Temperature Control for Hydrogel Bio-Printing

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***Abstract*—** Recently, there have been many attempts to develop low-cost and open-source bio-printers worldwide. To reduce cost and increase accessibility, the bio-printer should be developed with open-architecture hardware and programming by G-code command. One of challenges is a temperature control. In this study, a nichrome-wire sheet heater syringe and Peltier/copper heat-sink cold-bed were designed to be compact and light-weighted. Thermistor sensors were employed to control temperature on both components. From the tests, our temperature control system could stabilize the temperatures of both the syringe and the cold-bed within 2 ºC. No significantly unstable temperature during the printing process was observed. The printing procedure was designed and validated as it could create the dot of the gelatin/fibroblast cell bioink on a glass slide with high percentage of viable cells.

***Keywords*—** Bio-printer, Temperature control, Bioink, Gelatin

I. INTRODUCTION

Recently, there has been great attention on applications, within tissue engineering and regenerative medicine, such as replicating specific animal or human tissues on a microfluidic chip to investigate a mechanism of disease or test drugs [1]. The key technology to achieve such ultimate goal is an advanced fabrication technique to construct complex cell-cell structures to closely mimic the in-vivo micro-environment. One of the most emerging techniques recently is bio-printing; however, most commercialized bio-printers are expensive, ranging from $10,000 to over $200,000. Therefore, there have been many attempts to develop low-cost and open-source bio-printers worldwide [2]. Briefly, bio-printers must be designed to be compatible with cell-laden "bioinks," referred to biomaterials [3-4]. Parameters, such as heating temperature, surface roughness and shear stress in a printing nozzle, could certainly impact the viability of printed cells. Among them, temperature control is the most challenging, and should be precisely controlled during the printing process.

Bioink, compared to clay or gel, is more difficult to handle during the printing process since its viscosity is not as high as clay and very sensitive to temperature. During the printing process, bioink must be maintained warm in a syringe in a liquid form. To form an ink structure, bioink must be extruded out the syringe's tip and cooled down in an ambient environment. To make bioink a gel-like material, the cold-bed, where the printed bioink is placed, must be designed with low temperature, a couple degree Celsius above zero.

Regarding these issues, our developed bio-printer was controlled by off-the-shelf 3D printer control board with its latest firmware. Thus, we could enjoy its powerful G-code command and variety of components in its ecosystem. The printer was constructed with industrial grade linear stages, for high resolution and robustness to withstand loading of the syringe pump and temperature-control system, and driven by powerful stepper motors. To warm bioink with low-cost and light-weighted system, a heater syringe was developed using nichrome wire and insulator sheet. A packed cold-bed was also designed to be light-weight and compact as it could fit in the printer's movable stage. Cooling system consisted of Peltier plate, copper heat-sink and water bath. Thermistor sensors were used to measure temperature at the heater and cold-bed and send feedback signal back to the temperature controller. To demonstrate the efficacy of the developed printer, an array of bioink dots on a glass slide was printed. The experimental results, such as the printing efficacy of dot array including gelatin/fibroblast cell bioink and cell viability, were included in this paper.

II. PRINTER AND BIOMATERIAL

A. Printer Components

The bio-printer was in-house developed Cartesian robot (Fig. 1). The X-Y motion was driven by THK KR26 linear stage and NEMA 17 Stepper motors while the Z motion was driven by double NEMA 23 steppers with built in acme lead screw. MKS-GEN L control board with 3D printer firmware was used to control all functions of the bio-printer. The resulting speed and resolution in the horizontal plane were 12.5 mm/s and 0.63 μm respectively while the speed and resolution in the vertical direction was 8.5 mm/s and 0.4 μm, respectively.

In this design, the end effector could carry a 1-kg motorized syringe pump easily, and the bed could carry a 3-kg thermoelectric cooler. The control board was used to control the motion and temperature of syringe that was installed at the robot's end effector. The board was also able to control the lit of the UV lamp. Thus, we could control all its function by G- and M-codes [5] which gained benefits for the research purpose.

