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A microfluidic wound-healing assay for quantifying endothelial cell migration

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van der Meer AD, Vermeul K, Poot AA, Feijen J, Vermes I. A microfluidic wound-healing assay for quantifying endothelial cell migration. *Am J Physiol Heart Circ Physiol* 298: H719–H725, 2010. First published November 20, 2009; doi:10.1152/ajpheart.00933.2009.—Endothelial migration is an important process in the formation of blood vessels and the repair of damaged tissue. To study this process in the laboratory, versatile and reliable migration assays are essential. The purpose of this study was to investigate whether the microfluidic version of the conventional wound-healing assay is a useful research tool for vascular science. Endothelial cells were seeded in a 500-μm-wide microfluidic channel. After overnight incubation, cells had formed a viable and confluent monolayer. Then, a wound was generated in this monolayer by flushing the channel with three parallel fluid streams, of which the middle one contained the protease trypsin. By analyzing the closing of the wound over time, endothelial cell migration could be measured. Although the migration rate was two times lower in the microfluidic assay than in the conventional assay, an identical 1.5-times increase in migration rate was found in both assays when vascular endothelial growth factor ($VEGF_{165}$) was added. In the microfluidic wound-healing assay, a stable gradient of $VEGF_{165}$ could be generated at the wound edge. This led to a two-times increase in migration rate compared with the untreated control. Finally, when a shear stress of 1.3 Pa was applied to the wound, the migration rate increased 1.8 times. In conclusion, the microfluidic assay is a solid alternative for the conventional wound-healing assay when endothelial cell migration is measured. Moreover, it offers unique advantages, such as gradient generation and application of shear stress.

endothelial cell; migration; microfluidics; wound-healing assay; scratch assay

MIGRATION OF VASCULAR ENDOTHELIAL CELLS plays an important role in vasculogenesis and angiogenesis (6, 17, 30, 31). It is therefore also a critical process in the development of a number of diseases, such as cancer, rheumatoid arthritis, and certain retinopathies (7). Endothelial migration is affected by many intracellular pathways as well as extracellular stimuli, such as growth factors and mechanical stress (3, 27). To increase understanding of the process, chemical, physical, or biological factors are tested for their ability to stimulate or inhibit endothelial cell migration. To this end, solid and reliable in vitro assays must be available.

In the laboratory, a number of assays are used for quantification of endothelial migration (20). The two most widely used assays are the Boyden chamber assay and the wound-healing, or scratch, assay. The former assay works by seeding endothelial cells on one side of a permeable membrane that separates two culture compartments. Subsequently, the number of cells that migrate through the membrane to the other compartment is used as a measure of cell migration. The wound-healing assay works by growing endothelial cells in a confluent monolayer and physically introducing a wound by scratching the layer. Cells move into the artificially generated space to close this wound. The rate at which this happens is used to quantify cell migration. Both methods have the advantage that they are easy to use and only require standard laboratory equipment. The main advantage of the Boyden chamber assay compared with the wound-healing assay is that gradients of soluble factors can be generated by adding those factors to only one of the two compartments. On the other hand, the advantage of the wound-healing assay is that it is easy to combine with microscopy, which makes it possible to track individual cells or perform staining after quantifying migration. Microscopy is not possible in the Boyden chamber assay because the membrane is non-transparent.

The wound-healing assay is usually carried out in standard wells plates. Recently, however, a microfluidic version of the wound-healing assay was reported in literature (24). In this assay, the cells were cultured inside a microfluidic channel instead of a culture well. Because the closed-channel structure does not allow for any physical scratching of the cell layer, the wound in the cell layer was generated by introducing three parallel fluid flows into the channel. Two of these flows contained normal medium, whereas one of them contained the protease trypsin. Cells that were exposed to the trypsin-containing flow detached from the surface, whereas cells that were exposed to normal medium remained attached. Afterward, migration of cells into the artificially introduced wound could be followed and quantified just like in a regular wound-healing assay.

In the aforementioned study, the researchers focused on proof of concept. A fibroblast cell line and growth-factor stimulation were used to show the feasibility and usefulness of the microfluidic migration assay. Our aim for this study was to establish the reported microfluidic assay as a mature alternative for the conventional wound-healing assay. By evaluating the viability of endothelial cells, monolayer integrity, and cell migration rate, it will be shown that the microfluidic assay performs just as well as the conventional assay. Moreover, we wanted to highlight the advantages of using a microfluidic approach. By using the microfluidic assay, a stable gradient of growth factors can be applied to the wound. For the first time in literature, the effect of such a gradient on the wound healing rate will be described. Also, it will be shown that, in the microfluidic assay, endothelial cells can be easily subjected to shear stress while studying wound healing. Our study firmly establishes that the microfluidic wound-healing assay is a useful research tool in vascular science and will serve as a starting point for more studies by the vascular research community.

