time is 100 μs, compare to 3 μs in ink-jet apparatus.

Laser guidance cell writing uses optical forces from a laser to drive single cells onto glass substrates. Patterns could be created by altering the position of laser. However, it has limited application in tissue engineering because of its low efficiency, and inability to control the migration of cell [14].

Soft lithographic technologies include microfluidic channel flow patterning and microcontact printing, which is more widely used. This method creates patterns by first fabricating a polydimethylsiloxane (PDMS) stamp using patterned silicon master, then coating biomolecules on the surface of the PDMS stamp to make it suitable for cells to anchor. This technology offers high resolution, which usually at a scale of 2-500 μm [38].

Other methods like photolithographic patterning technology or dip-pen nanolithography are more often used in protein patterning or fabricating protein arrays. For photolithographic technology, it can also be used to create very fine patterns at a scale of about 5 μm [45]. Both techniques offer higher resolution [38, 45], but practically are less adopted in cell printing.

1.3 Current development of Cell printing systems

Most cell printing systems are based on inkjet printing technologies. Valve-based setup is also adopted by multiple groups.

As mentioned before, several research groups have developed a system involved a modified Hewlett Packard (HP) 550C (or similar) (Hewlett Packard, CA, US) or Canon (Canon, Tokyo, Japan) thermal inkjet printer and successfully applied to cell/ micro particle printing. However, they still use the original printing driver and use Microsoft Office software (Microsoft, WA, US) to generate patterns on the computer.

Several groups have adopted piezoelectric inkjet technologies. An open source project Piezoelectric Oligonucleotide Synthesizer and Microarrayer (POSaM) was developed to provide a customizeable DNA microarrays [46] and controlling system [47]. Other commercial manufacturers including Microfab (Microfab, TX, US) have printing products based on piezoelectric actuators to achieve computer-controlled, multi-nozzle printing apparatus [48]. However, the price of these designs can be as high as over $50,000.

1.4 Objectives

The goal of this project is to design and build an accurate, easy-to-use, affordable and customizable apparatus for cells printing. It will allow precise control over the printing device, and provide reasonable cell viability. This apparatus should fit for multiple printing materials with different components or viscosity. It should be durable, easy to clean and have low maintenance requirements.

2. The cell printing system

In this chapter, a comprehensive introduction of a cell printing apparatus will be introduced. This introduction includes a list of important components, the reason to choose them and for some parts the designing details were also given.

2.1 System components overview

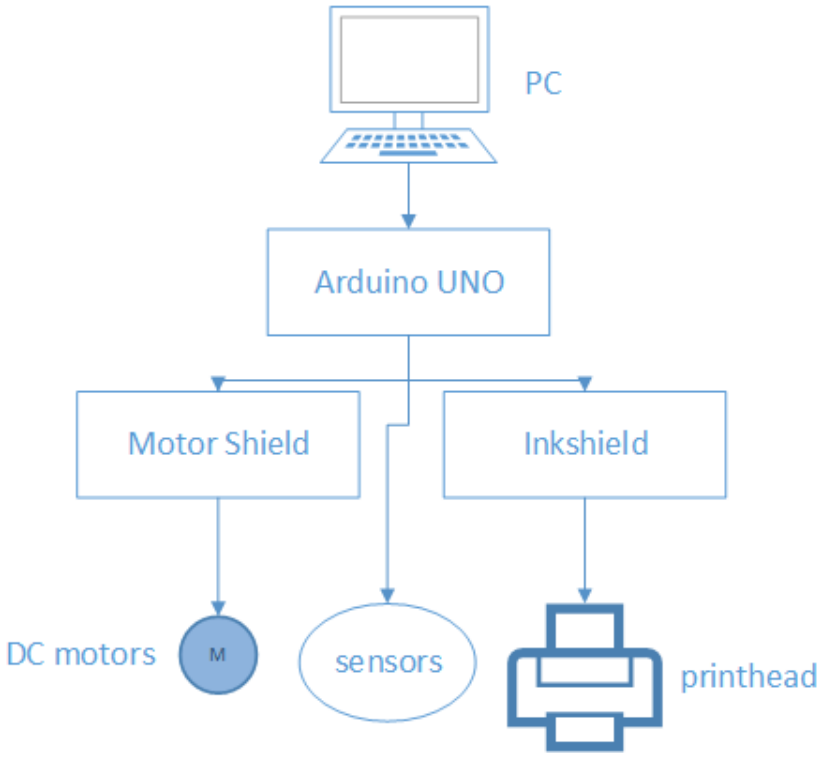

Figure 2.1 The structure of the system

The structure of this system is shown in Figure 2.1.

The entire printing system is based on a HP C6602 thermal inkjet cartridge/ printhead. HP C6602 is a single color cartridge with integrated printhead. It has 12 individually controlled nozzles and can provide a resolution of 96 dpi [49]. Proper modification was conducted to make this cartridge suitable for bio printing.

The system consists of a 2D moving stage made with 2 DC brushless motors and 4 photoelectric sensors. When use, the printing cartridge will be mounted onto the stage.

The controlling system contains: 1) an Arduino UNO board served as the motherboard and controller of the photoelectric sensor; 2) Inkshield to control the ink cartridge and 3) Arduino Motor Shield to control the DC motors.

A controlling software is developed in Arduino integrated Development Environment (Arduino IDE) to control the cartridge, the movement of the DC motors and the photoelectric sensor. A Graphic User Interface (GUI) is developed in Processing 2, a JAVA developing environment that usually works with Arduino IDE. This GUI allows user to control the nozzle on the computer through serial communication with the Arduino UNO board. Arduino IDE is a developing interface based on C++, Processing 2 is a developing interface based on Java.

2.2 The printhead

As mentioned above, a modified HP C6602 cartridge was used to do the cell printing. It's a thermal inkjet printhead with 96 dpi resolution. The printhead has 12 nozzles each with a diameter of around 60 μm. Major reasons for choosing this particular type of cartridge are as follows:

1) Based on the results of the hands-on tests, thermal inkjet printhead, compared to piezoelectric ones, is less throne to clogging and easier to clean.
2) The cartridge has an integrated printhead right at the bottom of the cartridge which means there's no need to clean both the cartridge and the printhead separately after each printing operation.
3) The cartridge has been "hacked". It can work with the Inkshield to achieve complete override to its original printer or driver. We don't need to modify a

printer to make it compatible to bio-printing like what the previous researchers did, nor have to use a third party software (Like Microsoft Office) to work with its original driver to conduct printing. By programming in Arduino Integrated Development Environment (Arduino IDE) a direct and complete control to this cartridge can be achieved.

The printhead need voltage pulse cycle to drive the micro-heater within. For HP C6602, normally, the heater in each of its 12 nozzles needs a 21V pulse for 6 μs to heat up the air bubble, then a 0.5 μs voltage drop to 0V and rest around 300 μs to cool down and get ready for next cycle [12].

Proper modifications needs to be done to make an original cartridge compatible for cell printing:

1) Remove half of the lid of the cartridge.
2) Remove the Sponge inside the cartridge and dump all the ink.
3) Remove the filter on top of the ink channel, the filter is a dual-layer metal net with mesh of around 40 μm in each layer. Tests have shown that the filter could block most of the cells from coming into the ink channel.
4) Flush and rinse the cartridge with Isopropanol and Deionized (DI) Water.

