A novel microfluidic platform for studying mammalian cell chemotaxis in different oxygen environments under zero-flow conditions

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The cell's micro-environment plays an important role in various physiological and pathological phenomena. To better investigate *in vivo* cellular behaviors, researchers have expended great effort in building controlled *in vitro* biophysical and biochemical environments. Because a cell's gaseous environment affects properties such as its division, metastasis, and differentiation, we developed a zero-flow based platform for studying mammalian cell chemotaxis behavior in different oxygen environments. This platform can construct a linear range of oxygen tensions within one chip (i.e., from 1.4% to 3.6% or 5.5% to 14.5%). To study cell chemotaxis behavior under varying oxygen environments, the chemical gradient direction is established perpendicularly to oxygen change within an observation area. Because the observation area is not subject to flow, shear force is of no concern. In addition, water flow around the cell chambers greatly reduces evaporation and makes long-term microscope imaging possible. In this study, we precisely measure the chemotaxis velocity of MCF-7 human breast cancer cells under different oxygen tension conditions towards CXCL12, which is a stromal cell-derived factor. We find that cell migration rates are not equivalent, even under two close oxygen tensions. We also observed that cells move faster towards high concentrations of chemoattractant when the oxygen tension is below 3% due to the increased expression of HIF-1 (hypoxia-inducible factor 1), which promotes a transition to the amoeboid rather than mesenchymal mode of movement. Our experiments demonstrate that this new microfluidic platform is useful for the quantitative study of mammalian cell chemotaxis under different oxygen conditions in the absence of shear force. We also shed light on the study of chemotaxis under other gaseous environments. © 2015 AIP Publishing LLC.

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INTRODUCTION

A major cause of tumor recurrence and treatment failure in cancer patients is metastasis,^{1–4} which encompasses epithelial mesenchymal transition, intravasation, survival in circulation, extravasation, and angiogenesis.^{5,6} Interestingly, different types of cancer preferentially form different metastatic lesions,^{7,8} and it has been proposed that metastatic cancer cells are directed to their target organs by soluble factors released by the target.^{9,10} For example, stromal cell-derived factor (SDF-1 α , or CXCL12) is constitutively produced by bone marrow, lymph nodes, liver, and lung.¹¹ Through binding to the CXCR4 receptor, which is upregulated in breast tumor

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cells,¹² CXCL12 leads to the attraction of breast cancer metastases to the target organs. Molecular studies have also shown that the expression of CXCR4^{13,14} and adhesion between cells in the tumor may be regulated by the hypoxia-inducible factor 1 (HIF-1),^{15,16} which is expressed due to the prevention of oxygen diffusion, which in turn is caused by sluggish blood flow through tortuous tumor vessels and the large size of the tumor.^{17–19} Thus, hypoxia can promote cancer cell metastasis. Most laboratory studies are carried out under atmospheric conditions. In the human body, however, cells encounter a wide range of oxygen tensions.^{20,21} For instance, the oxygen level in a normal brain ranges from 5% to 10%, and in alveoli is approximately 14%.²² To investigate the relationship between fine oxygen environment and cell behavior, we developed a microfluidic platform in which cell chemotaxis experiments can be performed in parallel under a range of oxygen tensions.

Microfluidics has become a crucial tool for cell biology research over the past decade.²³ Its advantages include the precise manipulation of fluid flow, fast response time, high detection sensitivity, low reagent consumption, and a high throughput.²⁴ The key benefit of the microfluidics technique for quantitative biological research is its ability to control parameters of the cell microenvironment at a high spatiotemporal resolution.²⁵ In living organisms, cells reside in a milieu of physical and chemical signals, such as shear force, cell-cell contact, growth factors, chemokines, temperature, oxygen tension, and pH. To better reproduce physiological and pathological phenomena in a microfluidic device, researchers have made great efforts to mimic cellular microenvironments.^{25–28} Many microfluidic-based devices designed to explore the effect of oxygen tension on cell migration have been reported.^{29–36} In one example, a three-dimensional microfluidic cell culture system featuring a controlled hypoxia environment was developed, a central 3D gel region acting as an external cellular matrix, and peripheral gas channel on each side establishing oxygen environment.²⁹ However, it was only used to study human cancer cells movement under two oxygen tension conditions (0% and 20%), and the movement of the cells are randomly walk rather than directional chemotaxis. Furthermore, spatially confined chemical reactions have been created on microfluidic chips to establish oxygen gradients,³⁰ and mixed chemical and oxygen gradients were developed to investigate cell migration in complex microenvironments.³¹ This microfluidic device consists of two PDMS (polydimethylsiloxane) layers with microfluidic channel patterns: the top layer generated an oxygen gradient, and the bottom layer created chemical gradient. Cell migration assay in the downstream was conducted under various combinations of chemical and oxygen gradients in the experiments. However, due to the constant medium flow, cells were exposed to continuous shear force, which is not a physiological condition for most cells *in vivo*. Moreover, studies have shown that even under low shear conditions, the direction of cell migration was influenced by flow.³⁷