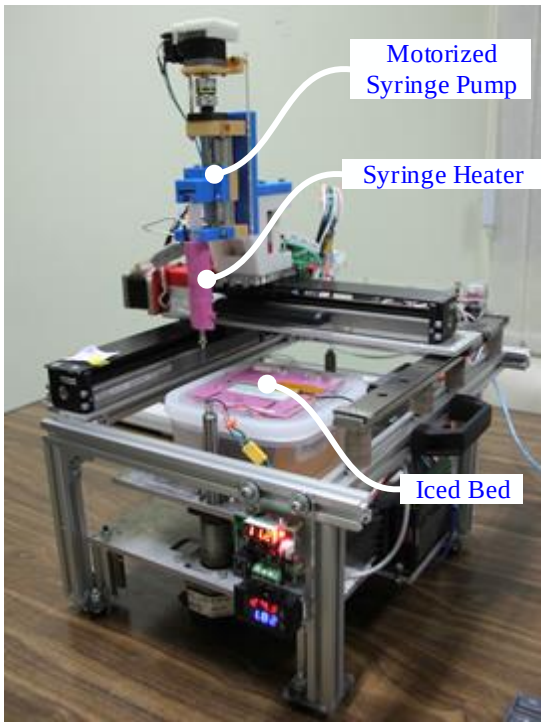


Fig. 1. Bio-Printer with 3D printer firmware

B. *Biomaterial*

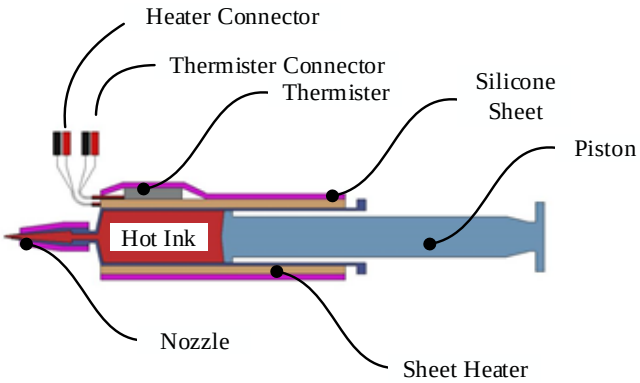
Bioink material in this investigation was hydrogel. The general preparation of the hydrogels is described as follows. In brief, gelatin (type A, 170 bloom, Sigma-Aldrich) was dissolved at 15% (w/v) in distilled water at 40°C under mild magnetic stirring for 1 hr. For encapsulating cells, fibroblast cell was used for this experiment. Mouse embryonic fibroblast (STO) cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic in a humidified atmosphere at 37°C with 5% CO_2. The medium was changed every 3 days. For cell encapsulation, gelatin solution 15% (w/v) in DMEM and STO cells were loaded with 1.5×10^6 cells/ml for experiments.

III. Main Components and Temperature Control

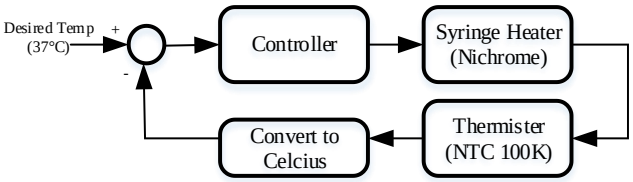
A. *Syringe*

During the printing, the syringe was heated to 37°C by the heating layer which was made by weaving 1800-mm nichrome wire (Ni80 26 AWG, approx. 16.0 Ohms) into a 0.15 x 70 x 125 mm^3 transparent PVC sheet, which was then wrapped around the syringe for 2 rounds. The insulating layer, made by 0.7 mm silicone sheet, covered the heating layer to block heat loss to the ambient. The NTC 100k thermistor, aiming to measure the heating layer's temperature, was installed between these layers. Noting that MKS-GEN L control board could interface the heater and thermistor directly. Its electronics and the temperature controller within the 3D printer firmware could control the temperature effectively.

The power to the heater was controlled by PWM technique [6] with 15 Hz, and the control law was dead-time control that was popular among 3D printer's hobbyist [5,7]. In the experiment, the heater was supplied with 24 V where the power was limited to only 25% of its full capacity. The heating capacity was enough to regulate the temperature of bioink inside the syringe during the process. Figure 2 shows the syringe and heater, and its control diagram.



(a) Syringe Heater-Thermistor



(b) Control diagram

Fig. 2. Temperature control for the syringe.

B. *Cold Bed*

For hydrogel printing, a printing bed, which was a glass slide, must be cooled at about 4°C. The allowable bed's temperature is only in a short range. If the temperature is below 0°C, water in gelatin freezes and cells are damaged. If the temperature is above 5°C, gelatin is less viscous and thus less resistance to stand in the gravity field.

