Size-Dependent Separation of Human Mesenchymal Stem Cells by

Applying Microfluidic Chip Filtration

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ABSTRACT

It is very important to separate the human bone marrowderived mesenchymal stem cells (hMSCs) with high multipotential to differentiation and to increase the efficacy of cell therapy. In this view, we have investigated to develop and validate a novel method for the collection of desired size of hMSCs by utilizing the microfluidic chip with main, side, and branch channels realizing the hydrodynamic filtration (HDF) principle. For the sizedependent cell separation, the PDMS-glass chip was designed to collect the smaller hMSCs at the foremost outlet of the branch channel. The separation performance was controlled by the number of branch channels and the ratio of the flow rate of a main inlet to that of a side inlet. Results of the size distribution of collected cells could be applied for sorting and isolating hMSCs. Further, microfluidic chip filtration has a key advantage capable of continuous operation without applying additional chemicals.

Keywords: microfluidic chip, stem cell, shear rate, cell separation, hydrodynamic filtration

1 INTRODUCTION

Human mesenchymal stem cell (hMSC) has long been a latent candidate for cell therapies and tissue repairing because of its regenerative properties and multi-potentiality. In addition, hMSC has been used as therapeutic strategies against age-related or other diseases [1]. hMSCs have been classified into three groups based on the shape: rapidly selfrenewing (RS) cells, spindle-shaped cells, and flattened cells. Haasters et al. [2] revealed that the smallest group (i.e., RS) derived from bone marrow has the great ability to become various types of cell.

The size-dependent property of hMSCs means that sorting out the RS cells from hMSCs population improves differentiation efficiency. There are many studies that deal with the cell separations. For example, fluorescenceactivated cell sorter (FACS) is a kind of flow cytometry that has been widely used so far. But, FACS is a labor intensive process with a high cost, and further utilizations of the collected cells are considerably restricted.

on the benefits of microfluidics and Based microfabrications, recent studies have been reported aiming the innovative manipulations of particles and living cells. Applying to passive and active separations has been the external fields. Note that realized bv the magnetophoresis and optical method correspond to the active separation, while the passive separation uses the inertia, dean flow, pinched flow, and the hydrodynamic filtration (HDF) [3]. Since mid 2000s, the HDF principle has been employed in the fractionation of the cells by using microfluidic chip [4-6]. The HDF does not need the chemical treatment, magnetic, and electric fields, which may affect cells.

The fractionation of stem cells is one of the most important procedures in the field of biomedicine to increase the efficacy of cell therapy. It should be pointed out that applications of the HDF to hMSCs have not been reported so far. In this study, the disruption of hMSCs against the shear stress is first tested by embedding the pillar structure inside the microchannel. It should be a prerequisite for the utility of hydrodynamic filtration. Then, we investigate a novel method for the size-dependent separation of hMSCs based on the microfluidic chip and the HDF principle. Rectangular channels for multi-modal separation are designed by introducing the principles of laminar flows. Unlabeled cells are obtained after the HDF, because the path of cells resorts only to the hydrodynamic force.

2 THE HDF PRINCIPLE

Our microchannel has a rectangular cross-section with the width W and the height H, as shown in Fig. 1. For a steady state laminar flow, the analytical solution for velocity profile is given by the pressure drop ΔP applied along the channel length L. The integration of velocity profile over the width and the height yields the flow rate Q [7] such that

$$Q = \frac{WH^3 \Delta P}{12\mu L} \left[1 - \frac{192H}{\pi^5 W} \tanh\left(\pi W/2H\right) \right] = \frac{\Delta P}{R}$$
(1)

where *R* is the flow resistance. In Fig. 1, S_{BM} stands for the branch point. S_{MI} and S_{MO} denote the inlet and outlet of the main channel, respectively, and S_{BO} is the branch channel

outlet. The widths of main and branch channels are W_{MO} and W_{BO} , and those lengths correspond to L_{MO} and L_{BO} .

At $S_{\rm MI}$, the laminar flow stream is divided into two streams: one toward $S_{\rm MO}$ (the gray-colored region) and the other toward S_{BO} (the white-colored region). Thus, $Q_{\rm MI} = Q_{\rm MO} + Q_{\rm BO}$. Note that there exists the dotted line dividing into two regions. It is a virtual boundary of the layers of fluid that will be diverted into the side branch. The distance between $S_{\rm MI}$ and $S_{\rm BM}$ is long enough for the streamline to become parallel to the main channel at $S_{\rm MI}$. A width of the white-colored region is termed as the cutoff width W_C , with the division ratio $\xi = W_C/W_{\rm MO}$. Similarly, the width of the gray-colored region is $W_{\rm MO} - W_C$.