Figure 2.2 shows the images of the cartridge.

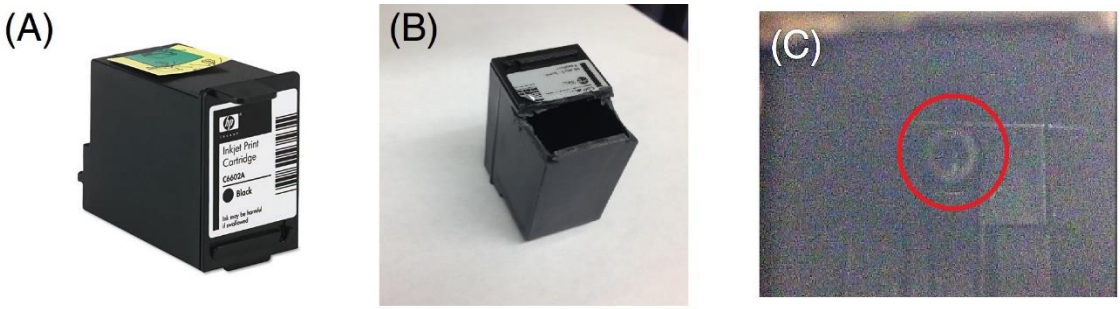

Figure 2.2 HP C6602 cartridge. (A) Original, (B) Modified,
(C) In the red circle: ink entrance with filter removed

To clean the cartridge before and after printing, put it into hot (~90 ℃) Deionized water for 10 min. Before each cell printing task, it is recommend to use 90% Isopropanol or Ethanol to spray on the inside and outside of the cartridge and then rinse it with Deionized water for 3 times [13, 14, 23].

2.3 The 2D moving stage and accessories

The system uses a XY stage of a microscope stage. The stage uses 2 customized Pittman (Amtrek-Pittman, PA, US) 8222 series brushless DC motor, along with 6 (4 used) PM-24 photoelectric sensors (Sunx sensors, IA, US). A customized mounting rack connects the stage with the cartridge.

The photoelectric sensor can detect if the X-axel or Y-axel is moving to the end of the rail. It sends pulse back to the Arduino UNO controller so with proper programming possible hardware damage or mal-operation could be avoided.

The mounting rack is manufactured by a Replicator 2 3-D printer (Makerbot Industries, NY, US). It's height-adjustable and specifically designed for 3-D printing to avoid possible large tolerance. With this device the cartridge can be placed at a

lower level for direct microscope operating and fluorescent imaging, the cartridge can also be set at a higher position and work with a culture dish tray to perform printing on a substrate. Figure 2.3 presents the mounting device. The cartridge and the mounting rack is also retractable.

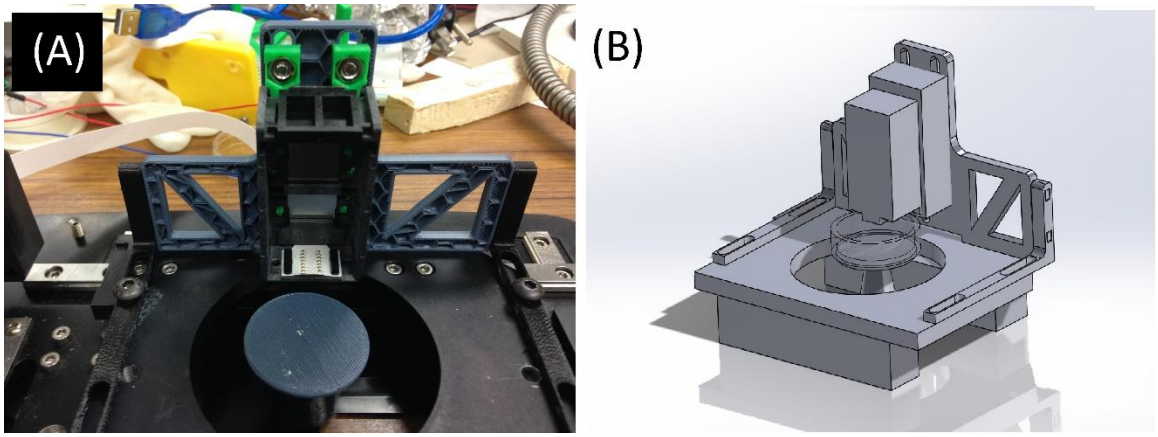

Figure 2.3 (A) Mounting device, assembly, (B) Solidworks sketch.

2.4 Controller and other hardware

The controlling part consists of 3 major components: 1) Arduino Uno controller; 2) Inkshield and 3) Motorshield. A "Shield" means it is an extended PCB that could installed on top of an Arduino board to realize some extra functions. Detailed introduction of all these 3 boards is addressed below.

2.4.1 Arduino Uno controller

Arduino Uno board is a microcontroller using an ATMega 328 (Atmel Corp., CA, US) single chip. It offers 6 analog inputs, 14 digital input/output pins. It connects to PC with a USB cord and supplied via a power jack.

In our case all analog pins are occupied. 4 out of 6 analog inputs are used to control 12 nozzles on the cartridge through the Inkshield. The rest 2 analog inputs pins take parts in controlling the DC motor.

4 of 14 digital pins, working with pull-up resistors, are used to receive signals from 4 photoelectric sensors. One of the rest digital pins is used to provide pulse signal for the cartridge, and another 4 pins participate in controlling the DC motor. So 9 out of 14 digital pins are used.

We used an independent 9V power supply to boost the hardware and the logic circuit, the USB port is used to send/ receive serial signals to/ from the computer.

2.4.2 Inkshield

Inkshield is an extended package developed to gain complete control over the HP C6602 cartridge. It has 2 major functions: raise the voltage and output multiple signals.

To provide a proper voltage for firing the nozzles, the Inkshield used a MC34063 Step-up/Down/Invert Switching Regulator. It can generate 20V with a 9-12V power source [50, 51].

Inkshield also used a CD4067 De-multiplexer to drive the nozzles. CD 4067 makes it possible to only use 4 analog pin on the Arduino Uno board to output 12 signals by creating a binary 4 bit address. The function of CD4067 chip is demonstrated in Figure 2.3. It uses 4 inputs to create up to 16 channels.

A	B	C	D	Inh	SELECTED CHANNEL
X	X	X	X	1	None
0	0	0	0	0	0
1	0	0	0	0	1
0	1	0	0	0	2
1	1	0	0	0	3
0	0	1	0	0	4
1	0	1	0	0	5
0	1	1	0	0	6
1	1	1	0	0	7
0	0	0	1	0	8
1	0	0	1	0	9
0	1	0	1	0	10
1	1	0	1	0	11
0	0	1	1	0	12
1	0	1	1	0	13
0	1	1	1	0	14
1	1	1	1	0	15

Table 2.1 The function of CD4067

The necessity of using it is because otherwise 12 of 16 digital pins will be taken up just to drive the nozzle, which would not leave enough pins for the sensors and DC motors. In Table 2.1, A to D indicates the input and selected channel on the right side shows there're 16 output channels.

2.4.3 Arduino Motor shield

Motorshield is used to control DC motors. It has 2 separated channels that each uses 4 pins (3 digital pins and 1 analog pins) to drive the motor. For each channel, the 1 analog channel controls the current, which means the speed of the motor. In the Arduino IDE the current value can be set from 0 (minimum) to 255 (maximum).