A heat pump is needed to force heat flow such that the surface of the bed is regulated at a desired low temperature. To this purpose, there are two commonly used devices such as compressor-based and Peltier modules. In our case, the installation space was limited, and the bed was movable, which could carry only a light load. Peltier module was then chosen since it could fit in our 3D printer's space and the system would not be too heavy. The challenge is that Peltier is non-linear, and its cooling capacity is a function of an electric current and temperature difference between the hot and cold surface [7,8]. High temperature difference could degrade the cooling capacity. The cooling performance that should be considered includes (1) the maximum temperature difference, (2) maximum coefficient of performance, and (3) maximum

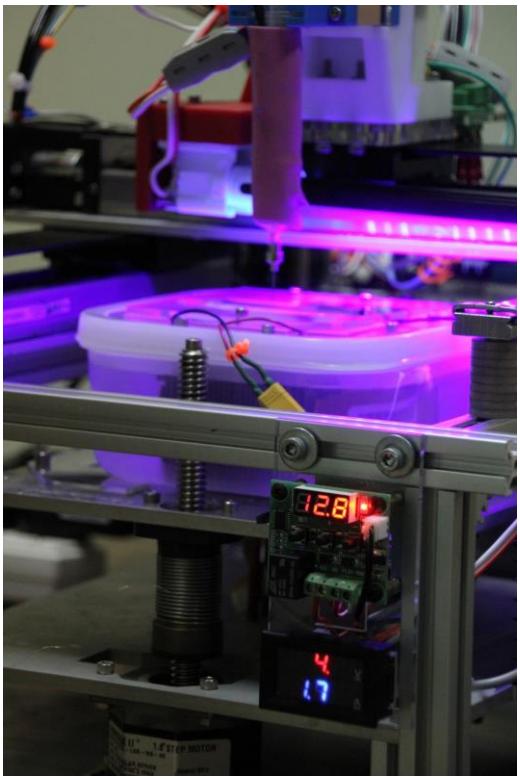
cooling power. Noting that cooling power degrades to zero at a certain level of temperature difference, and over-applied current also degrades the cooling power as well. We have found that the impedance of the Peltier module was not constant, and the current supply should be used to comfort the manipulation of cooling capacity.

For the cold-bed (Figs.3 a-c), a 1.1x 25x 75 mm^3 glass slide and the NTC10K thermistor were placed on the cold side of CP10-254-06 Peltier module. The hot side was placed on the Foxconn 344498-001 REV.B copper heat sink which was partially submerged in water. The Peltier module was subjected to 24 V and was estimated to draw current at 1.9 A and provided 5 W cooling capacity when the cold side's temperature reached 4°C. The water temperature was assumed to be at room temperature (25°C). Since the cooling performance was quite complex, the two-position temperature control was used at this stage of the project. The reference temperature was set to 4.0°C. The switch was "ON" and "OFF" at 4.0°C and 3.8°C, respectively.

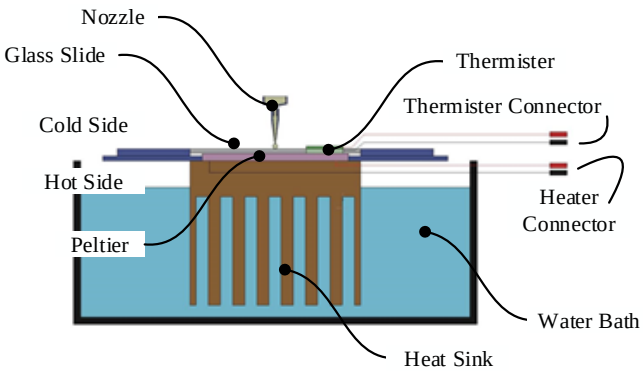
When the Peltier system is operated, the Peltier module pumps heat from its cold side to hot side, making the glass slide cooler and the heat sink hotter. The heat sink dissipates the heat to the water. As mentioned earlier, the cooling capacity depends on the temperature difference of the two sides of the Peltier module, the heat dissipation from water to the ambient is required for a long run to stabilize the system. In case that the water's heat dissipation is not good enough, the water becomes hotter and thus the temperature of the hot surface increases. In our case, the cold side is regulated at 4°C which means that the temperature difference between the hot and cold side becomes wider, and this might degrade the cooling capacity. The other concern is that when the hot surface is hotter, the Peltier module decreases its impedance and thus draws more current, by which its performance reduces. The system might end up overheated and fail to regulate to temperature of the cold side.

