CELL CYCLE SYNCHRONIZATION OF STEM CELLS USING INERTIAL MICROFLUIDICS

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ABSTRACT

Cell cycle synchronization is of paramount importance for studying cellular properties and biological processes involved in various stages of the cell cycle. Here, we present a microfluidics based approach to synchronize the cell cycle of a primary –cells, human bone marrow-derived mesenchymal stem cells (hMSCs), using inertial forces in spiral microchannels. The device operating principle exploits the relationship between the volume (and thus diameter) of a cell and its phase in the cell cycle, in order to fractionate hMSCs populations into synchronized subpopulations enriched in cell cycle fractions of G0/G1, S and G2/M phases.

KEYWORDS: cell cycle, stem cells, inertial forces, Dean flows, size fractionation

INTRODUCTION

Synchronizing cells at various phases of the cell cycle is essential for studying cellular properties, biological processes and elucidating genetic regulatory mechanisms involved in each responsible for cell proliferation. The cell cycle consists of four sequential phases, namely: G1 gap, S (DNA synthesis), G2 gap and M (mitosis). As a cell progresses through the cell cycle, it synthesizes new macromolecules, organelles and vesicles which fuse with the cell membrane leading to increase in cell diameter [1]. Thus, cell cycle synchronization techniques are important to enable the study of these processes and changes occurring in each phase. Cells are typically synchronized by inhibiting DNA replication in the S phase, via methods including hydroxyurea addition to the media [2], serum starvation. or contact inhibition [3]. The metabolism of synchronized cells is often modified using these techniques, leading to disrupted progressive cell growth and, in severe cases, inducing apoptosis. Counter-flow centrifugal elutriation and fluorescence activated cell sorter (FACS) are other commonly used cell synchronization techniques [4]. Although these techniques do not affect the metabolism of cells, the need for expensive equipment has limited their wide application.

In this work, a microfluidics based approach to cell cycle synchronization of MSCs using inertial forces in spiral microchannels is presented. The device operating principle exploits this relationship between a cell volume (and thus diameter or, more generically "size") and its phase in the cell cycle to synchronize human bone marrow-derived MSCs (hMSCs) populations. Microfluidics-based cell separation systems offers considerable advantages over conventional cell sorting techniques such as FACS and counter-flow centrifugal elutriation including reduced sample volume; faster sample processing which reduces analysis time; and high sensitivity and spatial resolution [5]. Cell cycle synchronization using microfluidics separation techniques such as dielectrophoresis [6], acoustophoresis [7], hydrophoresis [8] and hydrodynamic filtration [9] have also been explored recently. Although such devices are powerful tools for size-based cell separation, they tend to be complex and costly, and require an active energy source for separation that could potentially affect cell viability. Also, the low-throughput of these approaches has limited the wide application of those techniques within the biological community. Hence there is a clear need to develop a simple, high throughput and high purity technique for cell cycle synchronization.

DESIGN PRINCIPLE

Recently, size-based cell separation in microfluidic systems has been demonstrated based on principles of inertial migration. Under Poiseuille flow conditions in channels with rectangular cross-section, the balance between shear-induced and wall-induced lift forces equilibrates the suspended particles at eight distinct positions across the channel cross-section [10]. Apart from these lift forces, in spiral microchannels the inherent outward directed centrifugal force gives rise to two counter rotating vortices (Dean vortices), in the top and bottom half of the microchannel. These secondary Dean vortices exerts a drag force on the particles, entraining them within the vortices. The magnitude of this Dean drag force (F_D) varies with the particle size (d) and its position within the channel cross-section ($F_D \alpha d$). Particles flowing in spiral microchannels are thus subjected to both the inertial lift (F_L) forces and the Dean drag force. The interplay between these two forces reduces the eight equilibrium positions to just two near the inner channel wall, each within the top and bottom Dean vortex [11]. Since the inertial forces is largely dependent on the particle size ($F_L \alpha d^4$), particles of varying sizes flowing in the spiral microchannel equilibrate at distinct positions along the microchannel cross-section under the influ-

ence of inertial lift and Dean drag forces [12]. Using this principle, we have size-fractionated hMSCs into synchronized populations enriched in G0/G1, S or G2/M phase cells. Figure 1 illustrates the developed spiral microfluidic design.

