A PUMPLESS CELL CULTURE CHIP WITH THE CONSTANT MEDIUM PERFUSION-RATE MAINTAINED BY BALANCED DROPLET DISPENSING

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ABSTRACT
This paper presents a pumpless perfusion cell culture chip, where the constant flow-rate medium perfusion is achieved by the balanced droplet dispensing. Compared to the conventional pump-based cell culture chips, the pumpless cell culture chips have simpler structure without external pumps. The previous pumpless cell culture chips, however, result in the decreasing perfusion-rate due to the decreasing hydraulic-head difference, $\Delta h$, between well and drain. The present pumpless cell culture chip maintains constant $\Delta h$ by the balanced droplet dispensing, thus achieving the constant flow-rate medium perfusion. In the experimental study, the perfusion-rate was measured as 0.1~0.3 $\mu l/min$ with the deviation and error lower than 9.96% and 6.55%, respectively. At the constant perfusion-rate of 0.1 $\mu l/min$, H358 lung cancer cells showed the maximum areal-growth-rate of 5,105 $\mu m^2/day$, which is 5 times higher than 1,028 $\mu m^2/day$ of static culture. The present chip offers favorable cell-growth environment with the constant perfusion-rate without requiring external pumps, thus being useful for the cell-based drug screening.

KEYWORDS: Perfusion culture, Constant perfusion-rate, Droplet dispenser, Balanced droplet dispensing

INTRODUCTION
Since perfusion cell culture provides more accurate in-vivo-like microenvironment [1] compared to the static cell culture, the precise medium perfusion is necessary for cell-based biomedical research. The constant flow-rate medium perfusion is also known to provide favorable cell-growth condition [2] with the uniform mass-transport of nutrients and waste products. Thus, a simple and effective method to achieve the constant flow-rate medium perfusion is required. Recently, the pumpless perfusion cell culture methods [3] have been proposed to offer simple structure by eliminating the external pumps of the conventional pump-based perfusion cell culture methods [4]. The previous pumpless cell culture methods, however, result in the decreasing perfusion-rate due to the decreasing hydraulic-head difference, $\Delta h$, between well and drain (Fig.1a).

In this paper, we propose a simple pumpless perfusion cell culture method (Fig.1b) to achieve the constant flow-rate medium perfusion maintained by the balanced droplet dispensing. We also present the experimental measurement of the perfusion-rate and cell-growth characteristics of the fabricated pumpless perfusion cell culture chip.

Figure 1: Comparison of the pumpless perfusion methods: (a) the previous method, resulting in the decreasing flow-rate perfusion due to the gradually decreasing $\Delta h$; (b) the present method, resulting in the constant flow-rate perfusion due to the constant $\Delta h$ maintained by the balanced droplet dispensing.

Table 1. Dimension and fluidic resistance of the drain channel in Fig.2a
### DESIGN AND FABRICATION

The pumpless perfusion cell culture chip (Fig.2) is composed of two layers: a well layer and a droplet dispenser layer. The well layer has 4×8 well array, where 8 wells in each row are interconnected to a common drain port by the drain channel (Fig.2a). The droplet dispenser layer is placed on the well layer to supply medium droplets to the well (Fig.2b). A perfusion-flow is induced by the hydraulic-head difference, $\Delta h$, between the well-inlet and drain-outlet, thus eliminating a waste droplet at the drain-outlet. Since the perfusion-flow results in the negative gauge pressure in the well, the droplet dispenser generates a medium droplet as the amount of the eliminated waste droplet to recover the well pressure to zero gauge pressure. As a result, the present chip maintains the constant $\Delta h$ and achieves the constant flow-rate medium perfusion. Based on the fluidic resistance model, we have designed the branch channel to have 600 fold higher fluidic resistance than the main channel (Table 1) for obtaining high uniformity (>99%) of the perfusion-rates at 8 wells interconnected by the drain channel. Figure 3 shows the PDMS molding and bonding process for the prototype of Fig.4.

### EXPERIMENTAL RESULTS

In the experimental study, we measured the deionized water perfusion-rates at the 8 wells, interconnected to a common drain port, for the three different $\Delta h$ of 38, 76, and 114mm (Fig.5). The maximum error of the measured average perfusion-rate compared to the theoretical value was measured as 6.55% at $\Delta h=38$mm, while the maximum perfusion-rate deviation was 9.96% at $\Delta h=114$mm. The perfusion-rate error and deviation are resulted from the height deviation of the drain channels fabricated by the thick (220μm)-PR lithography process in Fig.3.

<table>
<thead>
<tr>
<th>Drain channel</th>
<th>Dimension [μm]</th>
<th>Fluidic resistance (Pressure/Flow-rate) [Pa·s/m³]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main</td>
<td>400 220 72,000</td>
<td>3.09×10¹¹</td>
</tr>
<tr>
<td>Branch</td>
<td>60 60 85,000</td>
<td>1.87×10¹⁴</td>
</tr>
</tbody>
</table>

**Figure 3:** Fabrication process for the present chip showing the cross-sectional view along B-B’ in Fig.2a.

**Figure 4:** Fabricated pumpless perfusion cell culture chip.

**Figure 5:** Experimental and theoretical perfusion-rates at the 8 wells in the fabricated chip for the varying hydraulic-head difference, $\Delta h$, in Fig.2b.
Next, we measured the cell-growth pattern at the three different perfusion-rates using H358 lung cancer cells of $5 \times 10^4$/well (Fig.6). At the constant perfusion-rate of 0.1μl/min, we obtained the maximum areal-growth-rate of cell colonies as 5,105μm$^2$/day (Fig.7), which is 5 fold higher than the value of 1,028μm$^2$/day in the static culture. Therefore, we demonstrate that the constant flow-rate medium perfusion using the present chip offers more favorable cell-growth environment than the static culture.

<table>
<thead>
<tr>
<th>Perfusion rate [μl/min]</th>
<th>Culture time [day]</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1.5</td>
</tr>
<tr>
<td>Static culture</td>
<td><img src="image1.png" alt="Image of cells" /></td>
<td><img src="image2.png" alt="Image of cells" /></td>
</tr>
<tr>
<td>0.099±0.005</td>
<td><img src="image5.png" alt="Image of cells" /></td>
<td><img src="image6.png" alt="Image of cells" /></td>
</tr>
<tr>
<td>0.21±0.018</td>
<td><img src="image9.png" alt="Image of cells" /></td>
<td><img src="image10.png" alt="Image of cells" /></td>
</tr>
<tr>
<td>0.30±0.030</td>
<td><img src="image13.png" alt="Image of cells" /></td>
<td><img src="image14.png" alt="Image of cells" /></td>
</tr>
</tbody>
</table>

**Figure 6:** Microscopic fluorescence images of the cells cultured in the fabricated chip for the varying perfusion-rates: scale bars in the images indicate 300μm.

**Figure 7:** Cell colony area measured and averaged from the 8 wells in the fabricated chip for the varying perfusion-rates.

**CONCLUSIONS**

In this paper, we present the pumpless perfusion cell culture chip, where the constant flow-rate medium perfusion is achieved by the balanced droplet dispensing. The proposed chip offers a favorable cell-growth environment with the constant flow-rate perfusion without requiring external pumps, thus being useful for the cell-based drug screening.

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**REFERENCES**


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