METHODS

Cell culture. Human umbilical vein endothelial cells (HUVECs; Tebu-Bio) were cultured in a humidified incubator at 37°C and 5% CO_2. The cells were overlaid with Endothelial Growth Medium-2

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(EGM-2; Lonza Benelux) in culture plates that were coated with 2 mg/ml partially purified fibronectin (Sanquin, Amsterdam, The Netherlands). When the cells reached confluence, they were detached with trypsin solution [0.05% (wt/vol) porcine trypsin, 0.02% (wt/vol) EDTA in PBS, Lonza Benelux] and replated in new flasks or used in experiments. The cells were used in experiments when they were between *passage 2* and *8*.

Microfluidic device fabrication. Microfluidic channels of poly(dimethylsiloxane) (PDMS) were prepared by pouring a 10:1 degassed mixture of base and curing agent of a Sylgard 184 elastomer kit (Dow Corning) on top of a silicon wafer with micrometer-sized SU-8 structures. These structures had the inverse shape of a long channel with dimensions 60 μm $\times$ 500 μm $\times$ 2 cm (height $\times$ width $\times$ length) separated on one side into three smaller inlet channels. After cross-linking of the network for 16 h at 60°C, the PDMS was peeled off of the mold, treated with oxygen plasma in a plasma sterilizer at 100 W for 1 min, and bonded to a glass coverslip by pressing the PDMS and glass together. Common theoretical analysis (33) of the fluid-flow profile inside the rectangular channel showed that wall effects were minimal and would only affect the row of cells closest to the walls.

Cell seeding. Before cell seeding, the microfluidic channels were coated for 2 h with fibronectin solution and then flushed with EGM-2. A $20 \cdot 10^6$ cells/ml cell suspension was prepared by harvesting cells from a culture flask with trypsin solution, pelleting the cells by centrifugation (300 *g*, 5 min), removing the supernatant, and resuspending in EGM-2. This cell suspension was pipetted into the channel, after which the microfluidic devices were overlaid with EGM-2 and incubated at 37°C and 5% CO_2. For the conventional wound-healing assay, the endothelial cells were seeded in fibronectin-coated 24-wells plates at a concentration of $40 \cdot 10^3$ cells/cm^2, overlaid with EGM-2, and placed in the incubator overnight.

Preparation of wounds. To prepare a wound, each of the three inlets of the microfluidic channel was connected to its own 5-ml syringe in a syringe pump using Tygon tubing. Two of the syringes contained endothelial basal medium (Lonza Benelux) with 2% fetal bovine serum (FBS; Lonza Benelux), and the middle syringe contained trypsin solution [0.05% (wt/vol) porcine trypsin, 0.02% (wt/vol) EDTA in PBS, Lonza Benelux]. The contents of these syringes were pushed through the microfluidic channel at a total rate of 3 ml/h for 15 min. After this, the pump was stopped, the channels were flushed with EBM-2 and 2% FBS, and placed in the incubator. When wounds were prepared in the conventional wound-healing assay, a single scratch was made in the endothelial monolayer using a micropipette tip. Subsequently, the cells were washed once with PBS and then incubated with EBM-2, 2% FBS at 37°C, 5% CO_2.

Quantifying cell migration. During the migration assay, the cells were taken out of the incubator every hour and placed under a Leica phase-contrast microscope with $\times$10 objective. Pictures of fixed positions in the wounds were taken with a Canon digital camera that was mounted on the microscope. Subsequently, the wound area in each picture was determined by outlining the wound and measuring the area using NIH ImageJ image analysis software (1). From the wound area, the average wound width could be obtained by dividing the area by the length of the analyzed region. The obtained wound widths were plotted against time in Microsoft Excel software, and a linear fit was generated for each dataset. The slope of the linear fit was used as a measure of cell migration.

Affecting cell migration with growth factors and shear stress. To study the effect of growth factor stimulation on cell migration, medium containing 100 ng/ml $VEGF_{165}$ (Sigma) was pipetted into the well or the microfluidic channel. Care was taken to introduce this medium into the channel at a low rate of several microliters per minute so as not to disturb the freshly prepared wound. In some experiments, a gradient of $VEGF_{165}$ was generated in the microfluidic channel. This was accomplished by connecting three syringes to their respective inlets. The middle syringe contained EBM-2, 2% FBS with 100 ng/ml $VEGF_{165}$, whereas the other two syringes contained only EBM-2, 2% FBS. The total flow rate through the channel was adjusted to 0.3 ml/h. The calculated shear stress (see below) on the endothelial cells caused by this flow is $<$0.2 Pa. Although a shear stress of this magnitude can be sensed by endothelial cells (25), it is not high enough to affect chemotactic migration (12).