In practice, there are three ways that we can operate the cold bed.

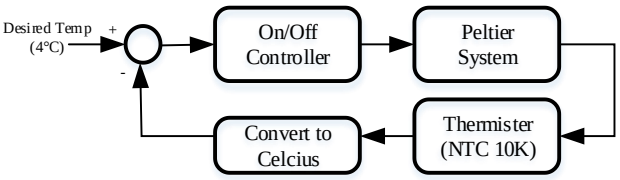
1. For a short operation, fill the bath with water at room temperature. The amount of water in the bath could absorb certain amount of heat, and the water temperature will slowly rise until the temperature is too high causing a breaking down of the system. The temperature difference is 21°C (assumed room temperature at 25 °C) at the beginning and rising when time passes.
2. For an accurate operation, fill the bath with ice water. The hot side temperature is thus constant at almost 0°C, and the temperature difference is almost zero. The Peltier module is operated at its maximum cooling capacity. If the loss is properly blocked, the power consumption is minimum, and the system can operate for a long period until all the ice melts.
3. For a long operation, the water-cooling system is used similar to Case 1. The heat in the water is forced to the ambient using a heat exchanger, and the water temperature is approximately kept to the room temperature. The temperature difference in this case is approximately 21°C and stable.



(a) Cold-bed and its temperature controller



(b) Schematics diagram of the cold-bed



(c) Control diagram

Fig. 3. Temperature control for the cold-bed.

The construction of the cold-bed is also very important for the precision of temperature control. In our design, the glass slide was placed directly on the cold side of the Peltier module. The contact was forced by the weight of the glass slide itself. The hot side was then placed on the copper heat sink. There was an acrylic plate to create little force on the Peltier module to the heat sink to eliminate the air gap. In this way, there was no unnecessary component to block the heat flow.

C. *Motorized Syringe Pump*

The motorized syringe pump was specially designed for a medical 10-ml syringe. The motorized pump was driven by a NEMA 11 stepper with one mm pitch ball screw, precision linear guide, and 3D printed structure such that the piston was precisely controlled with 0.63 μm resolution. In this design, the syringe pump was light-weight, but it provided very precise motion and created enough force to push/pull the syringe piston. The syringe pump was positive displacement type, thus the bioink could be extruded or drawn back to form a tiny droplet at the tip of the nozzle. The installing and detaching of a syringe was very easy with snapped fitting with a screw to lock the piston for the precision of movement. Figure 4 shows the mechanism and desired temperature at each location.

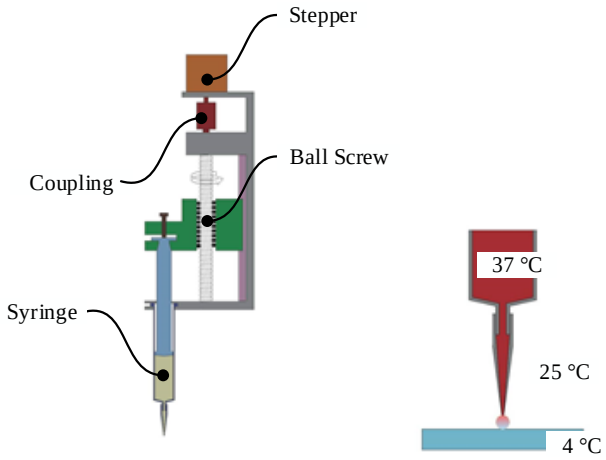


Fig. 4. Light weight syringe pump and its driving mechanism.

D. *Required Temperature for Hydrogel*

One of the difficulties in this work is to form a bioink dot from a warm droplet at the nozzle at about 30°C on the cold bed at 4°C. When the nozzle approaches the cold bed, the gap between the tip and the bed is less than 1 mm and the temperature gradient is quite high at this moment. The bioink is temperature sensitive material, and its viscosity varies during the process. Thus, to form a precise bioink dot, the temperature of both the syringe and the cold bed must be precisely stabilized. In addition, the motion of the nozzle tip must be precisely controlled as well.

E. *Temperature Data*

Temperature control devices of our bio-printer had been modified continuously during the project. Temperature performance shown in Fig. 5 is one example of temperature data regulated by the system in which the cold-bed is operated using the third way (Case 3) as mentioned earlier. Furthermore, both heater and thermistor were controlled by the 3D printer hardware and firmware. The heater on syringe was modified to be "clip-on-heater", which was easier to attach and detach around the syringe.

