CURIOSIS

APPLICATION NOTE

Cellpuri™ | Microfluidic cell concentration chip without centrifugation

1. Introduction

Separating and collecting cells is an essential procedure for most cell culture-based experiments, including media exchange, cell washing and removal of debris during subculture, seeding and thawing. To date, cell collection has been achieved by pelleting cells using centrifuge. Centrifugation is a method of separating and settling down particles from a solution based on their shape, size, density, and rotor speed. Rotation of rotor generates a centrifugal force upon the cells in the liquid medium and biological centrifugation is a process of using this centrifugal force to separate and purify the cell mixture. Because the cell will sediment at the rate proportional to the centrifugal force applied, sometimes the strong centrifugal force provided by the centrifuge can be applied to each cell in the sample, especially at a high rotor speed. An increase of the applied centrifugal field at the high rotation speeds can induce centrifugation-mediated mechanical stress in cells and produce subsequent cytokine involved in inflammatory process[1]. It has been also reported that the gravitational field by centrifugation causes changes in cellular traction forces and cytoskeletal rearrangement as part of the cellular mechano-response[2].

Curiosis Inc. has recently developed **Cellpuri**, a centrifugal force-free cell concentrating device that cuts down generated fluid shear stress into only a few seconds thus minimally affects the cells in terms of mechanical stress. Moreover, unlike centrifugation, a cell concentrate can be obtained without the need to carefully remove the supernatant after separation. The purpose of this study is to validate the performance of newly developed cell-enrichment chip in comparison to the conventional centrifugation method.

2. Materials and Methods

Cell Preparation

Seven types of adherent or suspension cell lines were used to test the performance of the **Cellpuri**. Jurkat, HL60, Raji and K562 cells were included in suspension cell test and NIH3T3, HeLa and MCF7 cells were used as adhesion cell line. Suspension cells were obtained from the culture vessel directly and adherent cells were gained from the culture vessel after trypsinization and addition of culture media. All type of cells were diluted at the concentration of $2x10^5$ cells/ml or $2x10^6$ cells/ml for evaluating the performance experiments. For measuring concentration of live and dead cells, the automated fluorescence cell counter, FACSCOPE (CURIOSIS Inc.) was used. HL60 cells were centrifuged at 200g for 10 min and the supernatant was carefully aspirated using a suction pipette to compare with the yield of centrifuge.

*Operation of the **Cellpuri***

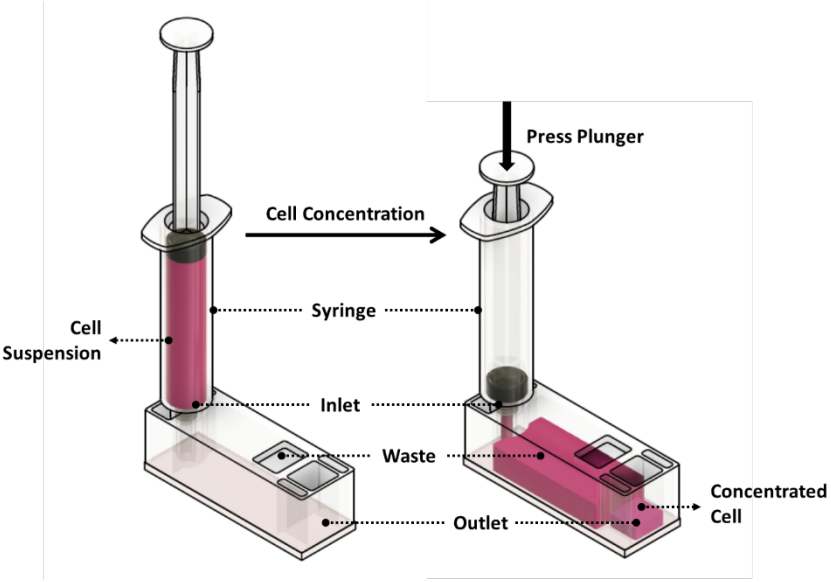

Figure 1. Illustration of the chip operation

As guided in the instructions for operation, prepared cells were filled into a syringe [Luer-lock type], then, the syringe tip was connected to the inlet of the **Cellpuri** and syringe plunger was pressed to inject the cell suspension into the chip using a vertical syringe pump (CURIOSIS Inc.) (Figure 1). After the operation is completed, the concentrated cells were collected at the outlet of the **Cellpuri** using a pipette. The cell-free medium and debris removed during the concentration was discarded as waste.

Measuring Enrichment and Yield of ***Cellpuri***

The cells are separated depending on their size, shape and deformability during the cell suspension passes through the chip. Enrichment and yield are important factors to consider when evaluating the performance of chip.