When the effect of shear stress on endothelial migration was tested, EBM-2, 2% FBS was pushed through the channel at a rate of 2 ml/h. A theoretical estimate for the shear stress on the endothelial cells can be given by using the calculation for wall shear stress in rectangular channels: $\tau = 6Q\mu/wh^2$, where τ is shear stress in Pa, Q is flow rate in m^3/s, μ is dynamic viscosity in Pa$\cdot$s, and w and h are the width and height of the channel in m. The medium viscosity can be approximated by the viscosity of water at 37°C and was set at $7 \cdot 10^{-4}$ Pa$\cdot$s. This means that the shear stress that was applied to the endothelial monolayer was ~1.3 Pa, a value that lies within the range of physiological values (26).

Fluorescence microscopy. All fluorescent microscopy studies were carried out using a Zeiss LSM 510 confocal laser-scanning microscope. Cell viability staining was performed by incubating cells in EBM-2 with 2% FBS and a 1:1,000 dilution of both components of a Live/Dead Kit (Invitrogen) for 30 min. Viable cells were identified by green calcein staining, whereas the DNA of dead or damaged cells was labeled with red fluorescent ethidium homodimer-1.

Staining patterns for CD144 were obtained by fixating the cells for 15 min with HistoChoice fixating agent (Sigma), incubating with primary anti-CD144 antibody (Santa Cruz Biotechnology) in PBS with 1 mg/ml bovine serum albumin (Sigma) and 0.1% Triton X-100 (Sigma) for 30 min and finally incubating with Alexa488-labeled secondary antibodies (Invitrogen) for another 30 min.

When actin filaments were stained for, cells were fixated with 4% paraformaldehyde for 20 min. Subsequently, the cells were incubated with 1 μg/ml phalloidin-FITC in PBS with 1 mg/ml bovine serum albumin and 0.1% Triton X-100 for 30 min.

For estimation of the shape of a growth factor gradient, the microfluidic channels were connected to three 5-ml syringes: two containing PBS and one containing the same solution with 25 μg/ml 10-kDa dextran-rhodamine B (Invitrogen). This dextran has a diffusion coefficient of ~85 μm^2/s (4). This value lies in the same range as the diffusivity of $VEGF_{165}$ (133 μm^2/s) (21) and therefore serves as a reliable control for estimating the growth factor gradient shape (5). The total flow rate through the channels was adjusted to 0.3 ml/h, pictures were taken, and the shape of the gradient was determined by plotting the profile with NIH ImageJ image analysis software.

RESULTS

Seeding and cell viability. When seeding endothelial cells for a migration assay, it is important to cover the total culture area. If the cell density is too low, cell migration rate will be affected. As can be seen in Fig. 1*A*, when seeding endothelial cells in a microfluidic channel at a concentration of $20 \cdot 10^6$ cells/ml, they cover the entire surface after 24 h. When working with microfluidic channels, it is important to realize that there is a very limited amount of medium present in the device (no more than 100 nl in the channel itself, plus several microliters in the channel inlets). Therefore, diffusion of nutrients and waste products is limited when the channels are not constantly perfused with fresh medium. Still, to keep the protocol as simple as possible, cells were cultured under static conditions. As a result, it was necessary to study whether cell viability is affected by long-term culturing without medium perfusion. To check cell viability, we used a calcein/ethidium homodimer live/dead assay. In this assay, living cells are labeled with green fluorescent calcein, whereas DNA of dead cells is labeled with red fluorescent ethidium homodimer.

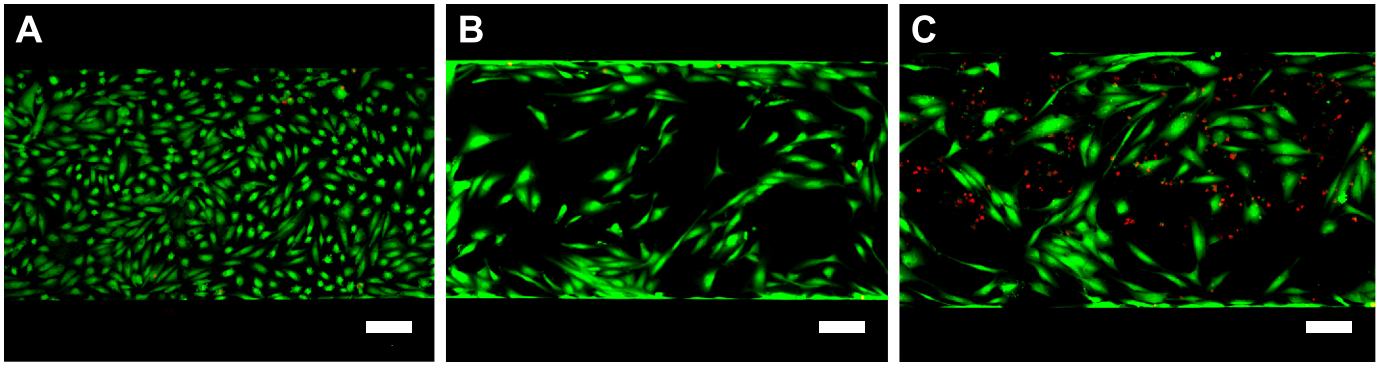

Fig. 1. Cell viability assay on HUVECs in microfluidic channels. Green fluorescence indicates living cells, whereas red fluorescence indicates dead cells. When cells are cultured inside a microfluidic channel without refreshing medium for 24 h (*A*), 48 h (*B*), or 72 h (*C*), viable cell confluency decreases over time. Scale bars, 100 μm.

