

# Effects of extrusion pressure and printing speed of 3D bioprinted construct on the fibroblast cells viability

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**Abstract.** In 3D bioprinting system, there are varies set of printing parameter involved that will affect the structure of bioprinted construct and cell viability. This study reports the effect of extrusion pressure and printing speed on the structure of construct and cell viability. This study contains two parts which first, to see the effect of the morphological structure of the construct by printing using Pluronic F127 and second, too evaluate the viability of the cell on the bioprinted construct by printing using fibroblast cell-laden Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) and Gelatin Methacrylate (GelMa). The construct was printed by using extrusion based 3D bioprinter with various ranges of extrusion pressure and printing speed. Based on the experiment, the result shows that printing at lower extrusion pressure requires a lower printing speed whereas printing at higher extrusion pressure requires a higher printing speed. For the cell viability of the bioprinted construct, printing at higher extrusion pressure will reduce the percentage of cell viability.

## 1. Introduction

Bioprinting is one facet of tissue engineering which has been emerging in recent years to build a three dimensional constructs containing biological cells [1]. 3D printing technique also has been primarily used to create acellular 3-Dimensional (3D) scaffolds and molds which then be seeded with cells post-fabrication [2]. Sacrificial ink likes pluronic F127 can be used for temporary support or to create channels, vessel or vasculature and can be washed away from the target structure which then the cell will take over the structure [3]. In general, bioprinting is the process that builds a 3D construct using computer-controlled 3D printing devices by depositing the cells and biomaterials precisely into geometries that create anatomically correct biological structures. It prints by outputting layer-upon-layer of living cells in hydrogels or viscous fluid, or cell-seeded microcarriers which is term as bioink [1]. 3D construct generated by 3D bioprinting technology can be used in regenerative medicine applications, and also as tissue models for drug screening or as a disease models [4].

In 3D bioprinting system, there are varies set of printing parameter involved, such as pressure and printing speed, that will affect the bioprinted construct. It is essential to understand how the system



parameters affect the structure of the printed construct and the viability of the cell on the bioprinted construct as cell are very sensitive to environmental changes. There are two parts of experimental in this study. The first part is to evaluate the effect of the extrusion pressure and printing speed on the morphology structure of the printed construct. The second part is to evaluate the effect on extrusion pressure on viability of the fibroblast cells on the bioprinted construct after printing process.

## 2. Experimental

### 2.1. *Effect of extrusion pressure and printing speed on structure*

Polyoxyethylene–polyoxypropylene–polyoxyethylene (Pluronic F127, BioBots) was allow to cool at 4°C before used in printing to make sure the material in liquid form and was transferred into a metal extruder and capped with 30 gauge needle to be used for printing. For printing process, commercial 3D bioprinter (BioBots) was utilized. The desired printing model was prior designed by using CAD software (SolidWorks). The .stl file was converted to .gcode file by loaded into repetier host (Slic3r) to adjust the preferred printing parameters. The 3D construct was printed at pressure of 60, 80 and 100 psi, and the printing speed from 2, 4, 8 and 12 mm/s. Morphology characterization of the extruded line was made by observing the 3D printed construct under the stereo microscope (Leica Zoom 2000) at 15x and 30x magnification.

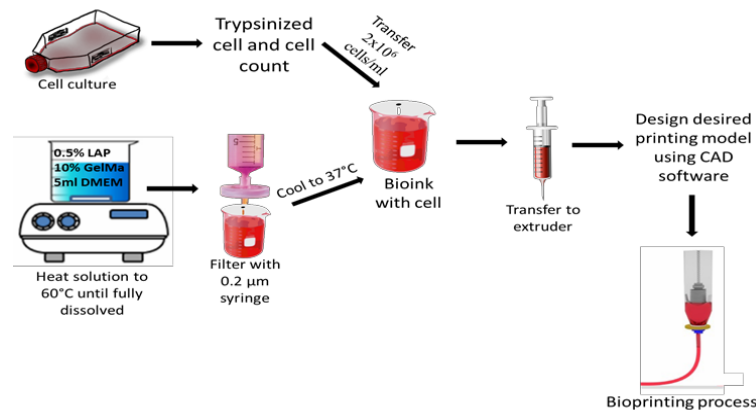
### 2.2. *Effect of Extrusion Pressure on Cell Viability*