Other 3 digital channels are responsible for direction, PWM (on/off switch) and Brake. It can achieve speed adjusting, emergency brake and inertia brake.

2.5 Software

The software system consists of 3 parts: 1) Inkshield driver; 2) Arduino program and 3) Graphic interface.

2.5.1 Inkshield driver

The driver connects the cartridge with the Arduino board. It distinguishes the order of printing and not-printing by reading input signals of 0 or 1. By working with CD4067 it also sends the order to the right nozzle.

2.5.2 Arduino IDE program

Several different Arduino IDE program were developed to cope with different printing tasks. User can create a printing task by input combinations of 0 and 1 as well as set up other printing parameters. Commonly used parameters includes:

1) Nozzle firing time: It determines how long the micro heater in each nozzle works to eject out the droplet;
2) Waiting time between each firing;
3) Repeat times of each printing tasks;
4) Waiting time between each tasks.

A typical Arduino IDE program contains a type database that allows you print 10 numbers, 26 English letters and multiple symbols and marks.

2.5.3 Graphic interface

The graphic interface is created in Processing 2, it communicates with the Arduino IDE program via serial port. The interface features a live input function which allows user to send words and symbols to the controller. It also provides advantages like emergency stop and reset printing task. Figure 2.4 shows the running interface.

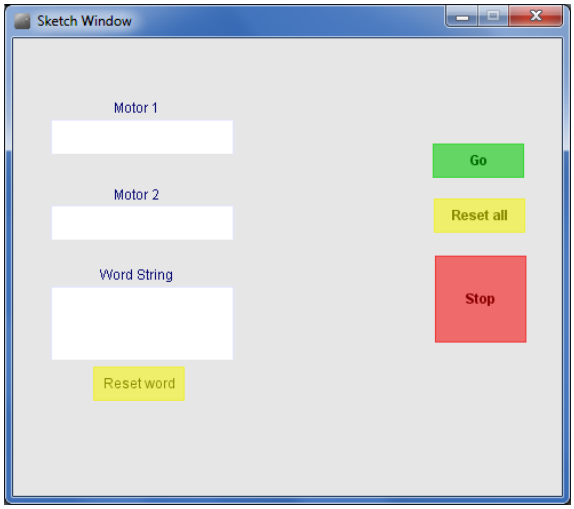

Figure 2.4 The control interface

3. Printing tests and cell damage evaluation

This part will demonstrate that this device is capable of printing cell culture substrate and viable mammalian cells. Two different cell damage/ viability tests are performed to reflect the overall cell damage right after printing and the individual cell viability and proliferation at 72h after printing.

In the printing tests, suspensions of Bovine Aortic Endothelial Cells (BAOEC) and Gelatin solution were printed directly onto multiple surfaces. The cell damage assay during the printing process was evaluated by a combined lactate dehydrogenase

(LDH). Individual viability was observed by cell staining, using Ethidium Homodimer-1 for dead cells and Calcein AM for live ones.

3.1 Materials and methods

3.1.1 Cell and other printing suspension preparation

BAOEC were cultured using modified Eagle's medium (MEM). Before printing, cells are removed from incubator and media is suctioned away. Cells on dish are washed 2-3 times with PBS. Then PBS is suctioned away and Trypsin is added. Cells incubated with Trypsin for 5-8 minutes in incubator. Once a large portion of the cells have detached from the culturing surface, culturing media is added to stop Trypsin effect. After which cells are transferred from culture dish to centrifuge tube and centrifuged at room temp for 4 min at 1.5 RPM. Centrifuge tube is removed once completed and the supernatant is suctioned away. Then use liquid media to re-suspend the cell to make the printable cell suspension ("Bio-ink"). After that the Bio-ink can be loaded into printer and tested. The final concentration of the BAOEC cells was roughly about 5,000,000 cells/ml if doing a single droplet observation. If doing a pattern printing or monolayer fabrication, 10,000,000 cells/ml or higher is recommended.

We also use 2% g/ml Porcine Gelatin solution at 25 ℃ to serve as an alternative printing material.

3.1.2 Printing Surface Preparation

Several different surfaces were used in the printing tests: regular glass slides for the Gelatin solution test, and two different hydrogel-based substrates were used respectively for cell viability test and pattern printing and culturing.

For viability tests, the substrates were made by 0.2% g/ml Porcine Gelatin in PBS. Add the suspension into the culture dish and incubate at 37℃ and 5% CO_2 for at least 2 hours to form a uniform layer of Gelatin. Remove the excess suspension from the culture dish before use.

For pattern printing, substrates is acquired by dissolving 10 g Porcine Gelatin in 50 ml DI water, the perform autoclaving at 121 ℃ for 30 min. After autoclaving add another 50 ml of MEM into the suspension when it's at around 45℃ [13]. By far a 100ml substrate of 10% g/ ml Gelatin solution was acquired. After shaking and mixing at 37 ℃ use about 100 μl of the solution to dispense onto a 35mm culture dish to form a 0.1mm uniform substrate layer, or similar volume in a glass coverslip located in a culture dish. Incubate the culture dish for at least 2 hours and take it out of the incubator at least an hour before use to let it cool down to the room temperature. The dishes have to stay in a clean chamber. When the substrate is in a "gel" like status it is ready to use.

3.1.3 Cell and Gelatin solution printing and culture

To conduct the printing properly, the inside of the cartridge has to be sprayed with 100% ethanol and wash both inside and outside with DI water for at least 3 times. If printing onto a culture dish, the printing has to be performed in a safety cabinet to prevent possible contaminations.

The cell suspension has to be shaken sufficiently to prevent cell deposition, which will cause an uneven cell distribution and may lead to the clogging of the cartridge. After shaking the suspension is ready to transfer to the cartridge. If the

volume of the suspension is less than 1 ml, direct pipetting the suspension into the entrance of the printhead located at the bottom of the cartridge is recommend. The printing surface (glass slides, culture dishes with coverslips) should be placed directly underneath the cartridge. The travel distance between the bottom of the nozzle and the substrate layer should be as short as possible to reduce spraying. The distance can be adjusted by three screws located at the back of the mounting device.

If doing a live/dead cell staining test, use the software in Arduino IDE to build a printing cycle with patterns, set the firing to standard 5 μs. Then use the culture dish coated with 0.2% g/ml Gelatin and print the cells on to the culture dish. Add MEM to the dish and then put the culture dish into the incubator that maintains at 37℃ and 5% CO_2 for 24 h. After 24 h the cells are ready to be stained.

For the substrate only printing (no cells involved), 0.02 g/ml and 0.04 g/ml Gelatin concentration, 3 μs and 5 μs firing time are both tested.

For cell printing, rectangular and round shaped pattern will be printed. For rectangular printing, use 5 μs of firing time (standard), waiting time between each printing 300 μs, and motor speed at 250. Perform the printing in a clean chamber while keeping the blower shut to prevent droplet drifting. The cells can be printed out following a same pattern several times (4-20) to achieve a higher cell concentration on the culture dish which will be helpful to the following viability test.

If doing a cell monolayer fabrication, which requires a much higher cell density, repeat printing for 500-2000 times is highly recommended. Repeat time varies based on the pattern.