Using this assay, we determined cell viability after culturing the cells inside the microfluidic channel for several periods. As can be seen in Fig. 1*A*, after culturing of HUVECs in the device for 24 h without refreshing the medium, the channel surface is still covered with viable cells. After 48 h, the number of cells in the channel has dropped dramatically (Fig. 1*B*). The remaining cells are still viable but have a stretched morphology. After 72 h, there are still some viable cells in the channel, but it also contains a lot of fragments of dead cells (Fig. 1*C*). Based on these results, we decided to perform the microfluidic wound-healing assay no more than 24 h after seeding of the endothelial cells in the microfluidic device.

Monolayer integrity. Another important parameter that needs to be controlled in a migration assay is the integrity of the endothelial monolayer (20). Endothelial cells grow in tight monolayers with extensive cell-cell contacts. These cell-cell contacts are important in endothelial cell signaling and functioning (8). It is therefore important that the endothelial cells have formed a confluent monolayer with tight cell-cell contacts before migration in a wound-healing assay is assessed. The integrity of the monolayer can be checked by staining for specific cell adhesion molecules, such as vascular endothelial cadherin (VE-cadherin; CD144). We performed fluorescent immunocytochemistry on HUVECs that were fixated at different time points after seeding to determine the cellular distribution of this adhesion molecule. As can be seen in Fig. 2, the staining of CD144 is localized exclusively at cell interfaces only after 18 h. It is therefore important to incubate overnight after seeding to allow the endothelial cells the time to form a confluent monolayer.

Wound preparation. Because the microfluidic channels are inaccessible for physical scratching, the wound was introduced by using three parallel fluid flows inside the channel. The stream in the middle contained the protease trypsin, whereas the streams on the sides contained medium with 2% serum. First, we checked whether three parallel streams could be generated. As is shown in Fig. 3*A*, the channel could be flushed with three parallel streams with negligible mixing. This is a result of the very low Reynolds number (~4) and short residence time of the medium (~0.7 s) in the microfluidic channel. The only mixing between the laminar streams takes place by diffusion, but because the whole volume of the channel is replaced more than once per second, there is not enough time for visible mixing to occur. After these parallel streams with medium and trypsin were applied to the endothelial monolayer, the middle of the channel was cleared from cells (Fig. 3*B*). In the conventional wound-healing assay, a wound was introduced in the monolayer of endothelial cells by scratching with the tip of a plastic micropipette (Fig. 3*C*). Both procedures result in similar wounds (Fig. 3, *B* and *C*).

Cell migration rate. Once a wound had been introduced in the endothelial monolayer, the migration rate could be quantified by taking pictures at fixed intervals. The wound size decreases over time, and the rate at which this happens was taken as a measure of endothelial cell migration. Microfluidic channels are compatible with phase-contrast microscopy, and it

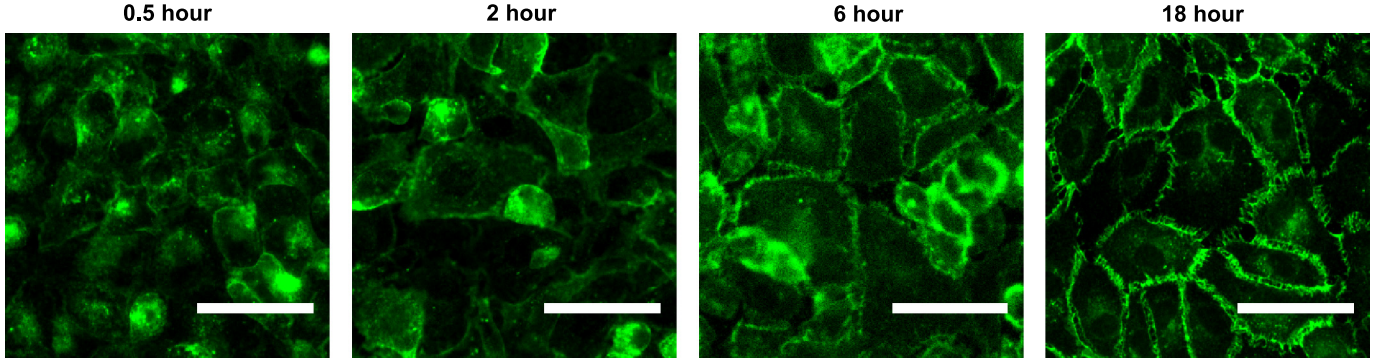

Fig. 2. Fluorescent staining for vascular endothelial cadherin at different time points after seeding. Initially, the staining is found predominantly in the cell body, whereas the staining is found almost exclusively at cell contacts after longer time periods. Scale bars, 50 μm.