Enrichment is defined as

$$Enrichment = \frac{Cell\ concentration\ at\ the\ Outlet}{Cell\ concentration\ at\ the\ Inlet}$$

Yield is defined as

$$Yield\ (\%) = \frac{the\ number\ of\ cells\ at\ the\ Outlet}{the\ number\ of\ cells\ passing\ through\ the\ chip} \times 100$$

$$= \frac{the\ number\ of\ cells\ at\ Outlet}{(the\ number\ of\ cells\ at\ the\ Waste\ area) + (the\ number\ of\ cells\ at\ the\ Outlet)} \times 100$$

3. Results and Discussion

Cell pelleting by centrifugation makes it possible to concentrate the cells by removing the medium mixed with the cells. It was attempted to demonstrate that **Cellpuri** efficiently concentrated the cells without spin-down of the cells. As a result of measuring the concentration efficiency of **Cellpuri** in adherent and suspension cell lines at two initial different concentration, it was confirmed that the enrichment of cells using **Cellpuri** was about 22.79 times on an average compared to the initial concentration. The suspension cells were found to be concentrated 23.52 times and 21.37 times in low and high concentration cells, respectively (Figure 2A). Enrichment of 24.55 times and 21.93 times was also observed in adherent cells at low and high concentrations, respectively (Figure 2B). Since there were no significant differences in enrichment observed in both suspension and adherent cells, **Cellpuri** is projected to be usable regardless of the cell type.

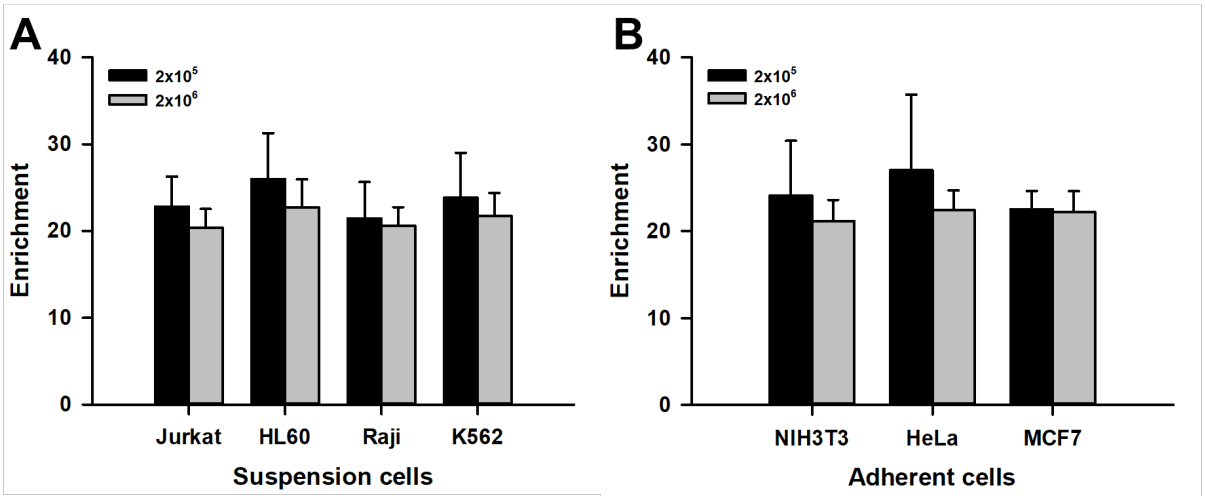

Figure 2. Cell enrichment by Cellpuri various cell lines at two initial concentrations (A) Jurkat (n=12), HL60 (n=10), Raji (n=16) and K562 (n=16) cells were used for suspension cell test at two concentrations. (B) NIH3T3 (n=10), HeLa (n=6), MCF7(n=9) cells were used for adhesion cell test at two concentrations. Error bars, s.d.

At this time, the yield of cells concentrated through the chip was verified to be an average of 94.92% in both adherent and suspension cell line (Figure 3A and 3B).

