CELL ROLLING CYTOMETER FOR CHARACTERIZING DYNAMIC ADHESION OF MESENCHYMAL STEM CELLS

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ABSTRACT

This paper reports a cell rolling cytometer (CRC) for controllably contacting and transporting cells in suspension using a three-dimensional microtopography coated with adhesion molecules, which enables quantification of cell-surface adhesion dynamics *via* transit time and lateral position at the single cell level. The cell rolling cytometer comprises slant ridges which not only enhance cell-surface interactions for cell tethering, but also direct the rolling of target cells. This approach enables simple, rapid quantification of cell adhesion to surface-bound molecules such as selectins at the single cell level.

KEYWORDS

Cell rolling cytometer, Adhesion molecules, Microfluidics, Mesenchymal stem cell.

INTRODUCTION

Cell rolling on vascular surfaces is the first, crucial step to recruit specific cells (*i.e.* leukocytes, cancer cells, and stem cells) from the bloodstream to a target tissue [1]. Quantitative assessment of cell rolling adhesion is critical for development of mesenchymal stem cell (MSC) therapies, where heterogeneous cell morphologies and donor-to-donor variations can result in variable cell adhesion that can potentially impact cell homing to target tissues [2]. While flow chambers have been employed for *in vitro* cell rolling adhesion assays [3], they lack a means to force cells to contact the surface and characterize only those cells that happen to interact with the surface. Microfluidic devices have recently employed mixing approaches using surface grooves to create circulating streamlines that enhance cell-surface interactions, resulting in higher cell capture efficiencies [4,5]. In these approaches, the cell adhesion occurred through long channels of $l > 4$ cm that are also not suitable for quantifying rolling cell adhesion as they can characterize cells only in a part of the channel within the field of view.

ASSAY PRINCIPLE

To overcome these challenges, we developed a cell rolling cytometer for controllably contacting and transporting cells in suspension using adhesion molecule-coated ridges, which enables quantification of cell-surface adhesion dynamics *via* transit time and lateral position at the single cell level (Figure 1). The device operation is based on "deterministic cell rolling" wherein a three-dimensional topography induces effective contact of cells with adhesion surfaces that support rolling of target cells, which alters their trajectories deterministically and results in cell separation [6]. We designed the adhesion channel with small dimensions (200 µm × 2,000 µm) to observe and characterize the trajectory of every cell flowing through the channel (Figure 1). Using the CRC, we examined the hypothesis that adipogenic and osteogenic MSCs have a different adhesion capability with undifferentiated MSCs.

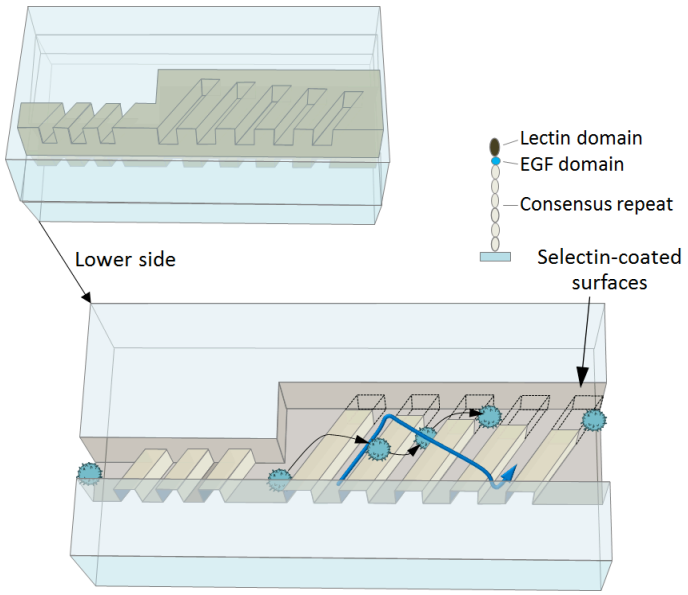

Fig. 1. Cell rolling cytometer. Schematic of the microfluidic device in which cells are controllably contacted with adhesion molecule-coated ridges and the adhesion of single cells is quantified *via* transit time, t_t and rolling trajectory, x. Specific adhesion interactions retard the cell and change its trajectory.

EXPERIMENT

The devices were fabricated in poly(dimethylsiloxane) (PDMS) using soft lithography, with h_t = 87.6 µm and h_g = 25.8 µm for HL60 cells, or h_t = 100.9 µm and h_g = 36.1 µm for MSCs, where h_t is the total channel height and h_g is the gap height between the top and bottom ridges (Figures 1 and 2). The whole channel was incubated with 1.5 µg/mL P-selectin for HL60 cells and 30 µg/mL E-selectin/Fc for MSCs and then washed with 1% BSA solution. HL60

978-0-9798064-5-2/µTAS 2012/$20©12CBMS-0001

cells were cultured in Iscove's modified Dulbecco's medium with supplements. Human mesenchymal stem cells (MSCs) were obtained from Lonza and cultured in MSCGM^{TM} medium (Lonza) according to the supplier protocol and used at passages 4-7. For adipogenesis and osteogenesis of MSCs, the medium was switched to adipogenic and osteogenic differentiation medium (Lonza), respectively. Histochemical staining was performed to examine cell differentiation. MSCs stained positive for Oil Red O when differentiated to adipocytes and were positive for alkaline phosphatase upon undergoing osteogenesis. A syringe pump was utilized to wash the device with 1% bovine serum albumin solution and to control the flow rates of cell suspensions.

