PIEZOELECTRIC INKJET-BASED SINGLE-CELLS PRINTING BY IMAGE PROCESSING FOR HIGH EFFICIENCY AND AUTOMATIC CELL PRINTING

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ABSTRACT

Research in cell printing by piezoelectric inkjet printer has been conducted, however, relatively reliable single-cells printing yield had not been obtained. We proposed a different approach to increase the yield in single-cells printing by utilizing piezoelectric driving method not used in recent inkjet printer devices, push-pull method. Furthermore, cell recognition system constructed by image processing algorithm was used to predict whether individual cell is present at the tip of inkjet head. Combining both methods, we successfully developed a single-cells printing system by piezoelectric inkjet printer with single-cells printing yield of up to 98%.

KEYWORDS: Piezoelectric inkjet printer, Single-cell printing, Image processing

INTRODUCTION

Single-cell analysis is playing an important role in the understanding of differences between individual cells [1, 2]. Several methods and devices have been developed to support researches in single-cell analysis [3, 4]. One of the emerging methods is cell printing by inkjet printer. Inkjet printer gained attention mainly because of its capability to print cells in high speed, up to several kHz. Additionally, there was also attempt to use inkjet printer to construct artificial 3D tissue in vitro, which shows inkjet printer potential role not only for study of cells patterning in 2D, but also in the field of tissue engineering [5, 6, 7, 8]. However, handling of single-cells using piezoelectric inkjet proved to be difficult, and only 87% of single cell printing results was obtained [9]. In this study, two basic piezoelectric driving mechanism for the purpose of single cell printing was investigated, and methods for system with high single-cells printing successful ratio was proposed.

SINGLE-CELL EJECTION METHOD AND SINGLE-CELL PRINTING EXPERIMENT

Simple inkjet head structure was used throughout experiments in this study (Fig. 1). SF9 and C2C12 cell line was used in this study to show our system capability to print viable insect and mammalian cells. Following method was used to perform single cell printing.

Cell position inside inkjet head was confirmed by observing the inkjet head with CCD camera, and a simple method to determine ejection area was used (Fig. 2). Ejection area is the area inside inkjet head, where cells are ejected. This area is roughly the volume of one liquid droplet. When single cell present inside the ejection area, it would be ejected onto the target position, otherwise the droplet would be ejected somewhere else. Using this simple method, single cell printing device was developed.

Additionally, piezoelectric element driving method commonly used in inkjet printer, pull-push method, and driving method not used recently, push-pull method, were both applied. Significant difference in the results of inkjet head mapping were obtained, as described in the next section.

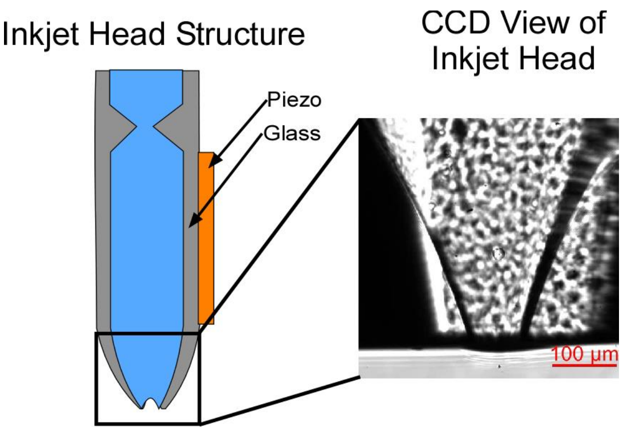

Figure 1: Schematic of piezoelectric inkjet head and the CCD view of the inkjet head used in this study.

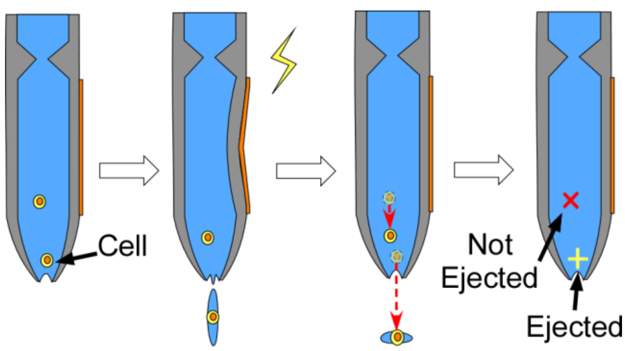

Figure 2: Inkjet head mapping method to determine ejection area inside the inkjet head. Red cross shows area where cells are not ejected, while yellow cross shows area where cells are ejected.