After printing, quickly place the 35mm dish with cells, along with another 35mm dish filled with DI water, with lid open, into a 100mm dish with lid closed. Then move the 100mm dish back to the incubator that maintains at 37℃ and 5% CO_2 to avoid a dry out. After 2 to 3 h of incubation, add DMEM to the culture dish carefully thus to minimize the potential damage to the original pattern.

3.1.4 Cell viability and staining

To conduct the damage assay, transfer 50 μL of supernatant into a well for each of the culture dishes which are planned to be tested. This volume of supernatant will act as the control group for the LDH activity assay. Then perform print test. Set the firing time to 6 μs. At least 250 μL of supernatant needs to be printed out. Transfer 50 μL of supernatant each to 5 wells on a 96 well plate. Repeat it after each replicate depression into the cultured cells. Then Prepare additional wells with: media controls, spontaneous LDH Activities (50 μL of media from culture dish prior to micromanipulator depression, one for each of the 4 cytotoxicity samplings) and maximum LDH Activities (10 μL of 10X Lysis Buffer after all depression tests in the culture dishes have been completed. One for each of the 4 cytotoxicity samplings). After that add 50 μL of Reaction Mixture to each of the wells and incubate in the dark at room temperature for 30 min. After incubation add 50 μL of stop solution. The absorbance at 490 nm and 680 nm was quickly read afterwards. Then the cytotoxicity percent can be calculated.

To perform the fluorescence cell staining to the cells on the culture dish, remove excess media from culture dish and wash with PBS 3 times. Then add prepared stain assay: Calcein AM of 0.1 to 10 μM, and Ethidium Homodimer-1 of 0.1 to 10 μM.

Incubate for 20 min before observing. Cells were incubated 24 h and 72 h before counting.

3.2 Results

3.2.1 Printing without cells

Two factors were observed to affect the droplet size: Gelatin concentration and firing time. The droplet size was measured by the diameter of Gelatin residue left out on the glass slide after water's evaporation in seconds. The results indicate droplets on substrate with higher Gelatin concentration and with longer firing time are bigger. Table 3.1 shows the average droplet size under different printing conditions. By adjusting concentration and firing time, droplet size can be controlled from around 80 μm to 140 μm. From our observation, the droplet cannot be formed with a firing time less than 3 μs. Figure 3.1 presents the image of the residue.

	2% Gelatin solution	4% Gelatin solution
Firing time 3 μs	91.55 μm	101.32 μm
Firing time 5 μs	145.93 μm	169.11 μm

Table 3.1 Average droplet size in different concentration and firing time

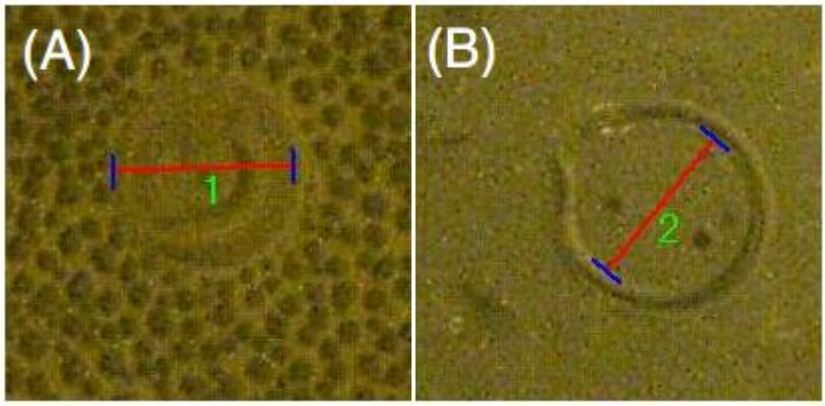

Figure 3.1 Gelatin residue images. Printing with 2% Gelatin solution and 3 µs (A); with 4% Gelatin solution and 5 µs (B). Distance 1= 88.54 µm, distance 2=170.04 µm

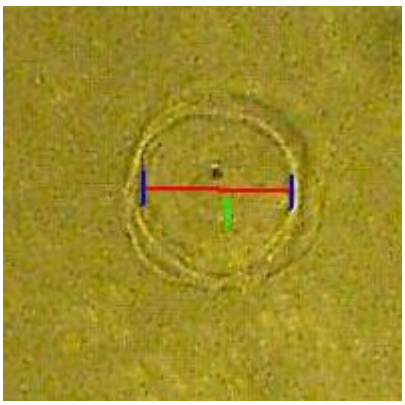

Figure 3.2 Gelatin residue images, after 30 times of printing. Distance 1=147.61 µm

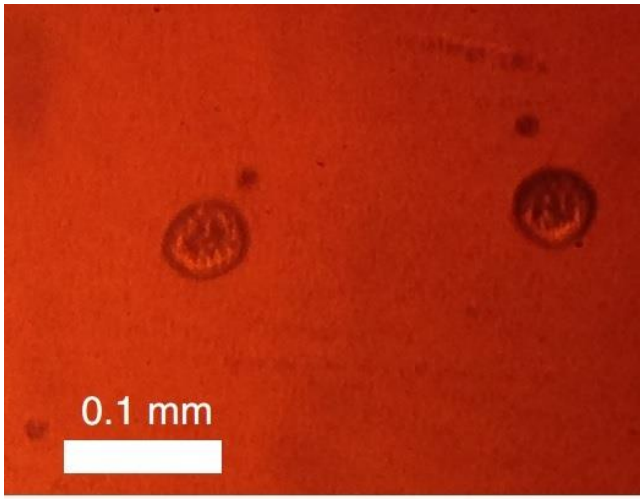

Figure 3.3 Droplets on superhydrophobic surface

The droplets produced by this apparatus also show high location repeatability. Figure 3.2 shows the Gelatin residue of 30 times printing with one nozzle with 10 min waiting period between each injection (2% Gelatin solution, 5 μs firing time). The residue, though piled up, is not randomly located but accumulated at the same spot.

Printing test on superhydrophobic surface was also performed. Superhydrophobic surface is fabricated usually by create nano-pillars on the glass slide or silicon. The nano-pillar will make the surface rough enough to form a large (>120 °) contact angle between water droplet and the surface. From the results, it is observed that the droplet size on the superhydrophobic surface is much smaller than on the normal glass. The average droplet size is about 50 μm, regardless of firing time. However, if firing time was set under 4 μs, the location repeatability would be largely reduced. Figure 3.3 presents image of droplets printed on a superhydrophobic surface. In addition, the water evaporation time on superhydrophobic surface is around one minute, which is longer than that on glass.

3.2.2 Cell printing results

The BAOEC cells were found in droplets printed out. With a cell concentration of 5,000,000 cells/ml and drop size of ~5.23×10^{-7}ml, most of the droplets contain 1 or 2 cells. Figure 3.4 shows a droplet with 2 cells in it.