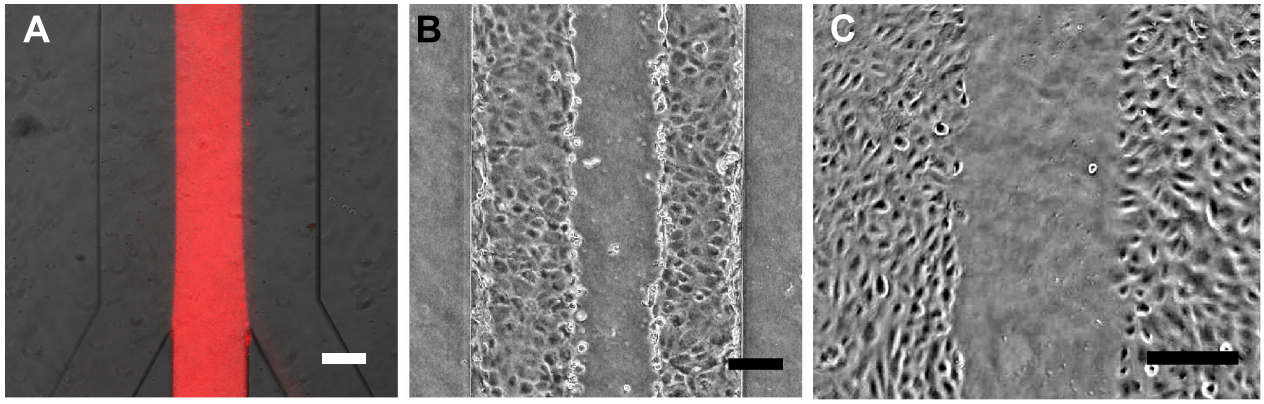

Fig. 3. Inducing artificial wounds in endothelial cell monolayers. *A*: fluorescent micrograph of a dextran-rhodamine B-containing fluid stream shows that no visible mixing of parallel fluid streams occurs in the microfluidic channel. Scale bar, 100 μm. *B*: micrograph of a wound that was prepared by treating an endothelial monolayer with parallel trypsin- and serum-containing fluid flows in a microfluidic device. Scale bar, 100 μm. *C*: micrograph of a wound that was prepared by scratching a monolayer in a wells plate with a pipette tip. Scale bar, 250 μm.

is therefore no problem to determine wound sizes (Fig. 4*A*). We found that the migration rate in microfluidic devices is stable over multiple hours, so this rate can be reliably quantified (Fig. 4*B*). We found that the average migration rate of HUVECs in the microfluidic wound-healing assay is 12 μm/h (Fig. 5). In the conventional wound-healing assay in wells plates, the average migration rate is 25 μm/h.

Growth factor stimulation. One of the most important applications of migration assays is to assess the effects of drugs and other factors on the process of endothelial migration. For the microfluidic assay to be a viable alternative for the conventional assay, the measured effects should be similar. Therefore, we compared the effect of stimulation with $VEGF_{165}$ on endothelial migration in both assays. As summarized in Fig. 5, stimulation with $VEGF_{165}$ led to an increase in migration rate from 25 to 35 μm/h in the conventional assay. In the microfluidic assay, these values were 12 and 18 μm/h, respectively. It is clear that the migration rate in the microfluidic assay is lower than in the conventional assay. However, in both cases, the addition of $VEGF_{165}$ led to a 1.5 times increase in cell migration rate.

A disadvantage of the conventional wound-healing assay is that it is impossible to generate and study the effect of gradients of growth factors. Endothelial cells do respond to growth factor gradients in determining the direction of migration (5). In the microfluidic version of the wound-healing assay, it is possible to generate these gradients. When growth factors are added to the middle fluid stream and the flow rate is sufficiently low to allow for mixing between the streams to occur by diffusion, stable growth factor gradients are realized inside the channel. To