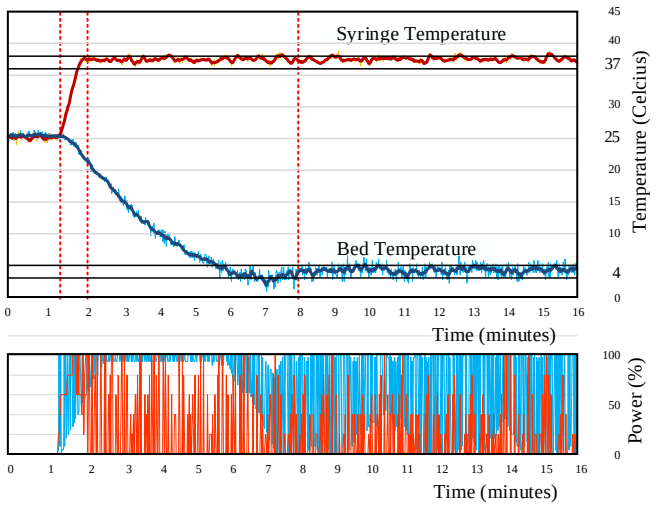


Fig. 5. Temperature of syringe and cold-bed, and required power.

From the results, the temperature of the syringe and the cold-bed were set at 37°C and 4°C, respectively. Initially, both the syringe and cold-bed were at room temperature of about 25°C. The controller was then started, at 1 min later. As a result, the syringe's heater and cold-bed's Peltier was both operated with their maximum capacity. Thus, the temperature of the syringe and the cold-bed changed. The syringe's temperature increased rapidly, while the cold-bed's temperature decreased slowly. The syringe's temperature rose at about 0.3°C per second while the cold-bed was cooled at about -0.1 °C per second. With this capacity, it took around 35 s for the syringe and around 7 min for the cold-bed to reach their desired temperature. When the temperature almost approached the desired temperature, the PID controller turned on and brought the temperatures to the desired level smoothly. With the current PID parameters, which were not well optimized, small overshoot in the bed's temperature was still occurred. After that, the PID controllers regulated both the syringe's and cold-bed's temperature at 37°C and 4°C, respectively.

Noting that the PID controller could manipulate the temperature in only one direction with this design. For the cold-bed, the controller can only lower the temperature when cooling is needed. Once, its temperature is below a desired temperature, the control is off, and then the temperature slowly rises towards the room temperature.

IV. Printing of Polka Dot

In order to make a success of bioink printing, the printing procedure must be well planned. Since system was operated by G- and M- codes, a desired sequence of operation and timing could be implemented. The sequence was designed as shown in Fig. 6, and could be explained as follows.

Prepare: We manually sucked up bioink by a syringe. The air bubble inside the syringe was removed by flipping it upside down, tapping the syringe to move bubble to the top and pushing gently on the plunger. Then, a smooth flow tapered tip gauge number 22 was attached to the syringe. Finally, the syringe was installed to a printer.

Flush: The controller extruded (flushed) a little amount of bioink outside the printing location. Tip of the nozzle was fully filled with bioink after flushing.

Position: The syringe was moved to the printing location. Bioink was regulated at 37°C inside the syringe.

Extrude: The droplet was formed by extruding 6.6 mm^3 of bioink. The large droplet was appeared at the tip.

Retract: Then, the syringe was half retracted back to reduce the size of droplet to 3.3 mm^3. The temperature of the droplet was somewhere between 37°C and 25°C (temperature at the syringe and ambient, respectively).

Touch: Next, the glass slide on the cold-bed (at 4°C) and the droplet at the tip were moved towards to each other. When they touched, the droplet was rapidly cooled to the bed temperature. At the temperature, the droplet of bioink became appropriately viscous to maintain its shape against the gravity. The touch process must be short as possible to make sure that both the syringe and the cold-bed could stabilize their temperatures.

Leave: The cold-bed and the tip were then separated, and the droplet was transferred to the glass slide. After that, the syringe was moved to next printing position and repeated the dot forming process.