RESULTS AND DISCUSSION

Using HL60 cells as controls, we first examined whether the cytometer could identify rolling from non-rolling phenotypes in terms of transit time and lateral position. While HL60 cells did not interact with BSA-passivated surfaces, they exhibited distinct trajectories on P-selectin coated surfaces (Figure 3A and 3B). With the CRC at 3.5 dyn/cm^2, we observed that rolling HL60 cells took ≈25 times longer than non-rolling HL60 cells to pass through the channel (t_t > 2.5 s) (Figure 3C). Gating in this region yields 86.9 ± 2.8% in the right two quadrants (n = 3). At shear stresses of 7.7 and 10.5 dyn/cm^2, the capture efficiency of a control flat chamber was significantly compromised (p < 0.05). In contrast, the CRC exhibits high efficiency in capturing cells and supports stable rolling in the range of shear stress (3.5 to 10.5 dyn/cm^2). We demonstrated the capability of the CRC to capture cells in the controlled location, the ridge region, which can be crucial for quantification of cell adhesion at the single cell level.

Next, we assessed MSC rolling adhesion on E-selectin-coated channels and found that 85.3 ± 4.0% of the MSCs exhibited robust rolling on these surfaces at 1.7 dyn/cm^2 (Figure 4). The rolling response decreases in a shear stress-dependent manner (Figure 4B). As expected, the CRC was highly effective at capturing cells, whereas significantly less capture was measured with the control flat chamber device (p < 0.05 for all shear stress conditions tested), suggesting that forced cell contact is crucial for initiation and assessment of rolling adhesion. Previously MSCs have been shown to exhibit poor rolling properties on a flat selectin coated surface [7]. Given that MSCs preferentially home to sites of inflammation that suggests there is a rolling mechanism [4], the CRC is an effective method to probe the rolling adhesion of a cell type that exhibits non-robust rolling properties

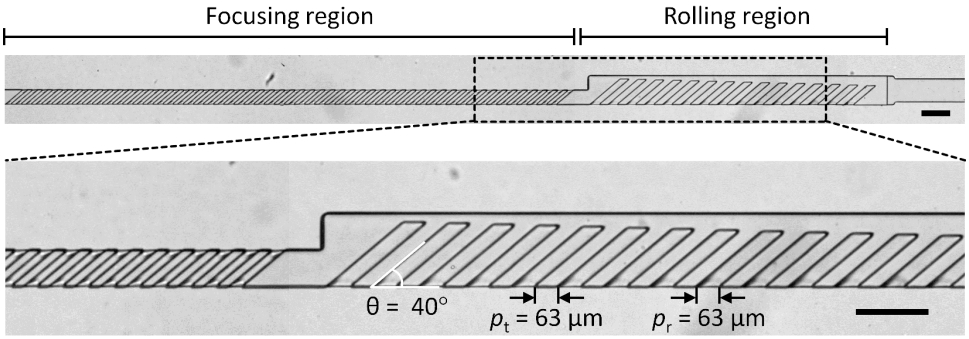

Fig. 2. Optical micrographs showing a cell rolling cytometer formed in PDMS. Scale bars, 200 µm.

