CELL Microsystems®

APPNOTE

Transmigration

Leukocyte transmigration through the endothelium under shear flow

Introduction

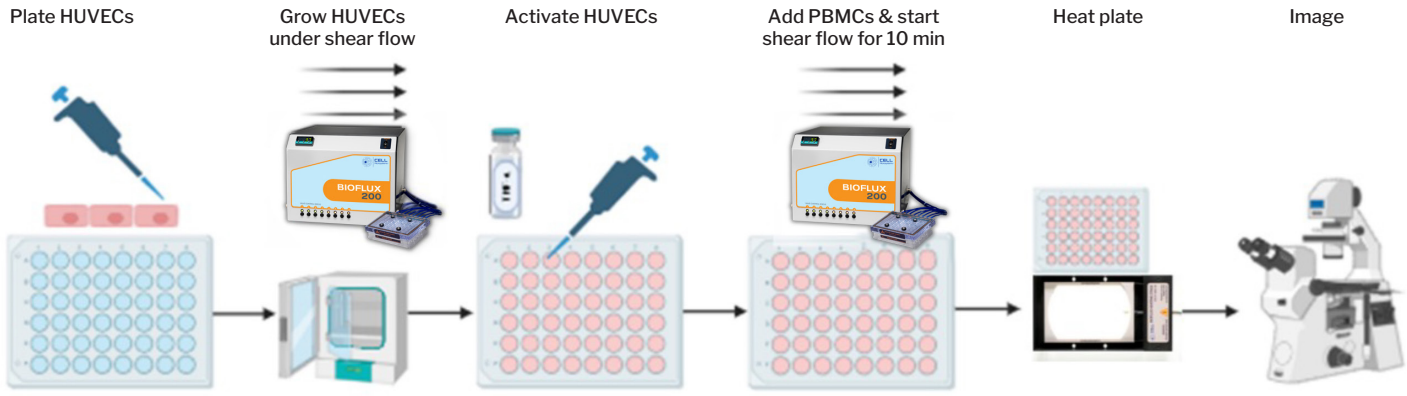
Transmigration of leukocytes from blood vessels into tissue is a vital process for normal immune function and the resolution of inflammation. However, in some disease states, such as atherosclerosis and some types of cancer, dysregulated transmigration of leukocytes contributes to chronic inflammation, subsequently increasing morbidity and mortality. Therefore, modulation of leukocyte transmigration is an important target for drug development.

BioFlux shear flow systems provide a high-throughput method to examine leukocyte transmigration in an in-vivo-like environment. In addition, because constant flow prevents the establishment of reagent concentration equilibrium across layers, BioFlux transmigration assays can be conducted over prolonged periods of time. Here, we present a model to study live cell transmigration in a physiologically relevant assay using primary endothelial cells and human peripheral blood mononuclear cells (PBMCs).

Key Highlights:

1. Image cell transmigration under shear flow conditions
2. Assess transmigration at multiple temperatures
3. Use one assay to examine cell adhesion, rolling, and transmigration

Transmigration Under Shear Flow BioFlux Workflow



Questions this App Note Answers

1. What is the procedure for assaying cellular transmigration under physiological shear flow?
2. Can experimental conditions, such as temperature, be controlled while imaging cells?
3. How can the distance of cellular movement be calculated?

Methods

Human umbilical vein endothelial cells (HUVECs) (Sciencell) were harvested at passage 4. HUVECs were subsequently grown in BioFlux plate microfluidic channels under 1 dyn/cm^2 shear flow at 37°C with 5% CO_2, to 100% confluence. HUVECs were then activated using 25 ng/mL recombinant human TNF-α (Invitrogen) for 6 hours under 1 dyn/cm^2 shear flow. Cryopreserved PBMCs (Cellular Technology, Ltd.) were prepared as follows: cells were thawed and transferred into a 50 mL conical tube, 5 mL CTL-Thaw (Cellular Technology, Ltd.) was diluted 1:20 in RPMI 1640, warmed to 37°C and slowly dripped into the cells over one minute to avoid shear-induced apoptosis. A second volume of diluted CTL-Thaw was added in the same manner over 30 seconds. Cells were centrifuged at 300 x G for 10 minutes and resuspended at 2 x 10^6 cells/mL in CO_2-independent media (Invitrogen) with 10% (v/v) fetal bovine serum.

