MICROFLUIDIC ASSAY TO STUDY TRANSENDOTHELIAL MIGRATION OF HUMAN LEUKOCYTE

Sewoon Han[1*], Ji-Jing Yan[2], Yoojin Shin[1], Roger D. Kamm[3], Young-Joon Kim[2], and Seok Chung[1]

[1]Korea University, South Korea,
[2]Yonsei University, South Korea,
[3]Massachusetts Institute of Technology, USA

ABSTRACT

Most of the study on transendothelial migration has been conducted with 2D in vitro assays, which are not appropriate for high-resolution imaging of cell-cell interaction, quantification of the cell migration ability in time-dependency, and investigation of 3D cell migration. For these reasons, the complex transendothelial migration cascades have hardly ever discovered. To overcome these drawbacks of several established in vitro essays, here, we introduce the hydrogel scaffold embedded microfluidic platform. In this study, we have demonstrated the transendothelial migration of human leukocytes interacting with hMVECs monolayer with the precisely regulated chemotactic gradient of inflammatory mediators.

KEYWORDS: Microfluidics, Transendothelial migration, Extracellular matrix (ECM), 3D cell migration

INTRODUCTION

Transendothelial migration of leukocytes is a key factor to understand pathogenesis of inflammatory disorders, but the related mechanisms still remain uncertain because of the complicated signaling network among leukocytes, endothelial cells, inflammatory chemoattractants, and other factors [1].

Here, we present a novel method to enable *in vivo*-like inflammatory models in a microfluidic device, and to quantitatively measure 3D transendothelial migration of leukocytes during the inflammatory response. This method allowed us to analyze the transendothelial migration behaviors under the influence of chemoattractants and to visualize cell dynamics, the interaction among the endothelial cells, leukocytes, and the related chemotactic components.

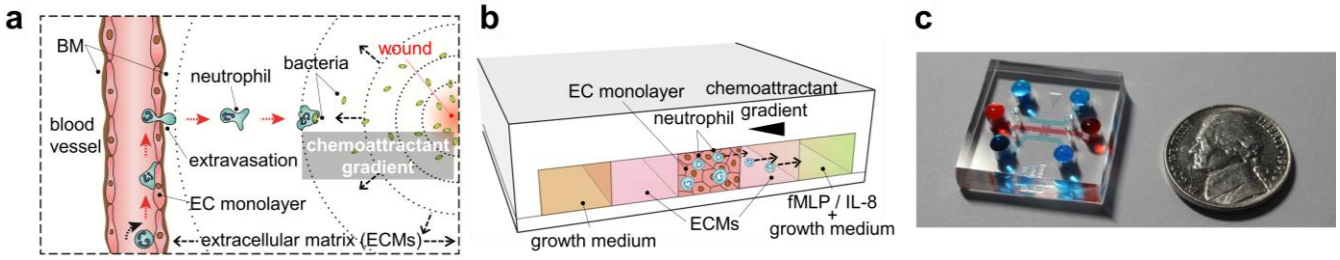

Figure 1: (a) Multistep cascades of transendothelial migration, (b). a schematic illustration of the microfluidic platform, (c). a photograph of the microfluidic platform

MATERIALS AND METHODS

Device preparation. The microfluidic device was fabricated by soft lithography. Briefly, SU-8-100 photoresist was spin-coated on a 4-inch silicon wafer. The coated wafer was then baked on a hotplate for 1 hour at 95℃. The microfluidic channels were patterned by a transparency photomask film and UV light. The UV-exposed pattern was developed in PGMEA photoresist developer. PDMS solution containing Sylgard 184 silicone elastomer base and curing agent mixture (10:1) was cured on the SU-8 100 patterned wafer for 1 hour at 80℃ in an oven. The inlets and outlets reservoirs of center/side channels were made by a 4mm biopsy punch. Also, the reservoirs for gel filling were perforated by a 1mm biopsy punch. After autoclaving the perforated PDMS and 24×24 mm glass coverslip, the patterned side of the PDMS was bonded onto the coverslip by oxygen plasma. Collagen type I, which was diluted to 2 mg/ml in 10X phosphate buffered saline and distilled deionized water, was injected into gel channels and gelated in a 37℃ and 5% CO2 incubator for 30 min. The pH of collagen determines gel stiffness to be adjusted by the volume of 0.5 N NaOH solution. The pH level of the ECM material was fixed at 11.0.