Figure 1: Schematic of the HDF of cell particles in microchip with a side channel. The region entering into the side channel is white-colored.

Cells are introduced to the main channel inlet $S_{\rm MI}$, and approaching the side where the branch channel is formed as they flow toward $S_{\rm BM}$ (which is called the cell focusing). Among the cells, the center of mass of the smaller ones is located in the white-colored region (such as the smaller sphere in Fig. 1), which is likely to enter the branch channel; the other cell is likely to pass the branch channel (as the larger sphere in Fig. 1). Due to the cell focusing, the cells with the radius less than W_C are expected to enter the branch channel.

The division ratio ξ also means the ratio of the flow rate at $S_{\rm BO}$ (i.e., $Q_{\rm BO}$) to the flow rate at $S_{\rm MI}$ (i.e., $Q_{\rm MO} + Q_{\rm BO}$), from which one can obtain

$$\frac{Q_{\rm MO}}{Q_{\rm BO}} = \frac{W_{\rm MO} - W_{\rm C}}{W_{\rm C}} \,. \tag{2}$$

Applying Eq.(1) into Eq.(2) and rearranging terms result in

$$\frac{W_{\rm MO} - W_{\rm C}}{W_{\rm C}} = \left(\frac{L_{\rm BO}W_{\rm MO}}{L_{\rm MO}W_{\rm BO}}\right) \frac{\left[1 - \frac{192H}{\pi^5 W_{\rm MO}} \tanh\left(\pi W_{\rm MO}/2H\right)\right]}{\left[1 - \frac{192H}{\pi^5 W_{\rm BO}} \tanh\left(\pi W_{\rm BO}/2H\right)\right]}.$$
 (3)

Here, the pressure drops $\Delta P_{\rm MO} = P_{\rm MO} - P_{\rm BM}$ and $\Delta P_{\rm BO} = P_{\rm BO} - P_{\rm BM}$ are set equal (i.e., $\Delta P_{\rm MO} = \Delta P_{\rm BO}$) on the ground that both $S_{\rm MO}$ and $S_{\rm BO}$ are open to the atmosphere ($P_{\rm MO} = P_{\rm BO} = P_{\rm atm}$).

3 EXPERIMENTALS

3.1 Channel Design and Chip Fabrication

We consider chip dimension for cell/particle separation with the cut-off cell size (W_C). Once L_{MO} , L_{BO} , W_{MO} , and Hare set, the width of the branch channel W_{BO} is obtained by applying the fixed-point iteration method. To this end, Eq.(3) is rewritten in the simple form

$$W_{\rm BO}^{C} = f\left(W_{C}, H, W_{\rm MO}, W_{\rm BO}^{G}, L_{\rm MO}, L_{\rm BO}\right).$$
(4)

Two kinds of chip design are considered in Fig. 2. The chip I consists of the main channel, side channel, and one branch channel, so that there are two inlets (I-M and I-S) and two outlets (O-1 and O-2) for the bimodal separation. The chip II has two branch channels with three outlets (O-1, O-2 and O-3) to achieve the trimodal separation. Their dimension is provided in Table 1.



Figure 2: Schematics of the microchip filtration for bimodal (chip I) and trimodal (chip II) separations of cell particles.

In either chips, cell suspension is introduced into the main channel inlet (I-M). The simple fluid is supplied into

the side channel inlet (I-S), which forms a side flow. The polydimethylsiloxane (PDMS) replica is bonded with glass cover slip using the oxygen plasma generator (CUTE-B, Femto Science, Korea) at 50 W for 10 sec.

Chip I	Channel	Branch		Main
	width (µm)	80		160
	length (mm)	3		1
	height (µm)	80		80
	$W_{c}(\mu m)$	15		
	Channel	Branch 1	Branch 2	Main
Chin	Channel width (μm)	Branch 1 80	Branch 2 80	Main 160
Chip II	Channel width (μm) length (mm)	Branch 1 80 3	Branch 2 80 0.67	Main 160 1
Chip II	Channel width (μm) length (mm) height (μm)	Branch 1 80 3 80	Branch 2 80 0.67 80	Main 160 1 80

Table 1. Structure and dimension of chip I and II.