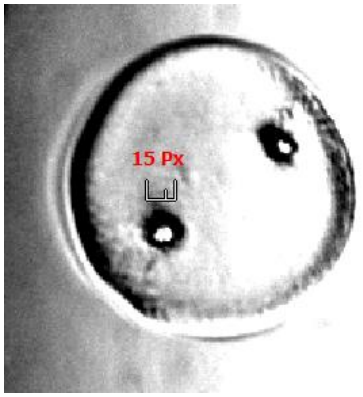

Figure 3.4 Single droplet with 2 cells, scale bar = 14.8 µm

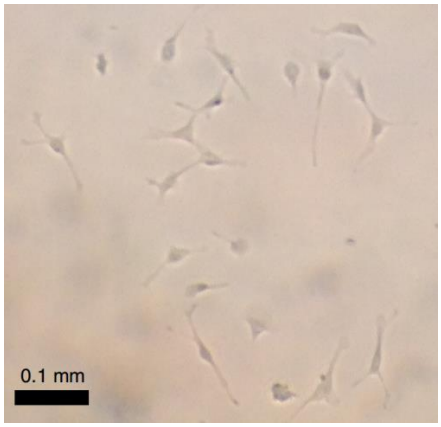

Figure 3.5 Printed Cells on culture dish

Cells were observed anchored and ready to proliferate after 2 days of culturing (Figure 3.5), which indicates a long term viability of this printing apparatus.

For pattern printing, dot matrix, round and rectangular shape round shaped pattern can be successfully fabricated.

The device can be used to print dot matrix. Figure 3.6 presets single droplet dot matrix on a superhydrophobic surface. In this test multiple nozzles were used to achieve a fast print speed and a high cell concentration of 10,000,000 cells/ml is used.

For round shape printing, a big dot with high concentration of cells can be fabricated on a 10% Getalin solution coated surface. The smallest diameter of the

circle we manage to make is about 600 μm. Figure 3.7 shows the image of a round pattern after 24 h of culturing. The cells, from the Fig. 3.7, are orientated toward the center. One possible reason is that when the cells are being printed out in a half-sphere droplet, there're more cells in the center part because for a half-sphere, center part is higher than the edge. The cells located in the center will move outward to the periphery of the droplet in order to find a place less populated, with more media so that they can settle. During their movement, the cell density gradient and elongation is formed.

For rectangular shape printing, the most common result we can achieve is a band-like shape. Figure 3.8 and 3.9 show related results: Figure 3.8 presents a corner of a rather large rectangular shape. Fig. 3.9 shows a part of a band shape. However the edge of this band is interrupted as to form an arrow-like edge.

The fabrication of cell monolayer is also conducted. Figure 3.10 shows a typical layer produced by this device, where the cells density is about 1056 cells/mm^2.

3.2.3 Cell viability results

From the results of the damage assay, on an average of a very small amount of cellular cytotoxicity was acquired: 4.48%. In all cases the percentage of cytotoxicity was below 10%.

For the individual cell viability counting, after 24 h of culturing 98.2% of all 495 cells were still alive (9 cells dead). For another group of cells, after 72 h of culturing 73.8% of all 309 cells were still alive (81 dead). Figure 3.11 presents an image of stained dead cells after 3 days.

Figure 3.6 Dot matrix on a superhydrophobic surface, scale bar = 0.1mm, grid on the top: 0.1mm × 0.1mm

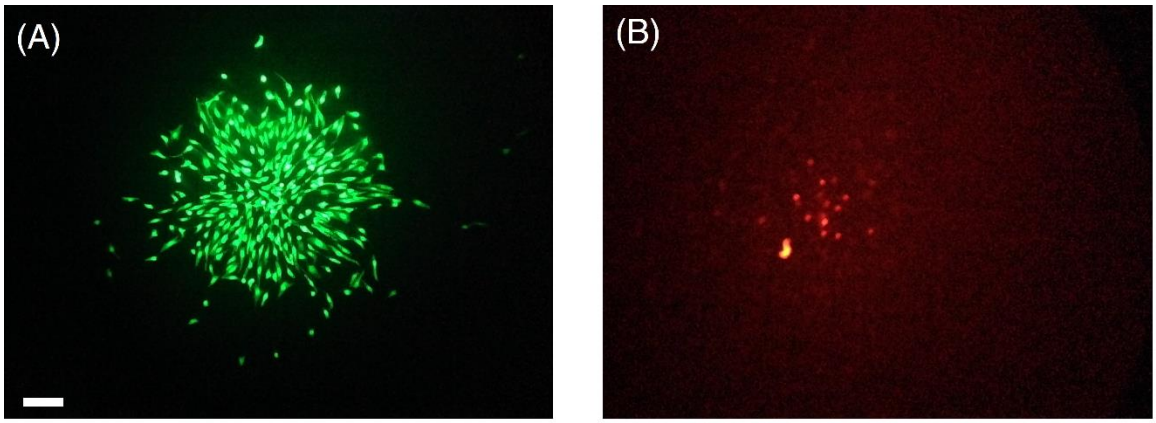

Figure 3.7 A round pattern. Live cells in the pattern (A), dead cells in the pattern (B), scale bar 0.1mm