Cell differentiation and culture. Human promyelocytic HL-60 cells obtained from Korean Cell Line Bank (KCLB, South Korea) were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum, 0.1mM nonessential amino acids, 50 U/ml penicillin and 50 μg/ml streptomycin at 37℃ under a humidified 5% CO2. To induce cells to undergo differentiation along the neutrophil lineage, aliquots of HL-60 cell suspension (500,000 cells/ml) were seeded onto tissue culture flasks and grown for 5 days in the presence of 1.25% DMSO. The differentiated HL-60 (dHL-60) cells were cryopreserved at a cell con-

centration of 3,000,000 cells/ml in FBS containing DMSO (10% v/v) until seeding into the microchannel. Cryopreserved human dermal microvascular endothelial cells (hMVEC) at the third passage, endothelial cell basal medium-2 (EBM-2), media supplements (EGM-2-MV SingleQuots containing human epidermal growth factor (hEGF), hydrocortisone, gentamicin, vascular endothelial growth factor (VEGF), human fibroblast growth factor-basic (hFGF-B), R3-insulin-like growth factor-1 (R3-IGF-1), ascorbic acid, heparin, and FBS) were obtained from Lonza. HMVEC were expanded with microvascular endothelial growth media (EGM-2MV; mixture of EBM-2 and EGM-2-MV SingleQuots, Lonza, NJ, USA) on tissue culture flasks for no more than 6 passages. These hMVECs were then cryopreserved at a cell concentration of 200,000 cells/ml in EGM-2-MV containing DMSO (10% v/v) and FBS (10% v/v) until use.

HMVEC seeding into the microchannel Prior to hMVEC seeding into the microchannel, the cryopreserved vial of hMVEC was thawed in a T75 tissue culture flask, then incubated at 37℃ in a humidified CO2 incubator until the cells reach 80% confluence. Thereafter, the hMVECs were detached with 0.05% trypsin/1mM EDTA solution. HMVECs were prepared at the density of 2,000,000 cells/ml and 50 µl of the cell suspension was filled into the center microfluidic channel. The device was then placed in the incubator for 30 min for cell attachment. Afterwards, 50 µl of media droplets were placed at all inlets and outlets of the microfluidic channels for removing non-adherent cells or cell debris. For obtaining confluent endothelial monolayer in the center microfluidic channel, hMVECs were cultured for 3 days. The growth medium in the all microfluidic channels was replaced daily with fresh medium.

DHL-60 cell seeding into the microchannel The cryovial of the DHL-60 cells was quickly thawed in a 37℃ water bath for as long as it takes to melt the last ice crystals. The DHL-60 cell suspension was prepared at the density of 1,000,000 cells/ml and 100 µl of the cell suspension was filled into the cell culturing channel. Then, 50 µl media droplets containing various concentrations of fMLP or IL-8 were placed at the inlets and outlets of the side channels

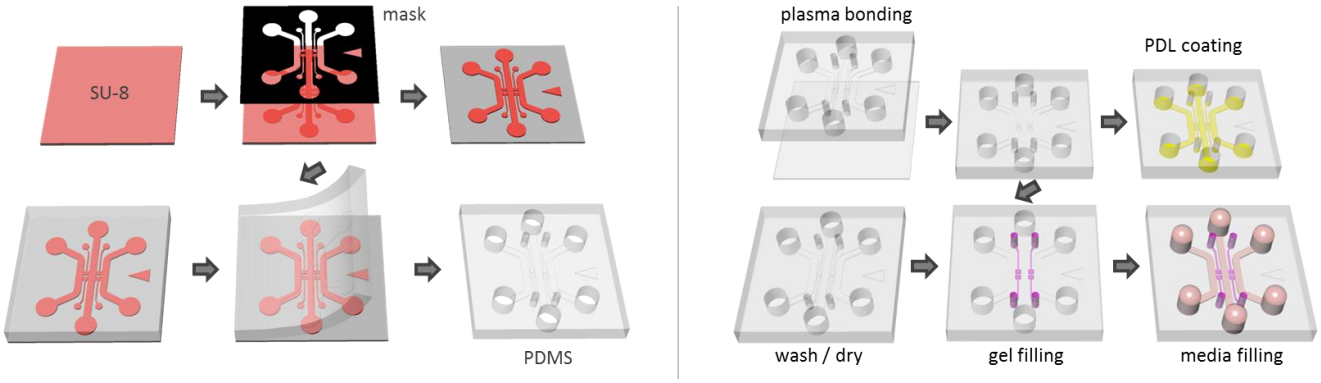

Figure 2: Schematics of soft lithography process (left), and preparation steps for the cell seeding (right)

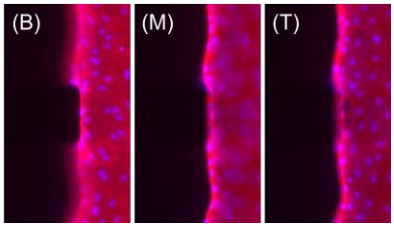

Figure 3: Z-sectioned immunofluorescence images of confluent endothelial monolayer. (B) bottom, (M) middle, and (T) top section of the microchannel