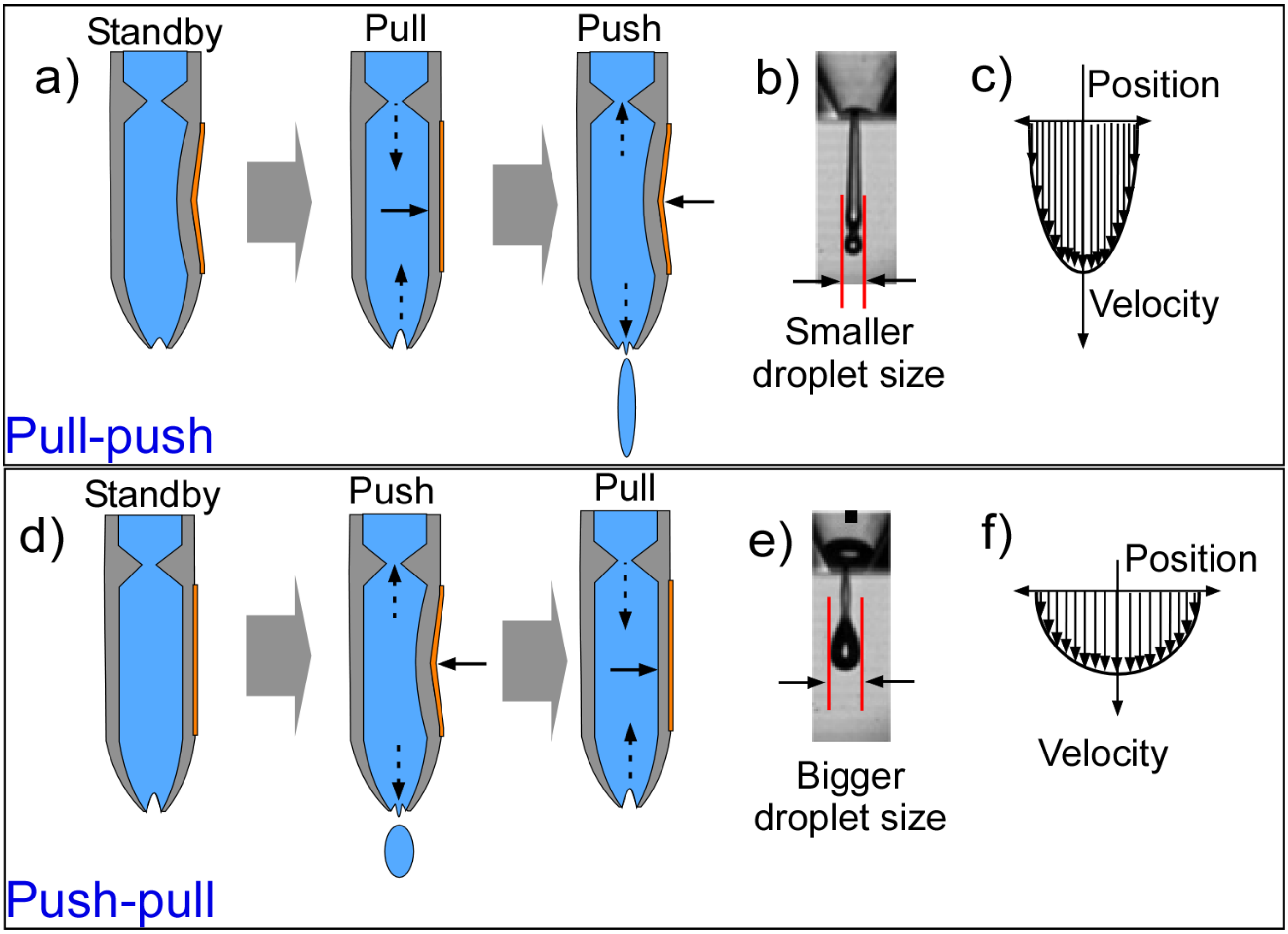

Figure 3: a) Piezoelectric element driving method, b) droplet photograph, and c) schematic diagram of velocity distribution inside inkjet head, for pull-push method. d) Piezoelectric element driving method, e) droplet photograph, and f) schematic diagram of velocity distribution inside inkjet head, for push-pull method.