Figure 1 shows an illustration on how the process of printing with cell. First, human skin fibroblast cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 mg/ml of penicillin and 10 mg/ml streptomycin and were maintained in the incubator at 5% CO<sub>2</sub> and 37°C. For bioink preparation, 0.5% w/v Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, photoiniator, BioBots) was mixed in supplemented DMEM until fully dissolved. Then 10% w/v Gelatin Methacrylate (GelMa, BioBots) was added to the LAP DMEM mixture solution and mixed at 60°C. The mixture solution of DMEM, LAP and GelMa were then be heated to 90°C and filtered with 0.2 µm syringe. The solutions then were allowed to cool down to 37°C prior to addition of fibroblast cells of concentration  $2 \times 10^6$  cells/ml. The mixture then were transferred to sterile extruder and capped with sterile 27 gauge tapered needle. Printing process was the same as printing with pluronic F127. The construct was print by extruding the line with 3, 4, 5, 6, 7, 8, 9, 10, 15, 30, and 45 psi with 4 mm/s printing speed under the sterile condition. The construct then will be evaluated for cell viability by staining the construct with Live/Dead assay kit (Ethidium homodimer-1 and calcein-AM, Thermo Fisher Scientific) and incubated it at room temperature for 30 minutes, then examined by using inverted fluorescence microscope. The number of live and dead cell then was calculated by using ImageJ software. The percentages of live cells were calculate as the number of green-staining cells divided by the total number of cells and the percentages of dead cells were calculated as the number of red-staining cells divided by the total number of cells.

## 3. Results and discussion

### 3.1. *Effect of extrusion pressure and printing speed on structure*

From observation, it can be clearly seen that the extrusion pressure and the printing speed affect the structure of the printed construct. At lower pressure (60 psi), the flow of the pluronic F127 was not continuous resulting beading of the material. At 80 psi, the flow of the pluronic F127 was continuous which create a more constant thickness of line. However, printings with 80 psi extrusion pressure at higher printing speed (8 and 12 mm/s) create a beading construct. Whereas excessive outpour was observed at constructs that printed at highest pressure (100 psi) with lower printing speed (2 and 4 mm/s). From this observation, we can state that printing at lower extrusion pressure require a lower printing speed whereas printing at higher extrusion pressure require a higher printing speed. Figure 2 shows the printed construct print at vary extrusion pressure and printing speed under a stereo microscope at 15x magnification.