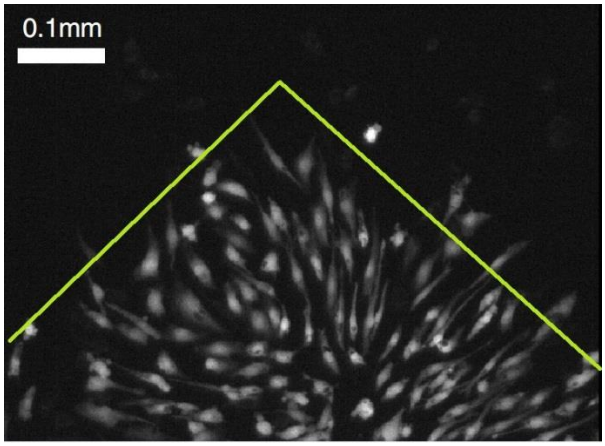

Figure 3.8 A corner of a rectangular shape, yellow line is a boundary indicator

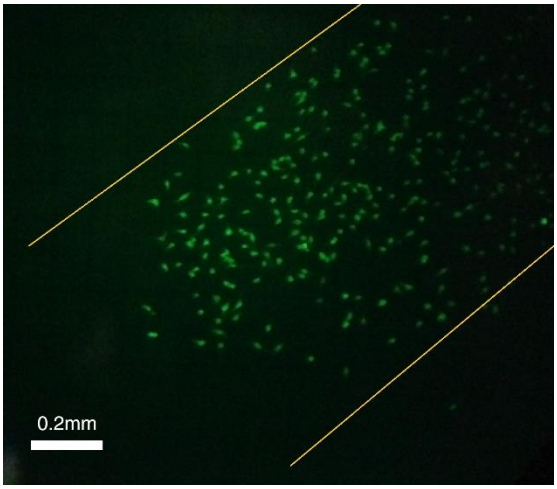

Figure 3.9 Part of a band pattern, yellow line is a boundary indicator

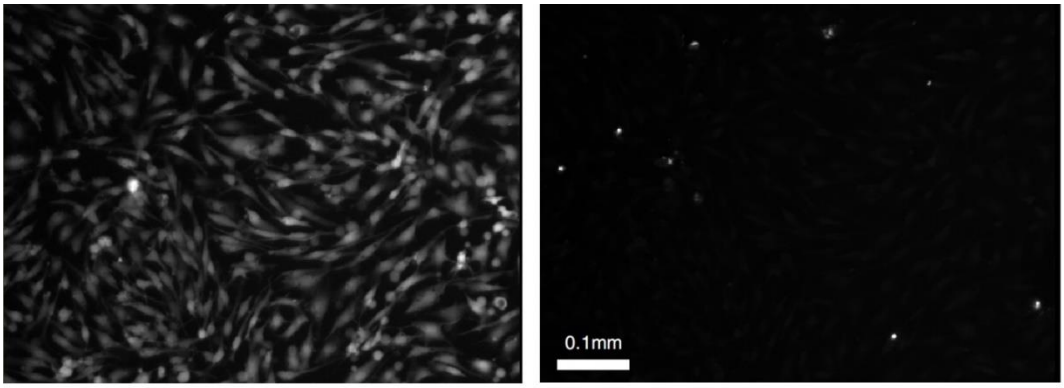

Figure 3.10 High density cell monolayer, live (left), dead (right), same scale

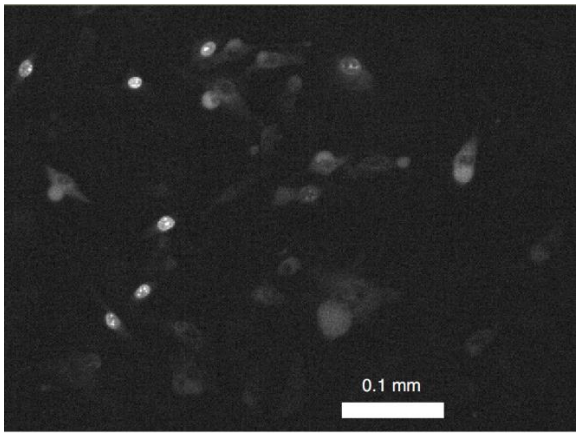

Figure 3.11 Printed Cells in culture dish, after 72 h, dead cells highlighted

3.3 Discussions

3.3.1 Substrate and cell printing

Both no-cell and cell printing have proved that this print apparatus is capable of performing bio-compatible printing. The result also asserts that a thermal inkjet based printer is able to deliver cells and the substrate it needs to grow on.

For substrate printing, the results confirmed that the firing time and Gelatin concentration influence the final droplet size. From previous studies the possible effect brought by firing time change hasn't been studied since previously people used a standard driver to drive the printer, as well as commercial office software (Like Microsoft Word or Powerpoint) to create a pattern to perform a printing [13, 16, 23]. In our case we find that if the firing time was reduced to 3 μs, the droplet size will reduced remarkably. Therefore it is believed that, at least for the HP C6602 cartridge, 3 μs is the minimal time that the micro heater needs to inflate and blast enough number of tiny air bubble in the nozzle so that droplets could descend and form a droplet. If the firing time is less than 3 μs, the micro heater couldn't heat enough air bubbles thus a integrate droplet can't be formed. For other thermal inkjet printers, similar threshold firing time should also exists and even for the piezoelectric inkjet printers, since the volume of the droplet is controlled by the deformation of piezoelectric actuators, thus by changing the charge voltage and the charge time a similar control over the droplet size should also be able to achieve. Different Gelatin concentration also has some impact on the droplet size. It is probably because that higher concentration of Gelatin could take more water with it when being ejected out.

These facts could help to increase the accuracy of single cell printing, where the droplet size could change the number of cells in one droplet.

As for the results on the superhydrophobic surface, it is expected that the droplet diameter would reduce due to a larger contact angle. Reducing of the diameter could in turn increase the resolution of the cell printing since it could detain the cell in a smaller region. It's also observed that the location repeatability will significantly reduce if the firing time is set to under 4 μs, it could because that the volume of droplets is not high enough so the droplets tend to move on the surface, rather than forming a big and steady sphere on the surface. As for cell culturing, many researches have shown that it's difficult and time-consuming to grow or adhere cell on a superhydrophobic surface. It takes up to 72 hours for Mouse 3T3 fibroblast cells to adhere on superhydrophobic surface [53]. People have taken advantage of this feature to tried to create surface with micro pattern formed by superhydrophobic and superhydropilic structures to define the cell growth area thus to achieve cell adhesion and patterning [54, 55]. They have created walls or gaps with superhydrophobic structure to separate the cells, and use super hydrophilic surface for cells to grow on. Some other researches have tried to change the surface roughness to increase the cell adhesion [56]. It will be easier for cells to adhere and grow on a surface with higher roughness.

For cell printing, the living cells in the ejected droplet proved that this set of device is capable for cell printing. By controlling the cell concentration, a single or dual-cells droplet can easily be produced. For patterning, the device is capable of producing round shape of different diameter and dot matrix. The concentration of the

round shape mostly depends on the repeat time. A typical 700 μm round shape usually requires at least 200 times of printing for a single nozzle. The drop size is smaller than previous researches [13], and has the potential to be further reduced. For matrix dot, the resolution is mainly depend on the motor setting.

It is also observed that this device is not easy to create a complete, easy-to-identify rectangular shape. The possible reasons are: 1) when performing rectangular printing, unlike circles, the cells won't really get a chance to be closely deposited, especially for the cells located at the edge of the pattern, which prolong the time that they need to adhere onto the surface. It also means that more cells could get flushed away when extra DMEM was added in after 2 h, this part will be addressed in detail at 3.3.3. 2) The cell concentration of the suspension is still not high enough. Even if performing with 10,000,000 cells/ml, only around 4 cells can be found in one droplet, which is far from enough in producing a cell monolayer. Creating a rectangular shape also needs more cells than circle does, which requires much more repeat time. 3) Some groups have tried to add Integrin [13] into the cell suspension, which could help cell aggregate and maintain its pattern.

3.3.2 Cell viability

From the result of the viability tests, the cell cytotoxicity in all cases is below 10%, which is very similar to other studies [13, 23], where a similar thermal inkjet printhead was applied, and is generally lower than other studies applying piezoelectric printhead [32, 33]. Also, from the result of the live/dead cell counts, a viability rate at 98% is higher than [33], and an over 70% rate is reached after 72 h, which is also close to [15].

3.3.3 Printing surface and operating procedure

Different printing surface (substrate) was tested to optimize the environment for cell growth and patterning. Various printing and culturing procedures were created. In early tests, cells were printed onto a thin substrate layer formed by 0.2% g/ml Porcine Gelatin PBS solution. When this substrate was used the culture dishes had to be filled with DMEM right after printing to avoid cell dehydration. However the adding of DMEM could flush away any original pattern so that eventually the cells would only spread randomly on the culture dish, so new substrate and operating procedure has to be created to solve this problem.

Eventually a modified substrate with 10% g/ml Porcine Gelatin mixed with 1x DI water and 2x MEM with a thickness under 0.5mm was used in all patterning tests. A substrate of this high Gelatin concentration will have much higher viscosity so as to become a "gel" like material in room temperature, which could: 1) Limit the cell movement so as to keep a more dense and tight pattern; 2) Slow down the water evaporation in the droplet. Also, the MEM in the substrate provides extra nutrition that normal 0.2% Gelatin PBS solution doesn't have. The substrate, first "gel" like, will liquefy slowly after being placed into the incubator since the humidity and temperature are both higher, to let the cell settle down at the bottom of the culture dish.

Results have shown that increasing the Gelatin concentration have an obvious effect on the cell density. Figure 3.12 presents the difference in cell density using 2 substrates with different concentrations. In Fig. 3.12 (A), cells spread out because the

low substrate viscosity, while Fig 3.12 (B) showing that substrate with high gelatin concentration can keep more cells at where they were, even after adding the media.

