FLUXION

APPLICATION NOTE

Transmigration

Leukocyte transmigration through the endothelium under shear flow

Introduction

Transmigration of leukocytes from the lumen of the blood vessels to the underlying tissue is a key process in immunosurveillance and in the response to inflammation and injury in the normal function of the immune system. However, in inflammatory diseases and cancer, transmigration of cells to underlying tissues is a gateway to metastasis and morbidity. The significance of transmigration of unwanted cells to underlying tissues and through the blood brain barrier from the vasculature in disease states makes it an important target for drug development. As such, transmigration is a prime target for inflammation and oncology drug discovery.

Here, we present use of the BioFlux System (Figure 1) to study live cell transmigration in a physiologically-relevant assay using primary endothelial cells and human peripheral blood mononuclear cells. The BioFlux System is a microfluidic platform (Figure 2) designed to run automated shear flow protocols for cell biology and microbiology experiments in high throughput.

Figure 1: The BioFlux System for live cell assays under controlled shear flow.

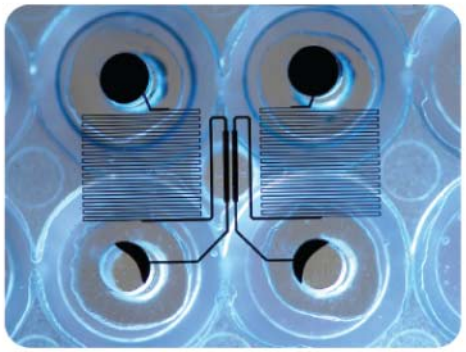


Figure 2: BioFlux Plate channels as viewed from beneath the well plate. Microfluidic flow cells are integrated into the bottom of an SBS-standard well plate. Each fluidic channel runs between pairs of wells and has a central viewing window for observation.

Methods

Human Umbilical Vein Endothelial cells (HUVEC), passage 4 (Sciencell) were grown in microfluidic channels to 100% confluence under 1dyn/cm2 flow in a 5% CO2 atmosphere. HUVEC cells were activated using 25ng/mL rhTNF-α (Invitrogen, Carlsbad, CA) for 6 hours under shear. Cryopreserved peripheral blood mononuclear cells (CPMBC) (Cellular Technology, Ltd., Shaker Heights, OH) were prepared for transmigration as follows: cells were removed from liquid nitrogen storage, thawed rapidly, and gently transferred into a 50 mL tube. 5mL thawing solution (CTL-Thaw, Cellular Technology, Ltd., diluted 1:20 in RPMI 1640) pre-warmed to 37 °C was slowly dripped into the tube containing the cells over one minute to avoid shear-induced apoptosis in cells. A second 5 mL volume of thawing solution was added to the cells in the same manner over 30 seconds. Cells were pelleted at 300 x g for 10 minutes and resuspended to 2 x 106 cells/mL in CO2-independent media (Invitrogen) containing 10% (v/v) FBS. PBMC were added to the activated HUVEC monlayers at 25°C at a shear of 1 dyn/cm2 for 10 minutes so that cells were able to attach to the HUVEC monlayers without penetrating the monolayer. A BioFlux Plate was placed on the BioFlux Heater Plate at 37°C to allow for transmigration to occur at 0.8 dyn/cm2 for either 15 or 30 minutes. Microscopy data were captured using a QImaging Camera (Surrey, B.C.) on a Nikon TS100 microscope. Migration distance measurements were made with the BioFlux Cell Tracking Module.

Results

Live cell imaging was used to study transmigration under flow in the BioFlux System. First, migration and transmigration were investigated using time-lapse image capture. Cells were observed both migrating and transmigrating (Figure 3) under shear flow at 37°C for 15 minutes. The average distance cells migrated during the time lapse recording was 31 µm (±4.45 µm, standard error for 25 cells). Cells in the process of transmigration, transitioning from above to below the HUVEC monolayer, moved a shorter distance, approximately 30µm, compared to cells engaged in slow rolling or active migration on top of the monolayer; maximum distances observed were 90 and 83µm respectively.