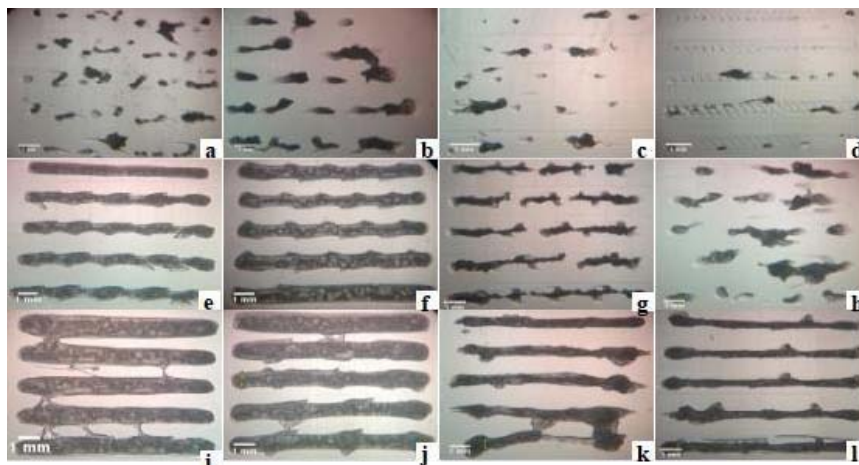


**Figure 1.** Illustration of bioprinting process

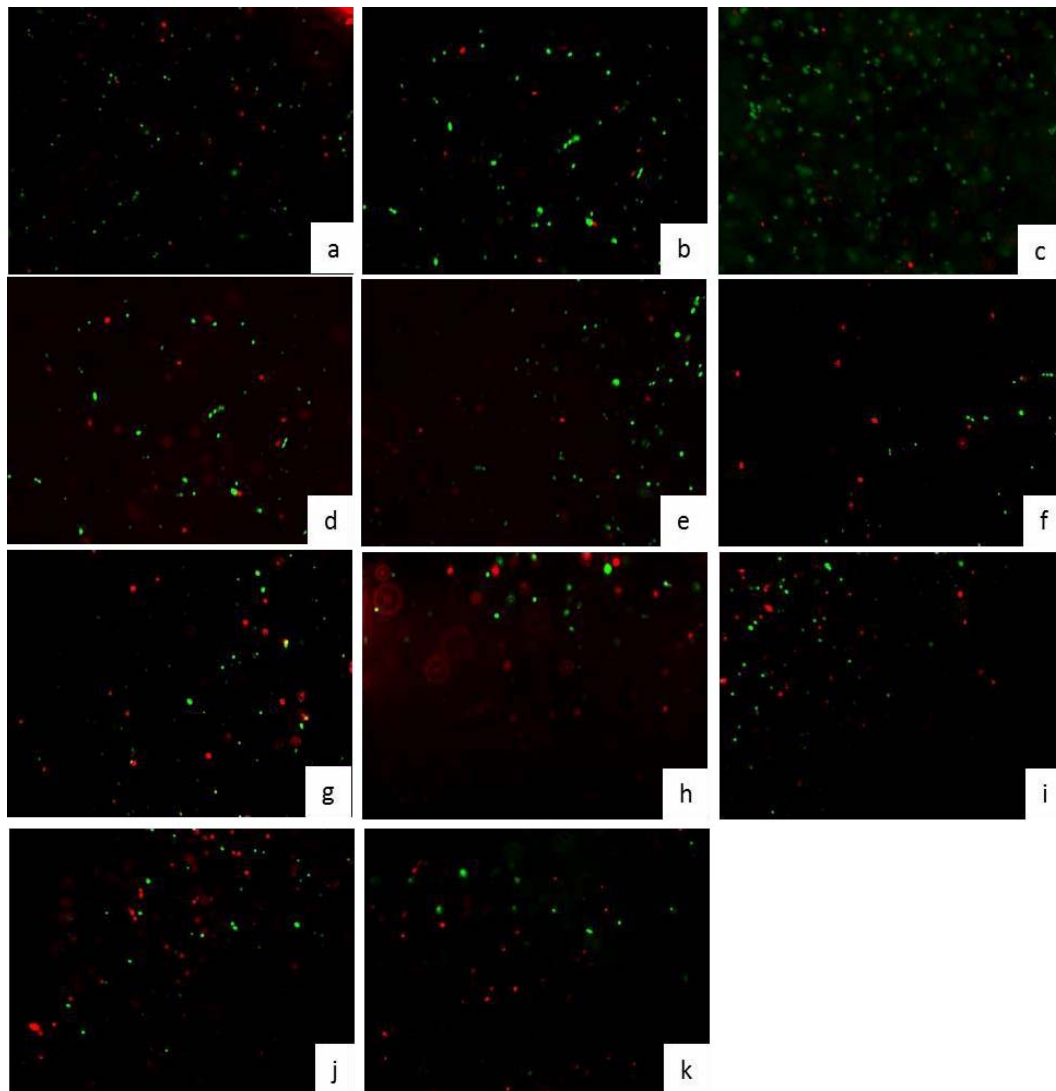
### 3.2. Effect of extrusion pressure on cell viability

Previous study shows that when printing with cell, it is essential to print at a lower extrusion pressure as print at high extrusion pressure will stress the cell [5, 6]. Because of that, we had changed the material use for printing from Pluronic F127 to GelMa as printing using Pluronic F127 requires high extrusion pressure. The constructs were print with various extrusion pressure ranging from 3 psi to 45 psi and printing speed of 4 mm/s as print at this speed will not produce excessive outpour and build-up of beading of the material.