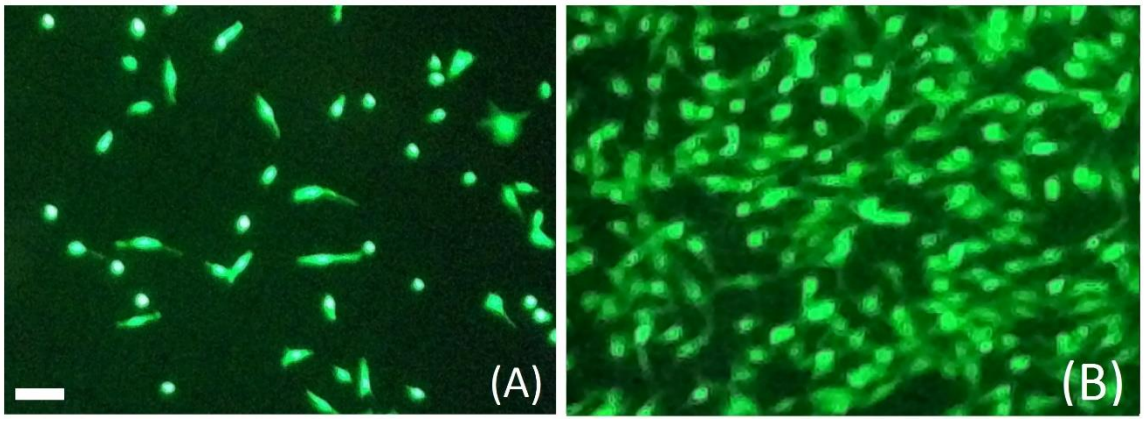

Figure 3.12 Cell density after 24h of culturing, both 500 firing times, concentration of cell suspension: 10,000,000 cells/ml. (A) Cell printed on 1.5% Gelatin substrate, (B) On 10% Gelatin substrate, (A) and (B) are on same scale, scale bar = 0.05mm

Apart from the substrate, operating procedure was also changed. Different from the previous process, we placed the 35mm culture dish, along with another open-lid 35mm dish that contains water, in a 100mm culture dish. This change could provide higher humidity in 100mm dish to slow down the evaporation process of the water in the printed droplet. With a higher humidity, cells can still be hydrated after as long as 2.5 – 3 h, which is long enough for them to adhere to the bottom of the dish to create a steady pattern that won't get flushed away by the extra media. Since the cells have to be left for 2 h, it's necessary to add DMEM in the substrate.

In summary, with a more viscous substrate and higher humidity, a better preservation of the pattern and cell density can be achieved.

4. Conclusion and future works

As addressed before, single cell printing is a crucial tool in tissue engineering, bioengineering and pharmaceutical research. The apparatus described in this paper provide a convenient, affordable and durable way to build a cell printer based on thermal inkjet printing technology.

For the hardware part, the modified ink cartridge is capable to perform micro droplet printing. With proper cell concentration the printhead can easily achieve single or double cell deposition. The printhead is durable, easy to clean and inexpensive. The 2-axle moving stage can drive the cartridge and accessories and realize pattern printing. Additional safety device could help to reduce human error.

The apparatus uses multiple software to properly drive hardware parts. A graphic user interface allows fast input and safer printing. A set of other programs is used to perform different tasks.

The test results revealed that this device offers similar or better printing quality than its predecessors. The device offers less than 10% of cell cytotoxicity in all cases, around 98% viability after 24 h of culturing, and more than 70% viability in 72h. All of these results are either better than or similar to previous results. This device also provides a good printing location repeatability. The uniqueness of this device is that it can fully control every adjustable parameter to find out relations between the settings and the output. In fact, some interesting facts were revealed during the tests of this device. A threshold firing time of 3μs was discovered, and interesting facts related to superhydrophobic surface printing clearly show it deserves a further study. The device can achieve some pattern printing, while the accuracy can still be improved.

Because of all the progress made in the cell printing, the cell printing technique sounds less fancy than it did in the ‘90s. However, there’re still plenty to do to improve this work.

First, a feasible way of culturing printed cell needs to be found. It’s crucial to make single cell anchor and proliferate while maintaining its pattern. There’s no such research for now that allows the pattern printed by single cells to grow, and while there is technique for single cell patterning through controlled surface microfabrication, there is not a technique for mass production. From the previous studies of single cell printing, droplets with single or double cells will be printed into wells with the media suspension [33], or combined with media droplets [34, 54]. Other researches seeks to produce a huge droplets results from multiple printing with a lot number of cells to create a comfortable environment for the cells to adhere onto the substrate [13, 35], however, by which means the pattern made by single cell will no longer be available. It could become possible if a pre-defined micro-pattern fabricated with superhydrophobic and superhydrophilic surface is used to act as walls and channels for the cell.

Secondly, the characteristics of how super hydrophobic surface interact with cells are also worth researching. Plenty of researches like [56- 58] have shown that cells are hard to grow on the superhydrophobic surface, however, it yet to be discovered that under some other conditions, like a cell suspension mixed with Gelatin or other chemicals, and a higher humidity, could them help the cells to adhere and grow?

Thirdly for the apparatus itself, to improve the accuracy of the device, replace the current motor with stepper motor is necessary. An interface with more function is also expected. Functions like costume printing process and nozzle test could be added into the current software. Also, the setup will support multiple printhead in the future, which will allow us to print cell suspension and other materials (like media or stain solution) simultaneously. This idea might also help to keep the cell hydrated so as to prolong their life.

References

[1] Drury JL, M. D. (2003). Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials*, 24(24):4337–51.

[2] Fuchs JR, N. B. (2001). Tissue engineering: a 21st century solution to surgical reconstruction. *Ann Thorac Surg*, 72(2):577–91.

[3] Barbara Lorber, W.-K. H. (2014). Adult rat retinal ganglion cells and glia can be printed by piezoelectric inkjet printing. *Biofabrication*, 015001.

[4] Newman JD, T. A. (1992). Ink-jet printing for the fabrication of amperometric glucose biosensors. *Anal Chim Acta*, 262(1):13–7.

[5] Hughes TR, M. M. (2001). Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. *Nat Biotechnol*, 19(4):342–7.

[6] Barbulovic-Nad I, L. M. (2006). Bio-microarray fabrication techniques—a review. *Crit Rev Biotechnol*, 26(4):237–59.

[7] Blanchard AP, K. R. (1996). High-density oligonucleotide arrays. *Biosensors Bioelectron*, 11(6–7):687–90.

[8] Roda A, G. M. (2000). Protein microdeposition using a conventional ink-jet printer. *Biotechniques*, 28(3):492-6.

[9] Sherwood JK, R. S. (2002). A three- dimensional osteochondral composite scaffold for articular cartridge repair. *Biomaterials*, 23(24):4739-51.

[10] Park A, W. B. (1998). Integration of surface modification and 3D fabrication techniques to prepare patterned poly (L-lactide) substrates allowing regionally selective cell adhesion. *J Biomater Sci Polym Ed*, 9(2):89-110.