3.2 hMSC Preparations

Adult hMSCs purchased from Cambrex (Walkersville, MD) are cultured in low glucose dulbecco's modified eagle's medium supplemented with 10% fetal bovine serum, 1% Penicillin/Streptomycin at 5% CO₂, 37°C. Cells are plated at a density of 4×10^4 cells/10 cm dishes and cultured for 48hr. For analysis of the cell size distribution, cells were trypsinized and added to the chambers of the hemocytometer. The viability of cells was checked with trypan blue, and images were observed by the microscope (TE2000-U, NiKon) with software (Image J, NIH, MD). The size distribution of hMSCs shows in the range of 8-40 μ m with an average of 24±5 μ m, as shown in Fig. 3.



Figure 3: Size distribution of trypsinized hMSCs (passage 15) used in this study.

3.3 Microfluidic Chip Filtration

Before operating the HDF chip for hMSCs separation, the PBS solution was carefully introduced into I-M and I-S for 15-30 minutes to avoid bubble generations. The cells were suspended at density of 10⁵ cells/ml in neurobasal media. The cell separation was always performed in a steady state condition with the fully-deveoped flow. The cell suspension was supplied into I-M at 0.2 ml/hr using a syringe pump (Elite 11, Harvard Apparatus), and the PBS buffer was introduced into I-S at 0.4 ml/hr using the another syringe pump (Fusion200). Here, total flow rate belongs to a proper range of shear stress. Teflon tubing was used with outer and inner diameters of 1.5 mm and 0.8 mm (Fig. 4).



Figure 4: Microfluidic chip and flow connections.

4 RESULTS

In our PDMS-glass microfluidic chip, each channel is designed by flow resistance in the network. The side channel at an inlet is merged to the main channel, thus the flow rate at S_{MI} is the sum of the flow rates at the main channel inlet and the side channel inlet. The side flow plays an important role in focusing the cells to migrate toward the side wall due to virtual boundary of fluid layers that will be diverted into the branch. This is called the cell focusing.

Prior to cell separations, the side flow effect on performance and validity of microfluidic chip filtration were tested by using model particles (Fig. 5). Particle suspensions are prepared with the latex bead mixture of fluorescent polystyrene of 15, 25, and 40 μ m diameters.



Figure 5: Side flow effect on separation performance in microchip filtration.

According to the shear stress effect in the hydrodynamic hMSCs disruption, in the region of lower shear rates less than 1,000 s⁻¹, cell disruption hardly occurs in short time, whereas high shear rate of 5,000 s⁻¹ provides the cell disruption of above 30 %. Thus, size-dependent hMSCs separations were conducted at shear rate of about 1,000 s⁻¹.







Figure 7: Fractional results of hMSCs obtained by trimodal separation chip. (a)-(d) are initatal, outlet 1, outlet 2, and outlet 3, respectively. Scale bars indicate $100 \mu m$.

We conducted two cases of bimodal and trimodal separations of hMSCs by using chip I and II, respectively. Histogram in Fig. 6 shows the average sizes of 15.9 ± 10.1 and 35.8 ± 14.5 µm so that the bimodal fractions are almost achieved. Histogram in Fig. 7 shows the average sizes of 17.2 ± 5.3 , 28.8 ± 5.4 , and 52 ± 13 µm at each outlet, where cells larger than 55 µm seem to be aggregated. It should be emphasized that the trimodal separation chip shows higher performance compared to the bimodal one, promising possible applications of the HDF with multiple branch channels for precise cell fractionations.

5 CONCLUSIONS

We observed the shear stress effect and the time evolution in the hydrodynamic hMSCs disruption. Higher shear stress can destroy of cell membrane. The separation of desired size of hMSCs can successfully be achieved by utilizing the microfluidic chip and the HDF principle. For continuous operation, two kinds of HDF chips were fabricated for bimodal and trimodal separations of the hMSCs. According to the focusing with a proper ratio between main and side flow rates, cell particles were diverted into the side branch by side flow. Fractional results obtained in this study confirm the validity of microfluidic chip filtration, which indicates possibility of isolating the hMSCs to increase the efficacy of cell therapy.

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