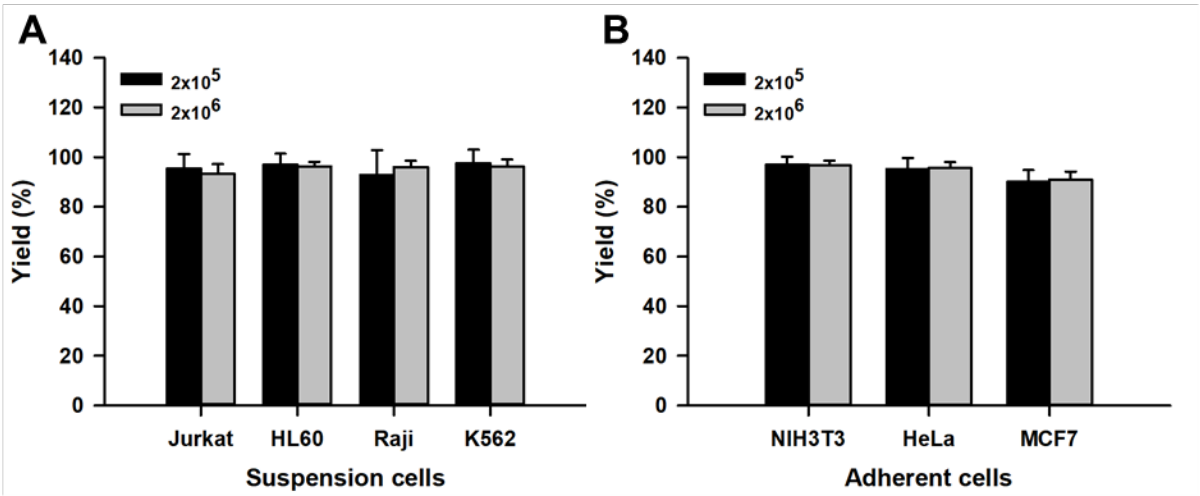

Figure 4. Yield upon enrichment of two initial concentrations of cells in various cell lines. (A) Jurkat (n=12), HL60 (n=15), Raji (n=16) and K562 (n=16) cells were used for suspension cell test at two concentrations. (B) NIH3T3 (n=10), HeLa (n=6) and MCF7 (n=9) cells were used for adhesion cell test at two concentrations. Error bars, s.d.

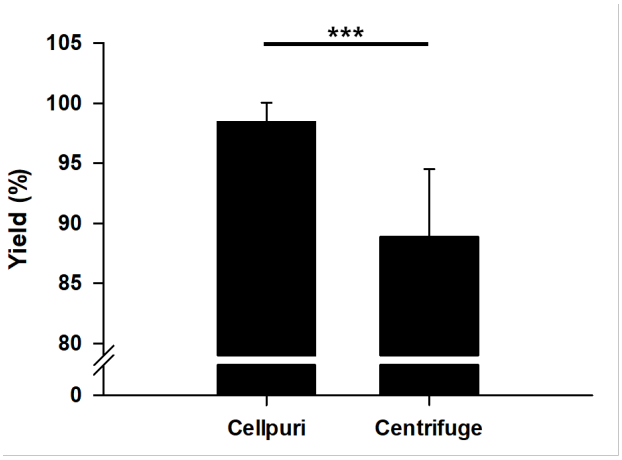

Figure 3. Comparison of yield in Cellpuri and centrifuge. P value (***p<0.001, unpaired t-test) indicates statistically significant difference in yield between chip and centrifuge. Error bars, s.d. (n≥10).

When pelleting the cells by centrifugation, culture medium supernatant should be removed by gentle aspiration, which nevertheless inevitably leads to cell loss. Therefore, the yield of HL60 cells during cell concentration using the **Cellpuri** and the centrifugation was compared. As shown in Figure 4, it was found that the recovery rate decreased in Centrifuge due to the greater loss of cells after the suction as compared to that of cell-enrichment chip which led to decline in the yield of centrifugation up to 88.87% compared to the cell-enrichment chip which is 98.44% (Figure 4).

According to the above results, **Cellpuri** can concentrate cells with a high recovery rate of 94.92% eliminating the hassle of removing the supernatants, so it is expected to have a better usability for cell concentration as an alternative to centrifugation. In addition, in order to concentrate cells through centrifugation, they are exposed to centrifugal force for more than 5 minutes, whereas in the case of **Cellpuri**, cells are affected only by shear force passing through the chip for only a few seconds. This denotes that with **Cellpuri**, cells are less exposed to mechanical stress and has minimal effect on cell functions compared to centrifugation method.

4. References

[1] HG Kim *et al*. Mechanical stress induces tumor necrosis factor-{alpha} production through Ca2+ release-dependent TLR2 signaling. *Am. J Physiol. Cell Physiol*. 295, 2 (2008)

[2] Eckert J *et al*. Hypergravity affects cell traction forces of fibroblasts. *Biophys. J*. 120, 5 (2021)

Description of **Cellpuri**

Cellpuri™ is a disposable chip that concentrates cells without the centrifugation process. The chip enriches cells using rheological phenomenon inside the microchannels where cell suspension passes through to filter out waste medium. The chip is composed of an inlet in which the cell suspension is loaded, outlet where the cell-enriched suspension is collected and cell free medium (waste) is filled at waste area. The size of **Cellpuri** is 76 (w) x 26 (d) x 23 (h) mm where it can process 2 ~ 20mL of cell suspension at the speed of 1.5 mL/min. Furthermore, **Cellpuri** can concentrate cells with 10~20μm in size and enriches more than 20 times with respect to the original cell concentration.