Thus, we introduce a microfluidic platform to quantitatively study the directional migration of mammalian cells under different oxygen microenvironments in zero-flow channels. Chemoattractant gradient direction is established by diffusion perpendicular to the direction of oxygen change, which allows us to investigate cell directional migration under various oxygen tensions. The migration behavior of a human breast cancer cell line (MCF-7) towards an attractant CXCL12 gradient under different oxygen tensions was measured in this system. We find that the oxygen tension exerts a threshold effect on the directional movement of the MCF-7 cell. When the oxygen tension falls below 3%, the cells' direct movement increases significantly. Analyzing a single cell's migration every 15 min under both hypoxia and non-hypoxia conditions, we find out the reason for high speed under hypoxia is that cells prefer to chemotaxis by amoeboid mode in such situation. Our experiments indicate that this microfluidic device provides a promising platform for integrating changes in both the gaseous environment and chemical gradient in the absence shear force to study cellular behaviors such as chemotaxis.

MATERIALS AND METHODS

Microfluidic device design

The microfluidic chip is shown filled with colored inks in Fig. 1(a). The device consists primarily of three parts: gas channels (yellow), water channels (blue), and cell/medium channels

(red). The gas channels are $1500\ \mu\text{m}$ wide and are located on two sides of the chip. By adjusting the oxygen concentration supplied by different gas mixtures to these gas channels, different oxygen microenvironments can be achieved across the chip. To avoid moisture loss due to gas flow, we designed wide gas channels to reduce the gas flow rate while maintaining stable oxygen concentrations. Moreover, we added water channels between the gas and cell/medium channels to prevent medium evaporation. Both the gas and water channels are $150\ \mu\text{m}$ high. Cell/medium channels contain 7×4 cell migration channel units that are supplied by a main medium channel. The oxygen tension in the chip can be made to vary from left to right, thus enabling the measurement of cell migration in 7 different oxygen environments, with four repeats for each condition. The seven migration channel rows are named “point1” through “point7,” from left to right. Every cell migration unit consists of four parts: a migration channel, a cell-loading cavity, parallel palisading channels, and a liquid-absorbing cavity³⁸ (Fig. 1(b)). The parallel palisading channels connect the cell-loading cavity and the liquid-absorbing cavity. They are $5\ \mu\text{m}$ wide and $5\ \mu\text{m}$ high, which is sufficiently narrow to gather cells into the loading cavity. The migration channel and cell-loading cavities are $20\ \mu\text{m}$ high, and the liquid-absorbing cavities and the main channel are $150\ \mu\text{m}$ high. To guarantee that the oxygen tension profile in the chip is not influenced by the ambient environment, we employed a PDMS-PVDC-PDMS sandwich structure (Fig. 1(c)). PVDC (polyvinylidene chloride) film has a relatively low oxygen permeability $3.5 \times 10^{-10}\ \text{cm}^2/\text{s}$, and its embedded fabrication procedure was demonstrated in our previous study.³⁹

Cell migration in different oxygen environments

Human breast cancer MCF-7 cells were purchased from the America Type Culture Collection (ATCC) and were cultured in an incubator at 37°C in 5% CO_2 , using Dulbecco's Modified Eagle Medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Invitrogen) and 1% penicillin and streptomycin (Invitrogen). Cells were dissociated with a 0.25% Trypsin-EDTA solution at 37°C for 3 min. After centrifugation, cells were washed in culture medium without FBS and re-suspended in culture medium with 1% FBS and 10 ng/ml Epidermal Growth Factor (EGF; Invitrogen). After degassing for 15 min at 10–100 Pa, we loaded a $1 \times 10^7/\text{ml}$ cell suspension into the cell/medium main channel of the chip. Due to gas absorption by the PDMS surface, the cell suspension is passively aspirated through the cell migration channel and into micro-cavities.⁴⁰ After approximately 10 min, cells had gathered within the cell-loading cavity, and both cell-loading cavity and liquid-absorbing cavity were

