

Experiments and Analysis of Drop on Demand Cell Printing

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Abstract: Cell printing is a promising technology in tissue engineering, with which the complex three-dimensional tissue constructs can be formed by sequentially printing the cells layer-by-layer. Though some cell printing experiments with commercial ink-jet printer show the possibility of this idea, there is a long way to implement cell printing in engineering. To get a better understanding of drop on demand piezoelectric cell printing, a cell printing experiment system with commercial piezoelectric ink jet printing head and configurable drive circuit is developed. And a series of cell printing experiment with yeast cell is performed to study the influence of drive parameters, cell solution density and viscosity of the solution on cell survival rate, droplet density and orifice status. The experimental results show that the cell solution density and its viscosity are key issue for cell printing with commercial printing head, and a large drive pulse width is helpful for printing the cell solution with a high cell density and viscosity.

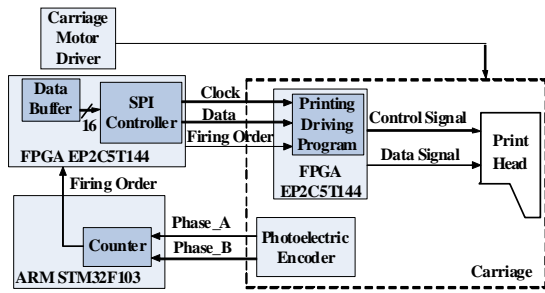
Key words: Cell printing, drop-on-demand, piezoelectric inkjet printing, yeast cell

INTRODUCTION

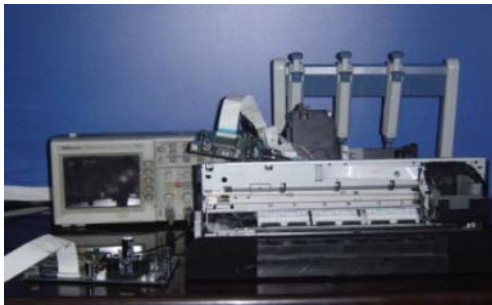
Cell printing technology is an alternative to classic biodegradable solid scaffold-based approaches and predicted to be promising in tissue engineering (Mironov *et al.*, 2003). It is based on the principle of the developmental biology that cells self-assembly into tissues, which is similar to the way embryonic-like tissues fuse into functional forms. By sequentially printing the cells layer-by-layer, three-dimensional tissue constructs can be formed. Although this goal is extremely difficult to achieve, it has been proved to be feasible (Mironov *et al.*, 2009). Preliminary cell printing experiments have been performed successfully, in which the droplets of various cells are printed to bio-substrates for constructing some patterns. This printing procedure can be implemented with laser-induced forward transfer (Lin *et al.*, 2009), including matrix-assisted pulsed-laser evaporation direct-write MAPLE DW (Wei *et al.*, 2007), and inkjet printing (Tao *et al.*, 2005).

Many research works for laser direct-write cell printing have been reported recently (Wei and Khalil, 2007; Tao *et al.*, 2005; Othon *et al.*, 2008). The advantage of this technology is that orifices are not necessary, therefore orifice clogging can be avoided, which makes it possible to transfer high viscosity biological preparation. However, the extremely localized heating causes a high temperature, and a high jetting velocity, typically 50~100 m/s. So the printed cells tend to be damaged by the heat transferred and the high pressure, typically 8~15MPa, in landing process (Wei *et al.*, 2009). Even for the yeast cells which have strong tolerance, there are still 10~55% cells

lose vitality during the printing process (Wei and Khalil, 2007). In addition, cell substrate is required to be prepared previously, so it is difficult to implement various cells mixed printing. Another cell printing method is based on Drop-on-Demand ink-jet printing technology, in which thermal bubble or piezo-electric actuators are used to print cell suspension. Xiaofeng Cui and Thomas Boland printed Human microvascular endothelial cells and fibrin to the fibrin substrate simultaneously to form a microvascular system with a HP thermal inkjet printer (Xiaofeng and Thomas, 2009). Yamazoe and Tanabe (2009) printed polyethyleneimine to the Albumin-based substrate forming patterns and words using ink-jet printing. Saunders Rachel E, Gough Julie E successfully printed Human Fibroblast Cells (Saunders *et al.*, 2008). Kim *et al.* (2010) printed Human Adipose-derived Stem Cells to polystyrene substrate with a FUJIFILM piezoelectric inkjet printer (Kim *et al.*, 2010). Thanks to its low jetting speed (about 5~8 m/s) and low pressure, a high survival rate can be achieved with this technology, especially for piezoelectric printing, in which no thermal bubble is produced. However, the applicable medium range, print parameters on the influence of cellular damage, and density of cell solution on the influence of printing are still open problems. Therefore, the influence of drive parameters, cell solution density and viscosity of the solution on cell survival rate, droplet density and orifice status are studied in this paper for better understanding of piezoelectric cell printing and designing new cell printing system.



(a) Structure diagram



(b) Experimental setup

Fig. 1: Cell print experiment device

MATERIALS AND METHODS

Experimental device: A home-developed cell printing experimental system with EPSON series F181010 piezoelectric printing head and programmable driver circuit is shown in Fig. 1. The printing head has 177 nozzles with a resolution of 90DPI and a droplet volume of 30 pL.

Materials preparation:

Bio-configured ink and bio-papers: At first, the quantitative filter paper with 30~50 μm aperture was used to filter out impurities from the yeast cell suspension, and then the processed suspension was diluted to obtain different concentration of bio-configured ink. Common malt extract medium was used to culture cell and as buffer medium. In addition, soy agar gel (2%) was coated on glass slide as another buffer medium, which is often called bio-paper.