COMPARISON BETWEEN PULL-PUSH AND PUSH-PULL METHOD FOR SINGLE CELL PRINTING

Fig. 3 shows differences between pull-push and push-pull method. Observation by CCD camera revealed that push-pull method produced bigger droplet compared to pull-push method, which showed difference in velocity distribution. Difference in velocity distribution correlated to the stability of particles ejection. Pull-push method resulted in high speed and narrow column of liquid droplet. On the other hand, push-pull method resulted in slower speed and wider column of liquid droplet.

Fig. 4 shows inkjet head mapping result for both pull-push and push-pull method. As can be seen from the figures, pull-push method had a mixed region. In such region, it could not be determined whether cell will be ejected or not. Push-pull method had negligible area of such region. Therefore, for single cell printing method described above, push-pull method was found to be more suitable.

IMAGE PROCESSING ALGORITHM FOR SINGLE CELL PRINTING

In the final step, using image processing software to calculate cells position inside the inkjet head, we constructed an automatic single-cells printing system. The image processing software automatically recognize cells inside inkjet head, and calculate its position. Cells recognition was performed with background subtraction method, and by beforehand examining size and shape characteristics of the target cells.

For the background subtraction part, codebook method was used, because it provides a fast and reliable tool to distinguish foreground object from background object [10]. In the next step, foreground object not only consists of single cells, therefore, single cells must be distinguished from other objects, for example, multiple cells, debris, etc. The size and shape characteristics of the target single cells was investigated beforehand, and based on this database, single cells was distinguished from other foreground objects.

Automatic cells recognition system provided us with tools to perform the single cells printing automatically. The last section described the experimental results of automatic single cells printing.

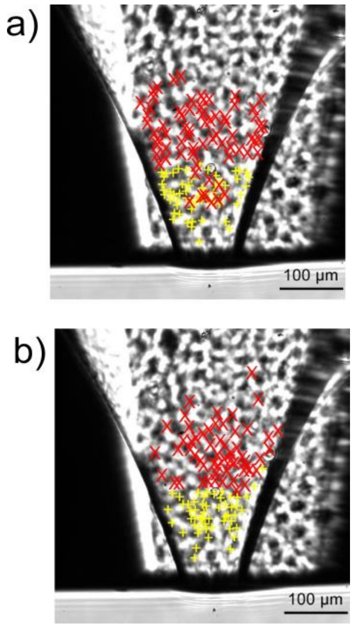

Figure 4: Inkjet head mapping results comparison between (a) pull-push method and (b) push-pull method.

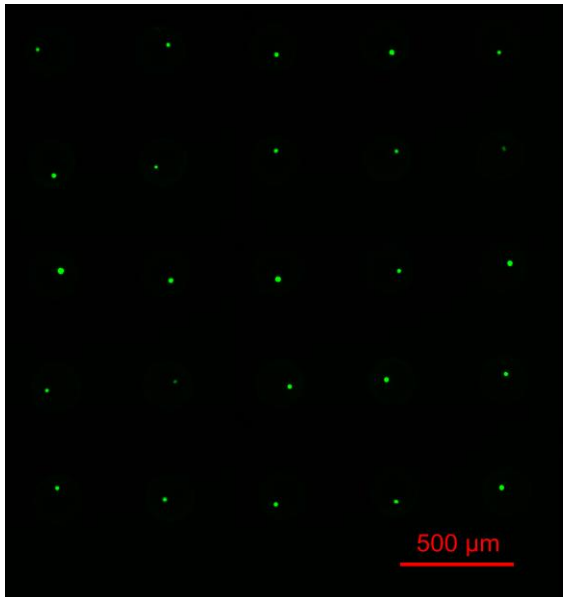

Figure 5: C2C12 single cell printing results for 5 x 5 grid pattern. Each dot was printed with a 500 µm distance. Cells were stained with SYTO Green to improve visibility.

RESULTS AND DISCUSSION

Fig. 5 shows the printing results of single cells in a 5 x 5 grid pattern. Cell nucleus was stained with SYTO Green. After printing, substrate was observed with fluorescence microscope for evaluation of single cells printing. Fluorescence observation shows cells were successfully printed one by one on this grid pattern. Additionally, ratio of successfully printed single cells to the number of printed droplets was found to be 98%. This results shows that our system is suitable for high efficiency printing of single cells.

CONCLUSION

In this study, drop-on-demand single-cells printing by piezoelectric inkjet printer and real time image processing software was developed, and 98% single-cells printed ratio was obtained. The piezoelectric driving method used in this system was the push-pull method, which recently is not used in commercial piezoelectric inkjet printer devices. Future study to increase the spatial resolution might lead to a new cell printing device, which will play an important role for study in the cell and tissue engineering.

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