PBMCs were then added to the activated HUVEC monolayers at 25°C at a shear rate of 1 dyn/cm^2 and allowed to attach for 10 minutes. The plate was then placed on a BioFlux heater attachment and incubated at 37°C for either 15 or 30 minutes at a shear flow rate of 0.8 dyn/cm^2. Transmigration microscopy data were captured at 30-second intervals using a QImaging Camera on a Nikon TS100 microscope. Migration distance measurements were made using the BioFlux Cell Tracking Module. 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.

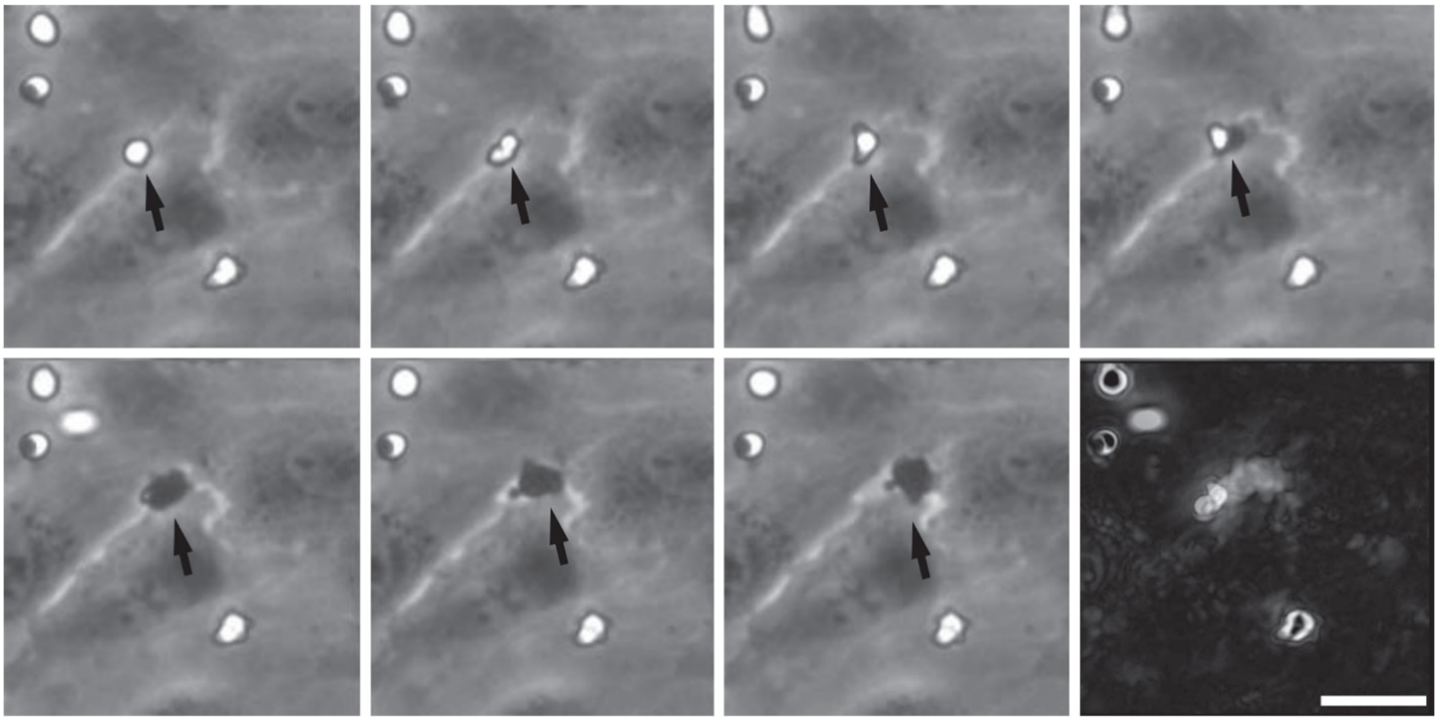


Figure 1. PBMCs undergoing transmigration. Images are shown chronologically from top left to bottom right. A Z-stack image using maximum intensity is shown to illustrate the trajectory of the cell being observed (bottom right panel). The observed cell moved a total distance of 24 µm. Scale bar = 20 µm.