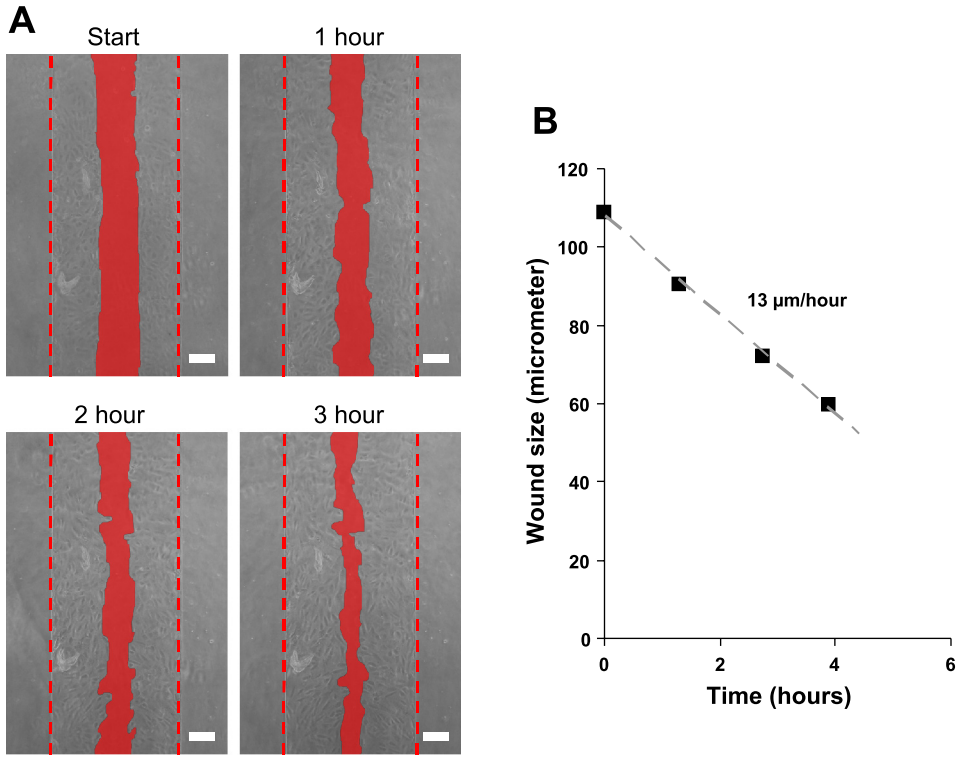

Fig. 4. Typical example of wound size analysis. *A*: micrographs of microfluidic channels at different time points after generating the wound. The wound area has been outlined and pseudocolored in red. Scale bars, 100 μm. *B*: plot of wound size vs. time. The slope of this plot is a measure of endothelial migration rate.

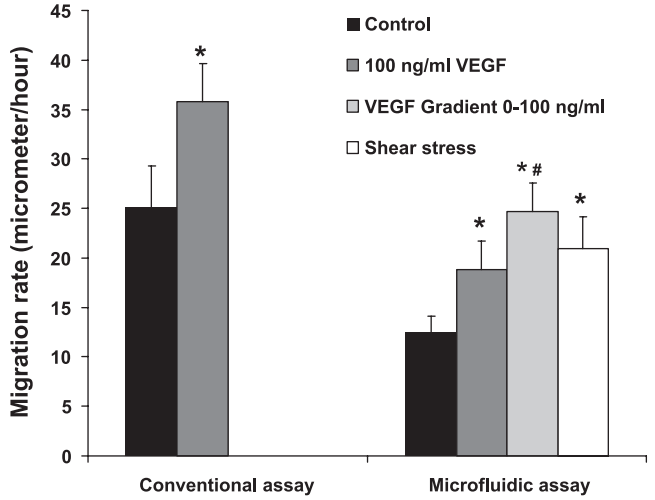

Fig. 5. Comparison of migration rates in different assays under different conditions. In the conventional and microfluidic wound-healing assay, migration was quantified under control conditions and in the presence of 100 ng/ml $VEGF_{165}$. In the microfluidic assay, migration was also quantified in the presence of a $VEGF_{165}$ gradient and while a shear stress of 1.3 Pa was applied without a $VEGF_{165}$ gradient. All bars are averages of three independent experiments. *Significant increase compared with control values (Student's *t*-test, $P < 0.05$). #Significant increase compared with 100 ng/ml $VEGF_{165}$ treatment (Student's *t*-test, $P < 0.05$).

assess the shape of such a gradient, a fluorescently labeled dextran with diffusivity similar to $VEGF_{165}$ was added to the middle stream. By checking the pattern of dextran with fluorescent microscopy and plotting the intensity of fluorescence, the shape of the gradient could be determined (Fig. 6). When a gradient with this shape was applied to a freshly prepared wound, the migration rate was significantly higher than when subjecting endothelial cells to stable concentrations of $VEGF_{165}$ (Fig. 5).

Shear stress application. In vivo, endothelial cells are constantly subjected to shear stress, which is caused by the blood flowing over their surface. When a conventional wound-healing assay is performed in wells plates, it is difficult to assess the effect of this stimulus. To perform such an assay, spinning discs need to be fitted onto the well plate (13). With the use of this equipment, a non-uniform shear stress can be applied to the monolayer. However, in the microfluidic assay, it is enough to connect the channel to a syringe pump after the wound is prepared. In this way, physiologically relevant shear stresses can be generated using flow rates from 200 μl/h to 2 ml/h. As is shown in Fig. 5, applying a shear stress of 1.3 Pa leads to a significant increase in migration rate. Moreover, cell morphology during migration is different (Fig. 7). Staining of actin filaments shows that cells migrate into the wound along with the direction of the applied shear stress. Under static conditions, the pattern of actin filaments and the direction of migration are more random.

