A NOVEL MICROFLUIDIC ASSAY FOR IN VITRO MODELING OF LEUKOCYTE-ENDOTHELIUM INTERACTIONS

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ABSTRACT

Due to the significance of the leukocyte-endothelium interactions in pathogenesis of disease and drug delivery, amongst many others, several in vitro models have been developed to study different aspects of the leukocyte adhesion cascade. Current in vitro models of the leukocyte adhesion cascade cannot be used for real-time studies of the entire leukocyte adhesion cascade including rolling, adhesion and migration in a single assay. In this study, we have developed and validated a novel microfluidic assay and used it to test the hypothesis that blocking of specific steps in the adhesion/migration cascade significantly affects other steps of the cascade.

KEYWORDS: Microfluidics, neutrophils, adhesion.

INTRODUCTION

Adhesion of particles in during in vivo experiments is highly dependent on microvascular environment comprising of unique anatomical and geometrical flow condition and cell-cell and cell-particles interactions. Classical in vitro static assays consisting of tissue culture dishes lack the in vivo complexity of microvascular environment. So, in vitro flow chambers are developed during the recent years to accurately reproduce in vivo conditions, e.g. bifurcations. Using the microfluidic channels, several investigators were able to characterize leukocytes adhesion. In this study, we have developed and validated a novel microfluidic assay and used it to test the hypothesis that blocking of specific steps in the adhesion/migration cascade significantly affects other steps of the cascade.

EXPERIMENTAL

Fabrication of microfluidic devices was achieved by soft lithography. The devices consisted of vascular channels in communication with a tissue compartment filled with chemoattractant (fMLP) via a 3 μm porous barrier. Human endothelial cells were cultured in the vascular channels and activated with TNF-α for initiation of the inflammatory response. Human neutrophils were subsequently flowed in the vascular channels under physiological shear conditions. We have validated this novel assay against in vivo mouse model using intravital microscopy.
**RESULTS AND DISCUSSION**

Different treatments show the variation of rolling behavior of neutrophils. The results are validated with mouse in vivo model. Numbers in Figure 1 panel B explains the difference in percent of activity compared to the control value.

**CONCLUSION**

We have developed and validated a novel bioinspired microfluidic assay that closely mimics physiological conditions of leukocytes rolling, adhesion/migration cascade observed in vivo during the inflammatory process. This realistic fluidic model allows for in vitro reconstitution of the microvascular environment using human cells and thus allows for screening of therapeutics on human cells. The microfluidic device has a number of other advantages including flow and morphologically realistic environment, ability to model adhesion and migration in the same system, quantitative real-time visualization, reduced reagents and use of disposable chips. Furthermore, this system can be further developed for high throughput analysis.

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**REFERENCES**


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