RESULTS

In order to characterize the inflammatory response of the dHL-60 to the fMLP gradient in the microenvironment, four different concentrations of the fMLP (0nM, 10 nM, 100 nM, and 1 µM) were applied to the side channel. The migration behaviors of dHL-60 cells were monitored by a phase contrast microscope at 1and 12 hours after being seeded, keeping devices in an incubator containing 5% CO2 at 37℃. Interestingly, a number of dHL-60 cells in the center channel (blood vessel channel) transmigrated toward to the condition channel with 10nM of the fMLP concentration in the existence of hMVEC monolayer. Moreover, FPR1 antibody, known as a blocker of the fMLP receptors on the surface of neutrophil, treated dHL-60

cells did show remarkably reduced chemotactic response. We confirmed the optimal concentration of fMLP on the transmigration behavior of leukocytes interacting with hMVEC monolayer.

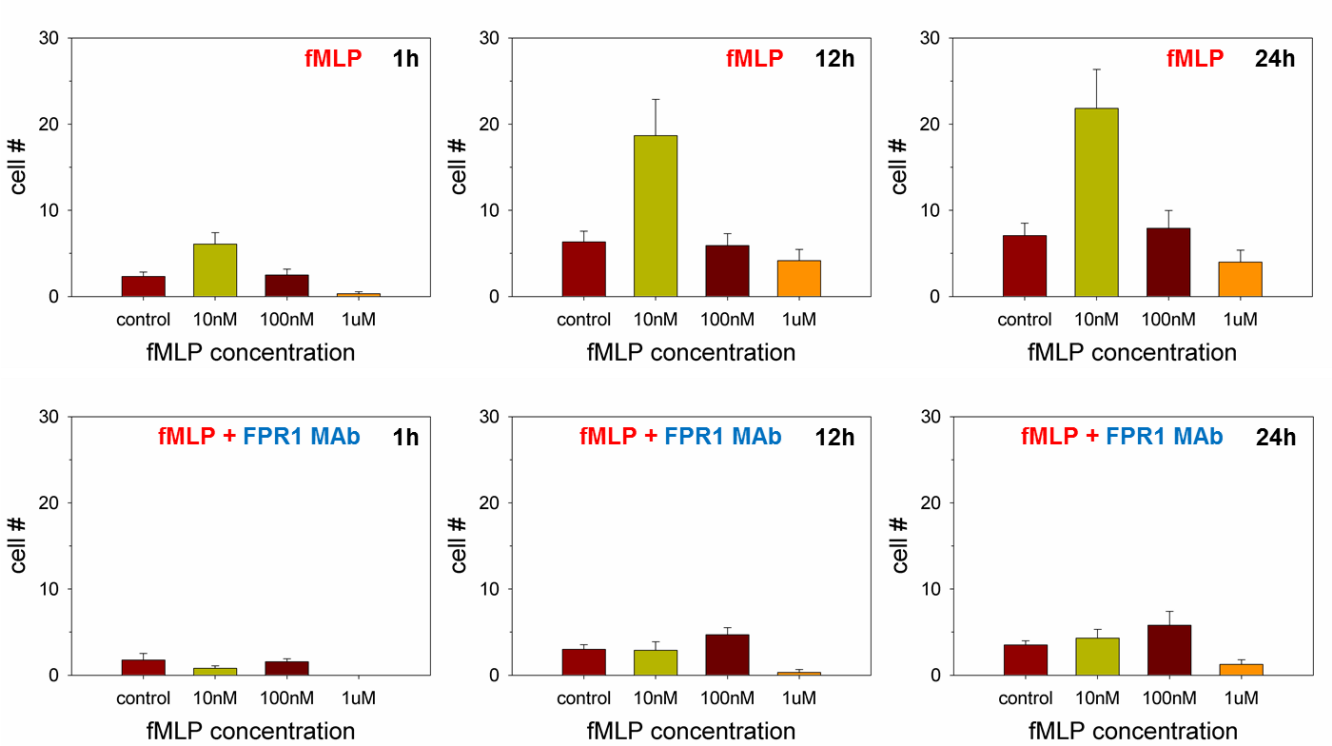

Figure 4: Total number of transmigrated dHL-60 cells into the collagen scaffold under the fMLP gradient (0, 10, 100, 1000nM) at 1h, 12h, and 24h respectively

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CONTACT

*Seok Chung, tel: +82-2-3290-3352; sidchung@gmail.com