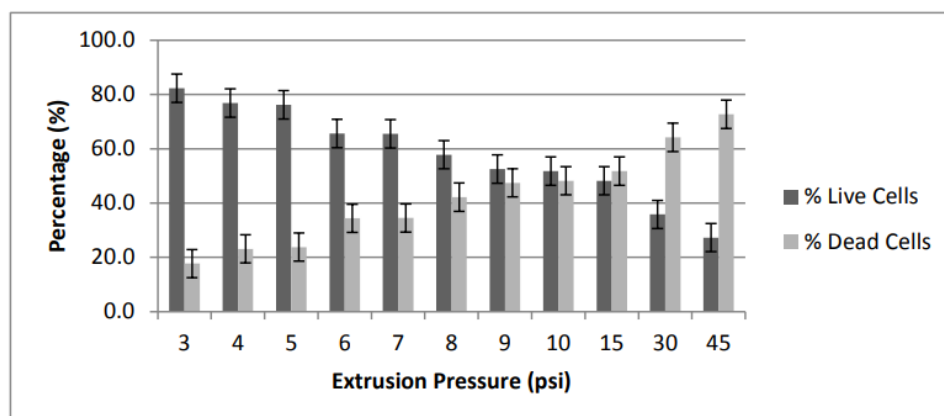
Cell viability is an important experimental endpoint in this study. The cell viability on the bioprinted construct was determined by staining the construct with Live/Dead assay kit (Ethidium homodimer-1 calcein-AM, Thermo Fisher Scientific) where green staining cell indicates live cell and red staining cells indicate dead cells (Figure 3). A major hallmark of viable cells is an intact plasma membrane and intracellular enzymatic activity [7]. Generally, a green fluorescence will be generated if the live cells are identified on the basis of intracellular esterase activity, while the dead cells are identified by the lack of esterase activity and non-intact plasma membrane which allows red dye staining [8]. The results shows decreasing of the percentage of live cells when print with increasing extrusion pressure. Whereas printing at higher extrusion pressures resulting in increase in number of dead cells (Figure 4). According to Kong et al., 2003, higher pressure will increase the shear stress in the nozzle, which subsequently damage the cell membrane and cause lower cell viability after extrusion. Normally, cells will respond to stress by enduring adaptation. However, when the cells are unable to adapt, it will cause injury to the cell and followed by cell death [6].



**Figure 2.** Image of the printed construct at 15x magnification, printing with 60 psi at 2mm/s (a), 4mm/s (b), 8mm/s (c), 12mm/s (d), 80 psi at 2mm/s (e), 4mm/s (f), 8mm/s (g), 12mm/s (h), and 100 psi at 2mm/s (i), 4mm/s (j), 8mm/s (k), 12mm/s (l).



**Figure 3.** The image of bioprinted construct after staining with Live/Dead assay kit printing with extrusion pressure of (a) 3 psi, (b) 4 psi, (c) 5 psi, (d) 6 psi, (e) 7 psi, (f) 8 psi, (g) 9 psi, (h) 10 psi, (i) 15 psi, (j) 30 psi, (k) 45 psi.



**Figure 4.** The percentage of cell viability when print at different extrusion pressure

#### 4. Conclusion

Herein, we have presented and described the effect of extrusion pressure and printing speed on the structure of the printed construct and the effect of extrusion pressure on fibroblast cell viability of the printed construct. Our initial finding show that printing at lower extrusion pressure require a lower printing speed whereas printing at higher extrusion pressure require a higher printing speed and suggest 80 psi of extrusion pressure and 4 mm/s of printing speed will produce the best printed construct structure when printed with pluronic F127 using extrusion-based bioprinter. As for cell viability, percentages of live cells are decreasing when printing at higher extrusion pressure because higher extrusion pressure will produce higher shear stress at the nozzle hence damaging the integrity of the membrane cell thus promoting toward cell death. Therefore, to print viable cell, it is essential to print at lower extrusion pressure.

#### 5. References

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