DISCUSSION

Based on the results in this study, it can be concluded that the microfluidic wound-healing assay is a good alternative for the conventional wound-healing assay in wells plates. Endothelial cells can be seeded in the microfluidic channel and form a tight monolayer of viable cells after overnight incubation. Wounds can be prepared by introducing three parallel fluid streams, one of which contains the protease trypsin. The rate of wound healing can be tracked over time and can be used to measure endothelial cell migration. We found the migration rate in the microfluidic assay to be about two times lower than in the conventional assay. This can be explained by the fact that the underlying substrates are completely different: in the conventional assay, cells migrate onto a tissue culture-treated polystyrene surface, whereas in the microfluidic assay the surface consists of trypsin-treated glass. In previous studies on endothelial cell migration, it has been found that both the average migration speed and the persistence of migration direction are higher on tissue culture-treated polystyrene than on glass (14–16). Moreover, the results of an experiment with the conventional assay in which we compared wound healing rates on both tissue culture-treated polystyrene and glass coverslips confirmed these differences (data not shown). It should be noted that these differences in migration rate are not a problem for the applicability of the microfluidic assay. Migration assays are used to study the effects of stimulants or inhibitors on migration rate. So, as long as the effects of adding drugs or growth factors are the same in both assays, the microfluidic assay is still a good alternative for the conventional assay. We tested this by adding the growth factor $VEGF_{165}$ during wound healing in both assays. The stimulatory effect of $VEGF_{165}$ on endothelial

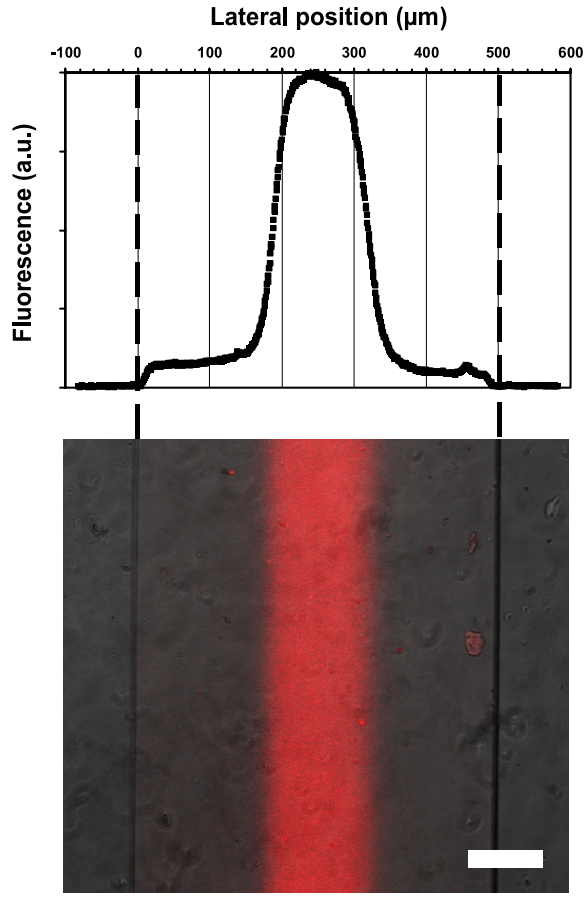

Fig. 6. Shape of a stable gradient in the microfluidic channel. *Bottom*: fluorescence micrograph of a microfluidic channel with rhodamine B-labeled, 10-kDa dextran in the middle stream and PBS in the other two streams. Scale bar, 100 μm. *Top*: plot of relative concentrations vs. lateral position in the channel. Relative concentrations were determined by analyzing the average fluorescence intensity of every lateral position in the fluorescent micrograph.