[11] Jan O. Orlandini v. Niessen, J, N. S. (2011). Development and characterization of a thermal inkjet-based aerosol generator for micro-volume sample introduction in analytical atomic spectrometry. *J. Anal. At. Spectrom*, 26, 1781-1789

[12] Gilliland M. (2005). Inkjet Applications. *Woodglen Press*

[13] Xu T, J. G. (2005). Inkjet printing of viable mammalian cells. *Biomaterials*, 26(2005) 93-99

[14] Roth EA, X, D. (2004). Inkjet printing for high-throughput patterning. *Biomaterials*, 25(2004) 3707-3715

[15] Xu T, G. M. (2006), Viability and electrophysiology of neural cell structures generated by the inkejet printing method. *Biomaterials*, 29(2006) 3580-3588

[16] Cui X, B. (2009), Human microvasculature fabrication using thermal inkjet printing technology. *Biomaterials*, 30(2009)6221-6227

[17] Xu T, R. Z. (2009), Inkjet-Mediated Gene Transfection into Living Cells Combined with Targeted Delivery, *Tissue Engineering: Part A*, 15(2009) 95-101

[18] Ilkhanizadeh S, T, H, (2007), Inkjet printing of macromolecules on hydrogels to steer neural stem cell differentiation, *Biomaterials*, 28(2007)3936-3943

[19] Watanabe K, M. M. (2003), Growth Factor Array Fabrication Using a Color Ink Jet Printer, *Zoological Science*, 20: 429-434

[20] Pardo L, W. B. (2003), Characterization of Patterned Self-Assembled Monolayers and Protein Arrays Generated by the Ink-Jet Method, *Langmuir*, 19(2003)1462-1466

[21] Xu T, P. L. et al (2003), Construction of High-Density Bacterial Colony Arrays and Patterns by the Ink-Jet Method, *Wiley InterScience*, 25 Nov.

[22] Goldmann T, G. (2000), DNA-printing: utilization of a standard inkjet printer for the transfer of nucleic acids to solid supports, *J. Biochem. Biophs. Methods*, 42(2000)105-110

[23] Cui X, D. R. (2011), Cell Damage Evaluation of Thermal Inkjet Printed Chinese Hamster Ovary Cells, *Biotechnol. Bioeng.*, 106(2010)936-939

[24] Narita T, S. H. T. (1993*), U. S. Patent No. 5,252,994*. Washington, DC: U.S. Patent and Trademark Office

[25] Derby B, (2008), Bioprinting: inkjet printing proteins and hybrid cell-containing materials and structures, *J. Meterials Chemistry*, 18(2008)5717-5721

[26] Nakamura M, K. T. W. et al (2005), Biocompatible Inkjet Printing Technique for Designed Seeding of Individual Living Cells, *Tissue Engineering*, 11(2005)1658-1666

[27] Lukacs G, M. H. (2012), Ink-Jet Printing: Perfect Tool for Cantilever Array Sensor Preparation for Microbial Growth Detection, *J. Sensors*, 2012

[28] Saunders RE, G. D. (2008), Delivery of human fibroblast cells by piezoelectric drop-on-demand inkjet printing, *Biomaterials*, 29(2008)193-203

[29] Miller E, F. W. et al. (2006), Dose-dependent cell growth in response to concentration modulated patterns of FGF-2 printed on fibrin, *Biomaterials*, 27(2006)2213-2221

[30] Campbell PG, M. F. et al. (2005), Engineerd spatial patterns of FGF-2 immobilized on fibrin direct cell organization, *Biomaterials*, 26(2005)6762-6770

[31] Phillippi J, M. W. et al. (2008), Microenvironments Engineered by Inkjet Bioprinting Spatially Direct Adult stem Cells Toward Muscle- and Bone- Like Subpopulations, *Stem Cells*, 26(2008)127-134

[32] Lorber B, H. H. M. (2014), Adult rat retinal ganglion cells and glia can be printed by piezoelectric inkjet printing, *Biofabrication*, 6(2014)015001

[33] Yusof A, K. S. S. et al. (2011), Inkjet-like printing of single-cells, *Lab Chip*, 11, 2447

[34] Faulkner-Jones A, G. K. G. et al (2013), Development of a valve-based cell printer for the formation of human embryonic stem cell spherid aggregates, *Biofabrication*, 5(2013)015013

[35] Demirci U, M. (2007), Cell encapsulating droplet vitrification, *Lab Chip*, 7(2007)1428-1433

[36] Pirlo R, D. K. G. (2006), Cell deposition system based on laser guidance, *Biotechnol. J.*, 1(2006)1007-1013

[37] Zhang SG, Y. A. L. et al. (1999), Biological surface Engineering: a simple system for cell pattern formation. *Biomaterials*, 20(13):1213–20.

[38] Kane RS, T. O. I. et al. (1999), Patterning proteins and cells using soft lithography, *Biomaterials*, 20(23-24):2363-76

[39] Tan W, D. (2003), Microfluidic patterning of cells in extracellular matrix biopolymers: effects of channel size, cell type, and matrix composition on pattern integrity, *Tissue Eng*, 9(2):255-67

[40] Bhatia SK, H. L. (1992), New approach to producing patterned biomolecular assemblies, *J Am Chem Soc*, 114(11):4432-3

[41] Liu VA, J. B. (2002), Engineering protein and cell adhesivity using PEO-terminated tribolock polymers. *J Biomed Mater Res*, 60(1):126-34

[42] Piner RD, Z. X. H. et al. (1999), "Dip-pen" nanolithography, *Science*, 283(5402):661-3

[43] Lee KB, P. M. S. et al. (2002), Pritein nanoarrays generated by dip-pen nanolithography, *Science*, 295(5560):1702-5

[44] Wilson DL. M. H. C-G. et al. (2001), Surface organization and nanopatterning of collagen by dip-pen nanolithography, *Proc Natl Acad Sci USA*, 98(24):13660-4

[45] Hickman JJ, B. Q. S. et al. (1994), Rational pattern design for in-vitro cellular networks using surface photochemistry, *J Vac Sci Technol A – Vac Surf Films*, 12(3):607-16

[46] Lausted C, D. W. K. et al (2004), POSaM: a fast, flexible, open-source, inkjet oligonucleotide synthesizer and microarrayer, *Genome Biol.*, 5(8): R58

[47] The Pogo- Posam Project, http://www.bioinformatics.org/pogo/

[48] MicroFab Technologies Inc., http://www.microfab.com

[49] Hewlett Packard Inc., http://h10010.www1.hp.com/wwpc/us/en/sm/WF06c/A10-12771-64199-69422-69422-43336-43338-43339.html?dnr=2

[50] http://www.onsemi.cn/pub_link/Collateral/MC34063A-D.PDF

[51] http://nicholasclewis.com/projects/inkshield/theory/

[52] Xu T, B. A. D. et al. (2012), Hybrid printing of mechanically and biologically improved constructs for cartilage tissue engineering applications, *Biofabrication*, 5(2013)015001

[53] Ishizaki T, S. T. (2010), Correlation of Cell Adhesice Behaviors on Superhydrophobic, Superhydrophilic, and Micpatterend Superhydrophobic/ Superhydrophilic Surface to Their Surface Chemistry, *Langmuir*, 26(11), 8147-8154

[54] Efremov A, S. W. S. et al (2013), Micropatterned superhydrophobic structures for the simultaneous culture of multiple cell types and the study of cell-cell commmunication, *Biomaterials*, 34(2013) 1757-1763

[55] Piret G, G. C. B. et al (2011), Culture of mammalian cells on patterned superhydrophobic/ Superhydrophilic silicon nanowire arrays, *Soft Matter*, 7, 8462

[56] Shiu J-Y, K. W. C. (2010), Observation of enhanced cell adhesion and transfection effciency on superhydrophobic surfaces, *Lab on a Chip*, 10, 556-558

Vita

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