Results

Transmigration was simultaneously assessed in 24 channels on TNF-α activated HUVEC monolayers. Images were captured after 10 minutes of perfusion at room temperature and again after 30 minutes at 37°C. After 15 minutes, live cell imaging showed that cells were observed both migrating and transmigrating (Figure 1) under shear flow. Average cell migration distance was 31 µm (SEM ± 4.45 µm for 25 cells). Maximum distances for slow-rolling and migrating cells were 90 and 83 µm, respectively. In fields of view 250 x 300 µm, 34% (± 13%) of cells transmigrated through the HUVEC monolayers, defined by a change in phase-contrast (phase bright to phase dark) and shape (spherical to amoeboid) (Figure 2A). Fields with lower numbers of attached cells had higher numbers of transmigration (Figure 2B). Cells in the process of transmigration moved approximately 30 µm.

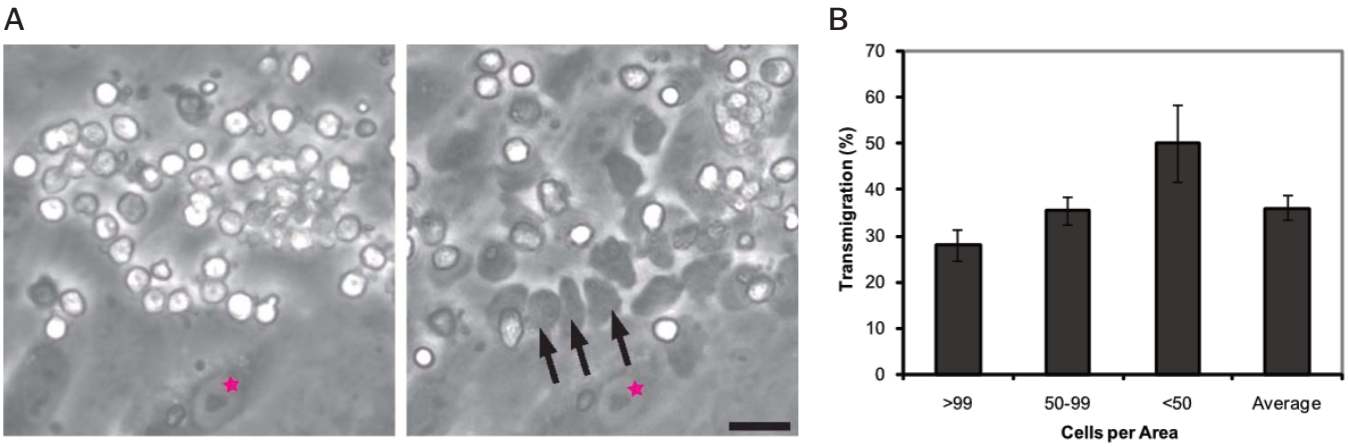


Figure 2. Adhesion and Transmigration. Basal levels of adhesion were measured by microscopy prior to shifting the temperature of the BioFlux plate to 37°C. Black arrows show representative transmigrated cells. Stars show a HUVEC nucleus for orientation (A) Scale bar = 20 µm. Percent transmigration (B). Error bars indicate standard error of the mean for selected populations.

Discussion

In this Application Note, we demonstrate that image analysis can be used to determine cellular migration and transmigration distances. The observed phenomenon of higher cell transmigration in fields with lower cell numbers attached could be a function of physical space for the cells to move or contact receptors required for transmigration. By utilizing the BioFlux microfluidic plates, 24 independent monolayers were examined by time-lapse microscopy in a single experiment. This capability for high-throughput is valuable for screening multiple compounds and/or conditions. We also show that the BioFlux Cell Tracking Module can be utilized for post-experiment image analysis to determine migration and transmigration distances over and through an endothelial monolayer. A BioFlux shear flow system is a simple and rapid method to study the dynamic process of transmigration using time-lapse microscopy under physiological shear flow.

For more information on BioFlux Technology,
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