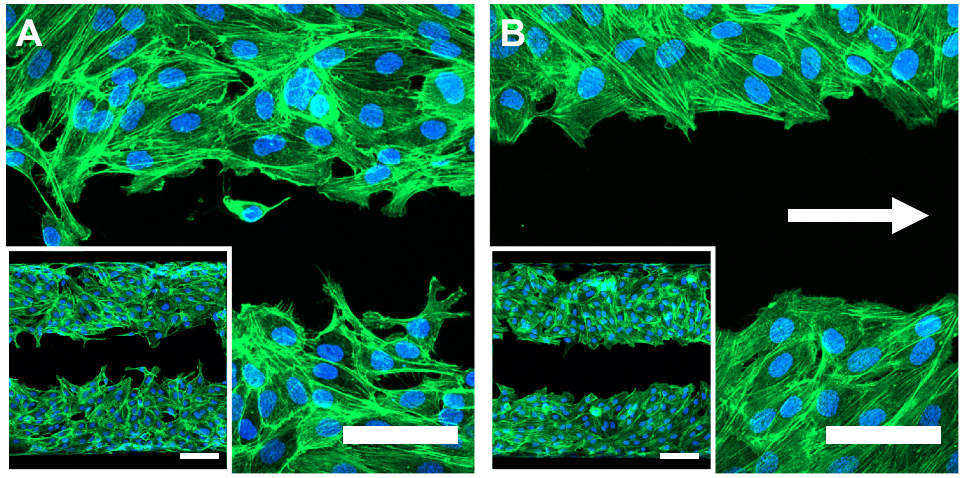

Fig. 7. Actin filament staining of migrating endothelial cells under static and sheared conditions. *A*: endothelial cells under static conditions migrate randomly into the wounded area. *B*: cells that are being sheared orient their actin filaments along with the direction of the applied stress (arrow). Scale bars of close-ups, 50 μm. Scale bars of *insets*, 100 μm.

migration is well established and has been shown numerous times in literature when a wound-healing assay was used (2, 10, 22). In our experiments, the effect of adding $VEGF_{165}$ was both qualitatively and quantitatively the same in both the conventional assay and the microfluidic assay. We found a 1.5 times increase in migration rate when this growth factor was added, confirming both the known effect of $VEGF_{165}$ and validating the microfluidic assay as an alternative for the conventional assay.

Gradients of VEGF are important in the embryonic development of new blood vessels as well as the formation of new blood vessels in adult tissue (29). To generate more mechanistic insight into these processes on a cellular level, in vitro assays can be very useful. Using a Boyden chamber assay, endothelial migration in gradients of VEGF can be quantified (28). However, in this assay, it is impossible to control the shape of the gradient or to visualize cells as they migrate into the gradient. Using microfluidics, it is possible to generate stable and tunable gradients of growth factors in devices that are compatible with microscopy (18). Recently, this property of microfluidics was used to study chemotaxis of individual endothelial cells in gradients of $VEGF_{165}$ (5). The main finding of this study was that directed migration can be induced by applying gradients of $VEGF_{165}$ and that the shape of the gradient dictates the strength of the chemotactic response. The steepest gradient, starting at 0 ng/ml and increasing up to 50 ng/ml $VEGF_{165}$ over a distance of 400 μm was found to induce the strongest migratory response. Our study is the first to assess the effect of a growth factor gradient on migration rate in a wound-healing assay. The gradients that we applied increased from 0 to 100 ng/ml $VEGF_{165}$ over a distance of 150 μm. Because the gradients form at the interface of two parallel streams, they are located exactly on the edge of the induced wound. We found an increase of 30% in migration rate when applying these gradients instead of a fixed concentration of $VEGF_{165}$ of 100 ng/ml to the endothelial cells. This increase is probably due to an increased VEGF-mediated activation of migration-inducing signal transduction pathways on the side of the cell that is facing the wound (23).

In vivo, endothelial cells are constantly subjected to shear stress that is caused by blood flowing over their surface. This shear stress is an important stimulus in endothelial cell functioning and has a big impact on blood vessel physiology (19). There are a number of reports in literature on the effects of shear stress on endothelial migration rates in a wound-healing assay (3, 11). The principal finding of these studies was that shear stress enhances endothelial wound healing in vitro. This stimulating effect on endothelial migration is mediated by an enhanced activation of the Rho family of small GTPases (11, 32). A secondary finding of these studies was that endothelial cells migrate along with the shear stress direction during wound healing. The increased wound healing rates are caused by this enhanced directional migration. Also in our study, we find that physiological levels of shear stress enhance wound healing. Moreover, morphological analysis of the migrating endothelial cells shows that they tend to orient their cytoskeletal components in the shear stress direction.

In conclusion, the microfluidic wound-healing assay for endothelial cells that we describe in this study is a good alternative for the conventional wound-healing assay. The microfluidic assay can be considered to be a highly useful laboratory tool for studying a lot of facets of endothelial migration. Moreover, the microfluidic version of the assay has some unique advantages compared with its conventional counterpart. Because of the small size, small amounts of cells and reagents are needed. This is especially useful when working with rare cell material (e.g., patient material) or expensive drugs. Furthermore, other cell-based applications of microfluidic technology are under active development (9). This means that, in the future, the microfluidic wound-healing assay may be parallelized or combined with other microfluidic analysis tools in the same device, the so-called "lab on a chip" concept (34). Another advantage of the microfluidic wound-healing assay is that it is compatible with high-magnification microscopy. Moreover, stable and tunable gradients of growth factors and drugs can be generated, and physiologically relevant shear stresses can easily be produced while studying migration. Given all these advantages, it will be interesting to see what other novel insights in vascular science may be produced using this new laboratory tool in the future.

GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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