Viability test: The methylene blue dye is a non-toxic dyestuff which is used to distinguish the live/dead yeast cells. The blue indicator turns colorless in the presence of active enzymes indicating living cells and vice versa. This reagent is made of two solutions, in which one solution is the mixture of 0.3 g methylene blue with 30 mL 95% ethanol and another is 1% KOH solution. Figure 2 shows

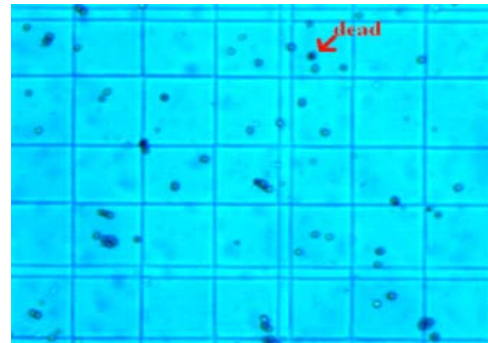


Fig. 2: Printed yeast cells

the printed cells under a microscope with the magnification of 160 \times .

Experiments and analysis:

Influence of drive pulse width on cell ejection: The yeast cell suspension was diluted to around 690,000 cells/mL by pure water. A rotational viscometer (NDJ-8S) was used to measure the viscosity of the cell solution, and the viscosity was 1.04 mpa.s (at 23 μs). To reduce the impact of cell droplets on the substrate, soy agar gel was used as buffer medium. The different drive signals with the pulse width from 10 to 30 μs were applied to analyze its influence on cell ejection. The methylene blue dye was dripped on each glass slide at a 1:1 volume ratio to dye the post-printing cells, and the live/dead yeast cells were counted with a hemocytometer after 20 min. Each sample was counted twice to get an average cell viability rate.

It can be seen from Table 1 that the post-printing cells have a rather high survival rate with the drive pulse width from 10 to 25 μs , revealing that the jetting process causes little damage on the living cells. In addition, a large pulse width is helpful to increase the concentration of the post-printing cell suspension and avoid orifice clogging. However, this printing head did not work even with ink when the drive width is large than 30 μs .

Influence of cell concentration on ejection: Different concentration of the bio-configured ink mentioned above was prepared to analyze its influence on cell ejection. As shown in Table 2, the concentration ratio of post-printing cell suspension to primary cell suspension declines with increasing concentration. Under the condition of low viscosity, there is no significant effect on printing process when cell concentration is changed.

Influence of solution viscosity on cells ejection: Different viscosity of bio-configured was prepared to analyze its influence on cell ejection. The concentration of the suspension was around 4,900,000 cells/mL. As shown in Table 3, the concentration ratio of post-printing

Table 1: Pulse width influence on cell ejection (viscosity: 1.04 mpa.s, Bio-ink concentration: 690,000 cells/mL, buffer medium: soy agar gel)

Pulse width (μs)	Droplet concentration (cells / mL)	Survival rate (%)	Orifice statu sin cell printing	Orifice statu sin ink printing
10	260,000	96	Serious clogging	Non clogging
18	300,000	96	Slight clogging	Non clogging
22	360,000	94	Non clogging	Non clogging
25	440,000	94	Non clogging	Non clogging
30	NA	NA	No jetting	No jetting

Table 2: Influence of cell concentration on ejection (viscosity: 1.04 mpa.s, pulse width:25μs, buffer medium: soy agar gel)

Bio-ink concentration (cells/mL)	Droplet concentration (cells/mL)	Concentration ratio (%)	Orifice status in cell printing	Bio-ink concentration (cells/mL)
690,000	440,000	63.8	Non clogging	690,000
1,240,000	780,000	62.9	Non clogging	1,240,000
4,850,000	2,900,000	59.8	Non clogging	4,850,000
8,630,000	3,790,000	43.9	Clogging	8,630,000

Table 3: Viscosity influence on cell ejection (Drive pulse width: 25μs, buffer medium: soy agar gel)

Viscosity (mpa.s)	Bio-ink concentration (cells/mL)	Droplet concentration (cells/mL)	Concentration ratio (%)	Orifice status in cell printing
1.04	4,850,000	2,900,000	59.8	Non clogging
1.16	4,900,000	950,000	19.4	Non clogging
1.24	4,950,000	900,000	18.2	Non clogging
1.35	4,900,000	147,000	3.1	Serious clogging
1.65	5,100,000	61,000	1.2	Hardly jetting

Table 4: Buffer medium influence on cells ejection (viscosity: 1.04 mpa.s, pulse width: 25 μs, concentration: 1,250,000 cells/mL)

Buffer medium	Post-printing cell viability (%)
None	33
Malt extract medium (Liquid)	93
Soy agar gel (Semisolid)	96

suspension to primary cell suspension declines with increasing viscosity. The orifices were frequently plugged up with high viscosity solution, especially for the case of 1.65 mpa.s.

Influence of buffer medium on cell viability: Two different substrates were prepared as the receiving substrate or bio paper. One was soy agar gel, and the other was malt extract medium. Viability test result for printed cell is shown in Table 4. It is found that the character of bio-paper is of great importance for constructing tissue with active cells.

CONCLUSION

Yeast cell printing experimental results show that the jetting process causes little damage to the living cells, revealing its great potential in tissue engineering. However, the orifice clogging is the key problem to be solved. A large drive pulse width is helpful to increase the cell density in droplets and to print cell solution with high viscosity.

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