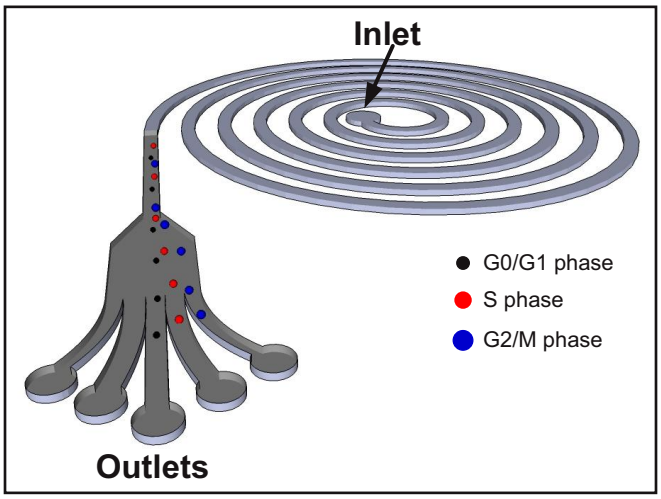

Figure 1. Schematic illustration of the spiral microfluidic design developed for cell cycle synchronization. Under the influence of inertial lift forces and Dean drag force, the hMSCs are size fractionated to obtain relatively pure populations of cells in the G0/G1, S and G2/M phase. The cells in the G2/M phase, due to the large size, equilibrate closest to the microchannel inner wall followed by cells in the S and G0/G1 phase.

EXPERIMENTAL PROCEDURES

The hMSCs were expanded at 37°C and 5% CO_2 in low glucose DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Unlike transformed cell lines and permanent cultures, hMSCs are more susceptible to 'contact inhibition' which would arrest most of the cells in the G0/G1 stage. Therefore, in order to obtain hMSCs in the S and G2/M phases, cultures at approximately 40-50% confluency were used in this study.

Spiral devices were fabricated in polydimethylsiloxane (PDMS) bonded to microscopic glass-slides (Figure 2A). The design consists of 500×200 µm (W×H) channels with 8-bifurctaed outlets to collect cells of varying sizes. During testing, hMSCs were introduced in the device at 2 mL/min flowrate. The flow was experimentally observed using an inverted epi-fluorescence microscope equipped with a 12-bit CCD camera. The microscopic images of the samples collected from the outlets were captured and the size analyzed and recorded. Following separation, flow cytometry using propidium iodide staining was performed to analyze DNA content [13].

RESULTS AND DISCUSSION

Figures 2B-C present optical micrographs and viability results of the control (unsorted) and sorted MSCs collected from outlets 2, 3 and 4. The cells were successfully separated on the basis of their size and the cell diameter was measured using Metamorph software (Moelcular Devices Inc.). In the control group of unfractionated MSCs, the mean cell diameter was 21.9 ± 3.5µm and the size distribution (approximately 14.4 – 33.9µm) was broader than that of sorted MSCs collected from each outlet. MSCs collected from outlet 2 had a mean cell diameter of 23.5 ± 5.6µm, and cells were significantly larger than those collected from outlets 3 and 4. The size distribution of the cells collected from outlet 3 was similar to that of the control cells. In contrast, cells in outlet 4 were smaller (approximately 15.5 ± 2.1µm) than those collected from the other outlets. Following separation, the cells were stained with trypan blue dye to assess their viability. As shown in Figure 2C, the viability of the sorted cells was similar to that of the control unsorted MSCs as more than 90% of the cells collected from each outlets excluded the dye; suggesting that the cells were sorted without incurring physical damage leading to cell lysis.

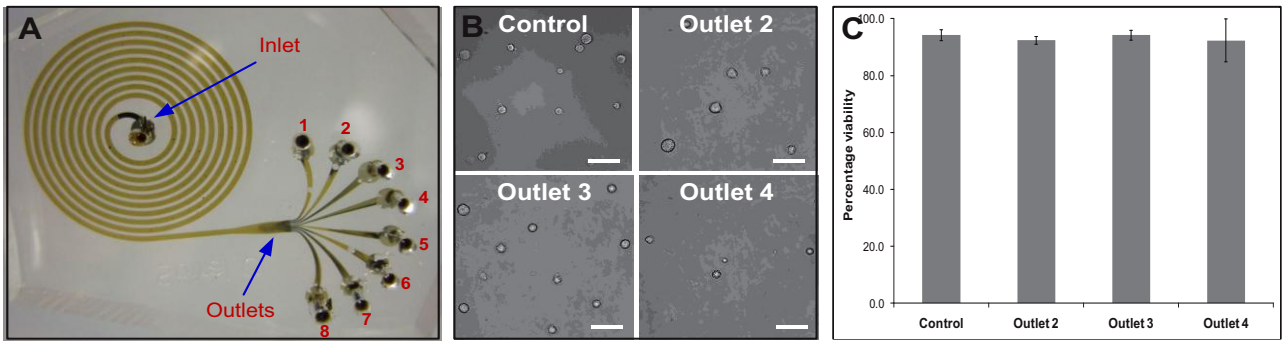

Figure 2. A. Photograph of the spiral microchannel with one inlet and eight outlets fabricated in PDMS. **B.** Optical micrographs of the size sorted cells collected from outlets 2, 3 and 4. bar = 50µm. **C.** Viability of sorted hMSCs verified using trypan blue exclusion assay. Results indicate that the shear experienced at very high flow rates (2 mL/min), do not compromise the cells achieving >90% cell recovery.