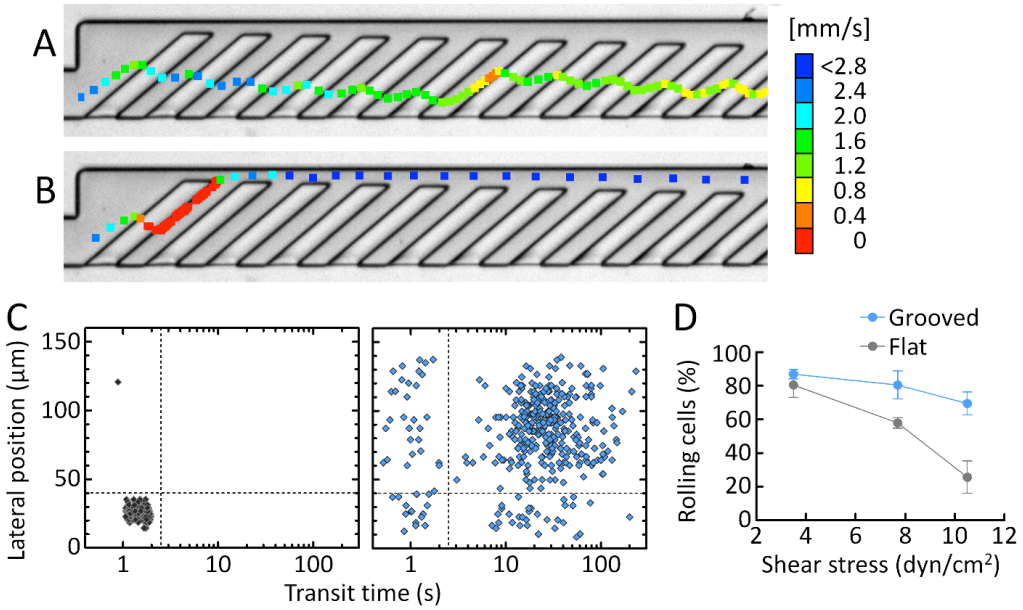

Fig. 3. (A, B) Trajectories of HL60 positive control cells that exhibit robust cell rolling properties in (A) BSA-passivated and (B) P-selectin coated channels. (C) Analysis of rolling adhesion of HL60 cells in (Left) BSA-passivated and (Right) P-selectin coated channels. (D) Efficiency of HL60 cell adhesion in the cell rolling cytometer and flat chamber device (n =3).

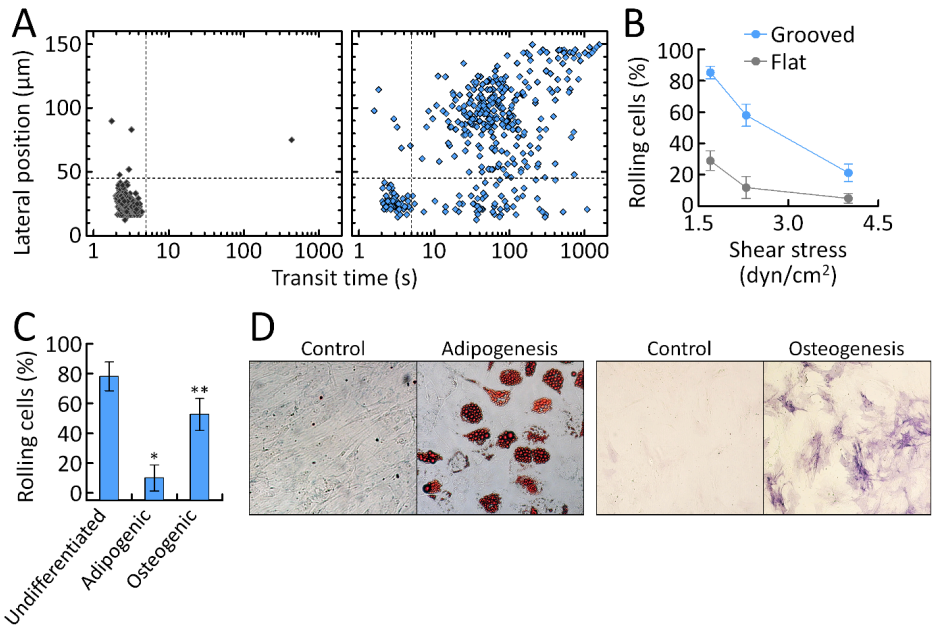

Fig. 4. (A) Scatter plots of the transit time and lateral position of 400 MSCs in IgG-passivated (Left) and E-selectin-coated cytometers (Right). (B) Efficiency of MSC adhesion in the cell rolling cytometer and the control device with the flat flow chamber. (C) Effects of MSC differentiation on MSC rolling adhesion. Error bars show standard deviations (n = 3). (D) Histochemical staining was performed to assess differentiated adipogenic (Left, Oil Red O stain) and osteogenic MSCs (Right, alkaline phosphatase stain).

Analyses for differentiated MSCs showed that the rolling response in both differentiated populations was significantly decreased relative to undifferentiated control group, suggesting that significant correlations exists between MSC differentiation (adipogenesis and osteogenesis) and rolling response (Figure 4C).

CONCLUSION

These results indicate that (1) the proposed cell rolling cytometer enables quantitative analysis of rolling cell phenotype by high capture efficiency, (2) E-selectin is an adhesion molecule mediating rolling adhesion of MSCs, (3) significant correlation exists between MSC differentiation (adipogenesis and osteogenesis) and rolling response. (4) rolling assay of MSCs mediated by E-selectin can be a potential, non-invasive means for MSC quality control.

ACKNOWLEDGEMENTS

We acknowledge support of National Institutes of Health grants HL-095722 and HL-097172 (J.M.K.) and NSF CAREER award 0952493 through the Chemical and Biological Separations program (R.K.). Devices were fabricated in the Microsystems Technology Laboratory at MIT.

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