Transmigration was then assessed simultaneously in 24 channels on HUVEC monolayers which were stimulated with TNF-α. CPBMC were added to monolayers under flow. Images were captured after 10 min of perfusion at room temperature and after 30 minutes at 37°C, a temperature permissive for transmigration. In fields of view 250 x 300µm, 34% (±13%) of cells transmigrated through the HUVEC monolayers as defined by a change in phase-contrast (phase bright to phase dark), and shape (spherical to amoeboid) (Figure 4A). Fields with lower numbers of cells attached had higher levels of transmigration (Figure 4B). This phenomenon could be a function of physical space for the cells to move or contact receptors required for transmigration.

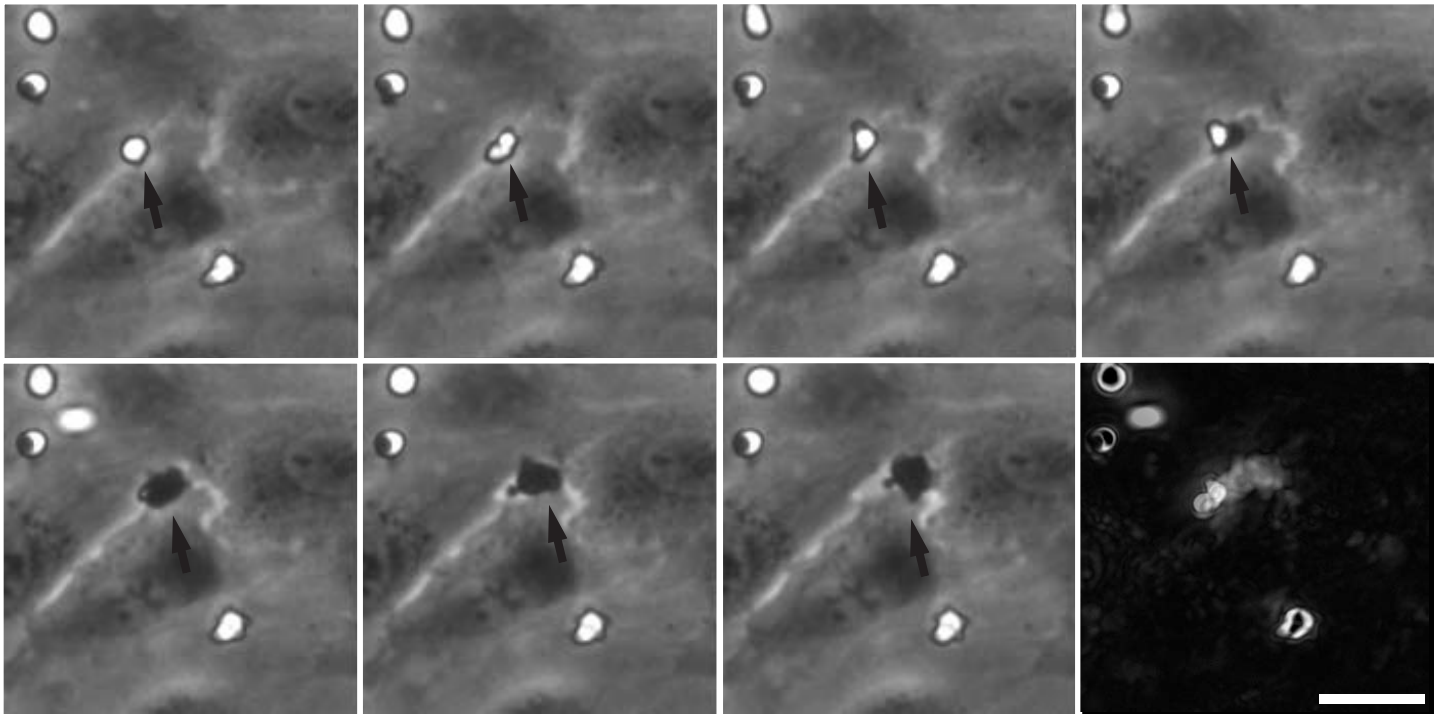
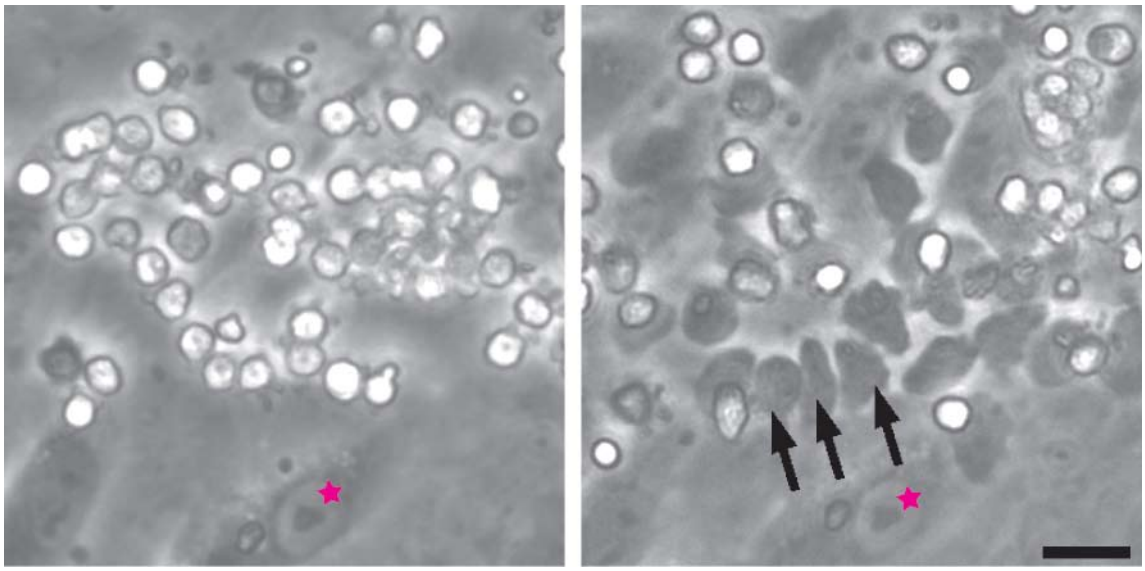