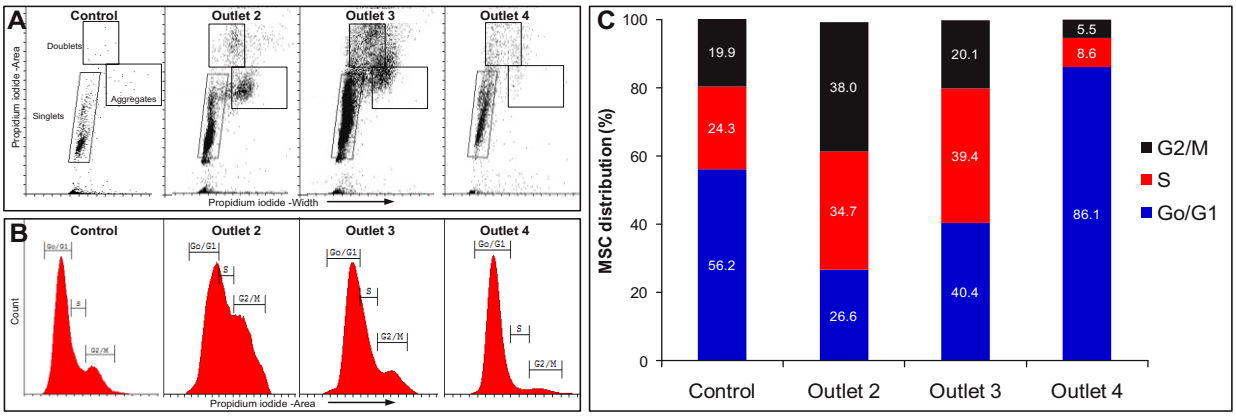

Figure 3. Cell cycle analysis results. **A.** Flow cytometry data showing the dot plots of propidium iodide fluorescence width/area for singlet, doublet and aggregate discrimination of the control and samples collected from outlets 2, 3 and 4. **B.** Histogram indicating the distribution of the DNA content of the sorted singlet cells in the G0/G1, S and G2/M phase after synchronization. **C.** Plot presenting the cell cycle population distribution of the synchronized hMSCs. The initial asynchronous cells have a G0/G1 to G2/M ratio of 2.8:1, while the small cells collected from outlet 4 show an enriched ratio of 15.7:1. Similarly, the larger cells collected from outlet 2 indicate a 4× enrichment in the G2/M population ratio.

Figure 3 presents the cell cycle analysis of the sorted hMSCs in the G0/G1, S and G2/M phase after synchronization. The relative populations of cells in different phases of the cell cycle in each outlet were determined by flow cytometric analysis based on their DNA contents. At day 3 of the MSC culture, 56.2% of the cells were found in G0/G1, 24.3% in S and 19.9% in the G2/M phase. After sorting, the cell population collected from outlet 2 had a combined 72.7% in the S-G2/M phases while 86.1% of the cells from outlet 4 were synchronized to the G0/G1 phase. As the size distribution of the cells recovered from outlet 3 was similar to that of the control cells, their respective DNA histograms were closely matched (Fig. 3B). These results indicate that the G0/G1 to G2/M ratio of 3:1 of the asynchronous sample is enriched to16:1 from the sample collected at outlet 4. Similarly, a fourfold enrichment in the G2/M population is achieved from the sample collected at outlet 2. These results are comparable with those reported using other microfluidic systems [6-9], though with significantly increased throughput and demonstrated maintenance of cell viability post-sorting.

CONCLUSIONS

To our knowledge, this microfluidics-based study is the first demonstration of synchronizing hMSCs into different phases of their cell cycle using size-based separation. The high flow throughput of this technique can fractionate ~15x10^6 cells/hr, while its passive sorting principle ensures ~100% cell viability. Finally, the device allows facile collection of synchronized cells after separation, and can find diverse applications in the biological studies of many other different primary cell types.

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