In this work, we proposed a technique to precisely form a droplet of bioink while the temperature of both syringe and cold-bed were maintained constantly. The droplet changed its phase from warm liquid to cold gel during the touching step. Then, the gel with the cells inside could stay on the cold-bed in the gravity field without freezing. From the observation as shown in Figs. 7a -b, we successfully printed an array of bioink dots with the developed hardware and printing technique. The amount of the bioink in each dot was precisely controlled by our syringe pump. The shape of bioink was precise as the volume of each dot was around 3.3 mm^3, and their average diameter was 2.8 ±0.3 mm.

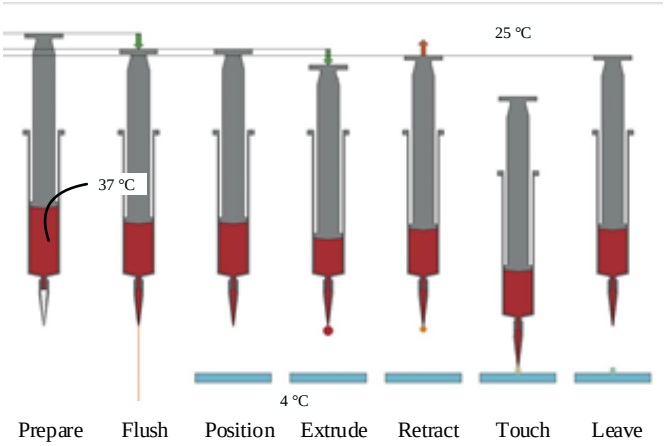
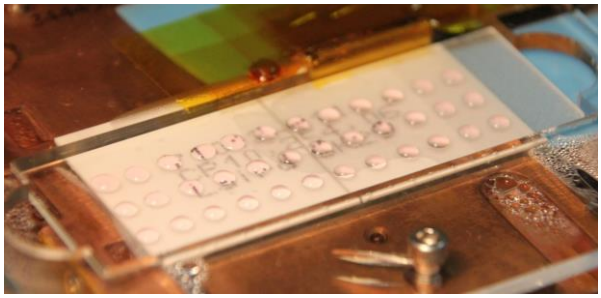


Fig. 6. Operational sequence to print a single polka dot.

(a) Movie during the printing process



(b) Three by ten array of dot printing

Fig. 7. Experiments for the polka dot printing.

V. Cell Viability

The combined LIVE/DEAD assay reagents (Calcein AM and Ethidium homodimer-1) were added into the cell encapsulation before and after printing and then incubated for 15 min at room temperature. By visual inspection, the numbers of viable cells are about the same before and after printing. This suggests that most of the cells survived the printing process.

VI. Conclusions

Bio-Printer was successfully developed with open architecture hardware and able to be programmed by G code. It was a liquid deposition modeling printer with a motorized syringe pump and temperature circuits for the syringe and cold-bed. All of them was specifically designed to well match with bio-printing applications. With the current system, the temperature was stable during the print process during which the syringe and the cold-bed were maintained warm at 37°C and cold at 4°C, respectively. The temperature fluctuation was less than 2°C during the printing process. The proposed printing procedure was also validated in the way that it could form the dot of the gelatin/fibroblast cell bioink on a glass slide. The resulting bioink dots were appeared to be uniform in size, and most printed cells survived the printing process.

Acknowledgement

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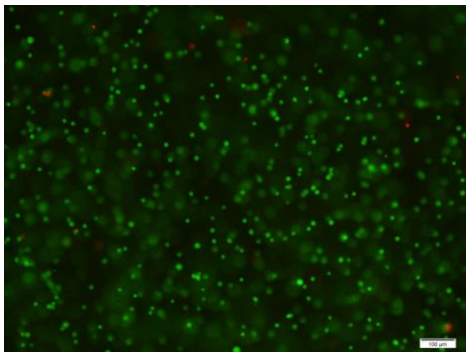
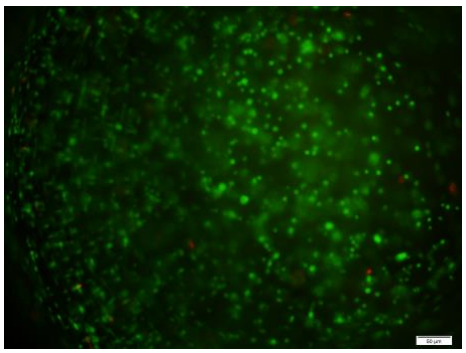


(a) Before printing (control)



(b) After Printing on the glass slide

Fig. 8. Experiments for gelatin/fibroblast cell printing (green: live, red: dead).

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