Figure 3. Time-lapse montage of a CPBMC undergoing transmigration.
Images were recorded at 30 second time intervals at a shear flow of 0.8 dyn/cm2 (data shown chronologically from top left to bottom right). A Z-stack image, using maximum intensity, is shown to illustrate the trajectory of the cell being observed (bottom right panel). This cell moved a total distance of 24µm. Scale bar =20µm.

Transmigration

Leukocyte transmigration through the endothelium under shear flow

A



Adhesion at room temperature

Transmigration at 37˚C

B

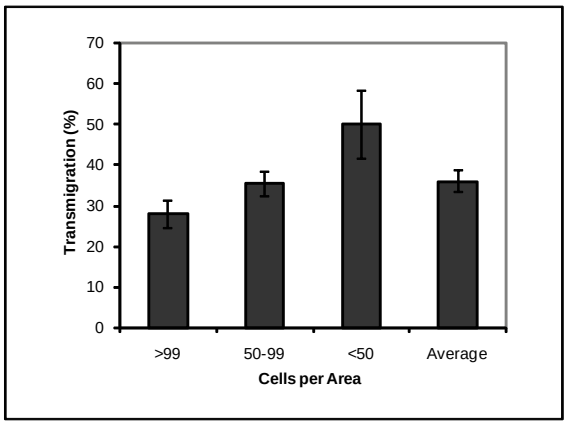


Figure 4. Adhesion and transmigration. (a) Basal levels of adhesion were measured (by microscopy) prior to shifting the temperature of the BioFlux Plate to 37°C for 30 minutes to allow for transmigration. Representative transmigrated cells are marked (black arrows). A HUVEC nucleus is marked for orientation purposes (star). Scale bar = 20µm. (b) Percent transmigration was calculated by dividing the number of transmigrated cells (phase dark) by the number of adherent cells (phase bright) at room temperature for each microscopic field. Error bars indicate standard error of the mean for selected cell populations.

Summary

The BioFlux System was used to study transmigration under shear flow. The shear flow is necessary to maintain physiologically-relevant conditions for this biological process which occurs in blood vessels. We demonstrated that the BioFlux System can be used to study the dynamic process of transmigration using time-lapse microscopy under flow and post-experiment image analysis to determine migration distance. The system was also used to examine 24 different monolayers at one time, which is useful for determining IC50 concentrations under shear flow or screening multiple compounds under the same experimental conditions.

FLUXION

384 Oyster Point Blvd., #6
South San Francisco, CA 94080

T: 650.241.4777
F: 650.873.3665
TOLL FREE: 866.266.8380

www.fluxionbio.com