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Microfluidic device as a novel cell transmigration assay

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Leukocytes circulating in blood transmigrate through vessel walls in a multistep process induced by chemoattractants [1]. The current method for cell migration studies is a static, membrane based end-point assay called a Boyden chamber that enables cell quantification after migration, but no means are available to track the process [2]. Contrary, in an alternative assay called a shear flow chamber, the cells cannot truly transmigrate through a layer of endothelial cells, as there is a solid support underneath [3]. The microfluidic migration assay presented here combines the principles of both assays to enable observation of the entire transmigration process under physiological flow conditions.

The microfluidic device, fabricated by micromilling in two poly(methylmethacrylate) (PMMA) plates, is composed of two straight channels physically separated by a polycarbonate membrane, assembled together by thermal bonding (Fig.1). Continuous flow of cells and chemoattractant solutions at 1.67×10^{-3} m/s in both channels assures that a stable chemoattractant gradient is formed across the membrane. This was validated with Comsol simulations (Fig.2). The cells transmigrate through the membrane from the top channel (cell channel) to the bottom channel (chemoattractant channel). To mimic the natural environment of migrating cells, cell adhesion proteins were injected to the cell channel after bonding for coating of the membrane and channel walls. The behavior of labeled Jurkat cells and purified lymphocytes in the devices coated with fibronectin (Fn) or vascular cell adhesion molecule-1 (VCAM-1) was recorded. Figure3 illustrates the cell velocity change with time.

Microscopic observations of Jurkat cells migrating on a Fn coated surface demonstrated tight adhesion to the surface followed by crawling to transmigrate through a pore. In a separate experiment, Jurkat cells (Fig.3) and lymphocytes were first rolling on the membrane coated with VCAM-1 before adhesion and transmigration. At average, cell velocity during rolling was 150×10^{-6} m/s, which is significantly lower than the flow velocity calculated at 5 μm distance from the channel wall (475×10^{-6} m/s). Such slow cell movement demonstrates weak bonds formed between cells and VCAM-1, which are broken due to shear flow. Further decrease in cell velocity is interpreted as a strong adhesion of cells to the membrane, induced by chemoattractant activation of cell surface integrins. After transmigration through the pores when the cells join the chemoattractant flow in the other channel, the velocity of all analyzed cells rapidly increases.

The presented microfluidic migration device imitates the physiological flow conditions for reliable cell transmigration studies. It can be applied in basic research or drugs development as it offers a significant reduction of sample volume used, it is inexpensive, easy to fabricate, disposable and amenable for high throughput analysis.

REFERENCES:

1. "_Tools for anti-inflammatory drug design: In vitro models of leukocyte migration,_" E.K. Frow, J. Reckless and D.J. Grainger, Medicinal Research Reviews, **24**, 267, (2004)
2. "_The chemotactic effect of mixtures of antibody and antigen of polymorphonuclear leucocytes,_" S. Boyden, The Journal of Experimental Medicine, **115**, 453 (1962)
3. "_Leukocyte Trafficking. Molecular Mechanisms, Therapeutic Targets, and Methods_", A. Hamann and B. Engelhardt, Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Chapter 19-21, (2005)
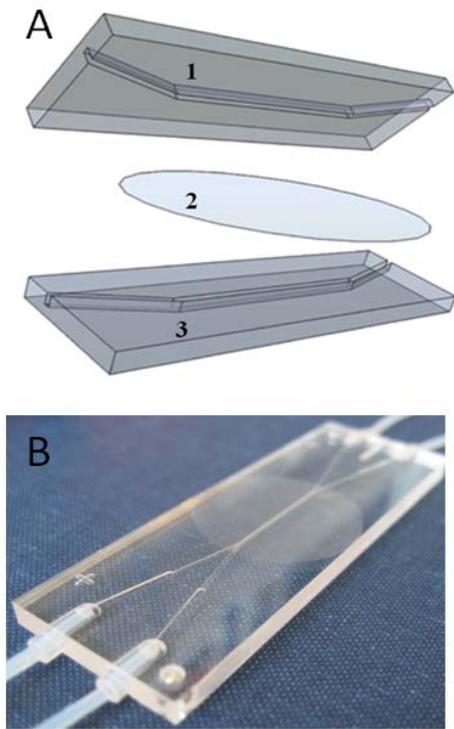
Figure 3 Graph of Jurkat cell velocity as a function of time on a VCAM-1 surface. The initial behavior of a slightly decreasing velocity indicates rolling. This is followed by a region of constant cell velocity, the length of which is different for each cell, and which occurs due to adhesion. The dashed line indicates the transmigration though pores (normalized as time 0). After transmigration there is a rapid linear increase in cell velocity, as the cells exit into the chemoattractant channel and simply follow the flow.

Figure 1 A schematic drawing of the three elements of the device (1-cell channel, 2-membrane, 3-chemoattractant channel) during assembling (A). A complete device for cell migration studies (B).
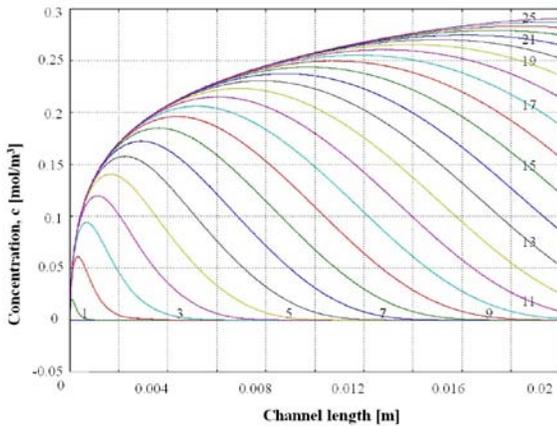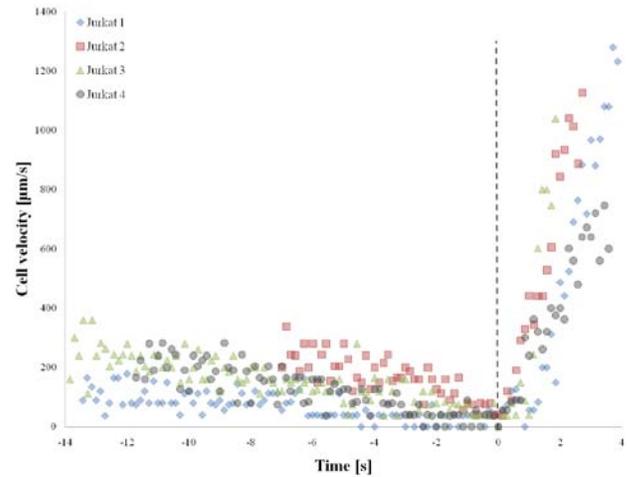

Figure 2 Simulation of the chemoattractant concentration gradient formed across the membrane in the device. The concentration is evaluated 5 μm above the membrane and changes with time and channel length.