FIG. 1. Microfluidic device capable of generating a chemical gradient under different oxygen environments. (a) Photograph of the fabricated device filled with colored inks. (b) A schematic of a chemical gradient in the migration channel following the addition of an attractant. (c) A cross-sectional view illustrating the PDMS-PVDC-PDMS sandwich structure of the chip. (d) Numerical simulation of the oxygen profile in the microfluidic device after supplying nitrogen on the left side and a 20% (or 5%) oxygen gas mixture on the right side.

fully filled by cell medium solution. Then, the microfluidic chip was returned to the incubator (37 °C, 5% CO₂) for 4 h to allow cells to adhere to its surface. We then flush away any residual cells in the main channel with the same culture medium supplemented with 1% FBS and 10 ng/ml EGF, then replace the main channel with culture medium containing 1% FBS, 10 ng/ml EGF, and 600 ng/ml CXCL12 (Invitrogen). Thus, a CXCL12 gradient begins to form in each 7 × 4 migration channel. We mount this chip on a BioStation IM (Nikon) observation stage, which provides a humidified atmosphere and is maintained at 37 °C for long-term time-lapse monitoring. Given that most mammalian tissues exist at 3%–9% O₂ and hypoxia generally occurs below 3%,⁴¹ 3 oxygen gas mixtures were used in our experiments: 0% oxygen gas mixture contains 95% nitrogen and 5% CO₂; 5% oxygen gas mixture contains 5% oxygen, 90% nitrogen, and 5% CO₂; and 20% oxygen gas mixture contains 95% air and 5% CO₂. When we independently supply the gas channels with 0% and 5% oxygen or 0% and 20% oxygen, a low/high oxygen tension profile forms in the chip three hours later. In addition, we supply both sides with a 20% oxygen gas mixture; thus, we can measure cell migration velocity under atmospheric conditions similar to ambient conditions in the laboratory. We captured 20× photographs of each migration channel every 15 min after a 3-h incubation period in the gas. The manual tracking function in ImageJ is used to record the trajectory of every migratory cell in the channel. We then calculate the average speed of every motile MCF-7 cell towards high CXCL12 concentrations under different oxygen conditions.

Chemical gradient validation

We used fluorescein (Sigma-Aldrich) to calibrate the generation of chemical gradients in the migration channel. After filling the cell/medium channel with water, 100 μM fluorescein was added to the main medium channel and fluorescence images were immediately captured using a 4× objective lens and QuantEM512SC CCD (Roper Scientific) every 15 min. We used the ImageJ software to measure fluorescence intensity along the central axis of the migration channel and to generate a time-dependent fluorescence gradient establishing curve. Because fluorescein and CXCL12 have different diffusion constants and because the diffusion time is inversely proportional to diffusion constant, we can estimate the establishment and duration of the CXCL12 gradient based on the fluorescein gradient curve.

Oxygen profile validation

To experimentally validate the oxygen profile within the device, the oxygen-sensitive fluorescent dye ruthenium tris(2, 2'-bipyridil) dichloride hexahydrate (RTDP) (Sigma-Aldrich) was used as an indicator to measure oxygen tension at every migration channel. We filled the cell/medium channel with RTDP solution, and flowed water through the water channels at 40 μl/h; the gas conditions changed over time, as shown in Table I. The fluorescence intensities at every liquid-absorbing cavity were recorded above the migration channel every 15 min using a QuantEM512SC CCD (Roper Scientific). Regions of interest (ROIs) were designated as 90 μm × 90 μm regions in the middle of the observation area. Then, the oxygen tension at every site was calculated based on the Stern-Volmer equation

$$\frac{I_0}{I} = 1 + K_q[O_2],$$

where I is the fluorescence intensity of RTDP molecules in the presence of oxygen, I_0 is the intensity in the absence of oxygen, and K_q is the Stern-Volmer quenching constant. We use I_0 , I_5 , and I_{20} , which are the fluorescence intensities measured at both 3 and 6 h under low and high oxygen tensions, for calibration. Under low oxygen tension, the Stern-Volmer quenching constant can be calculated as follows:

$$K_q = \frac{I_0 - I_5}{I_5[5\%]} - 1$$

TABLE I. Gas conditions for oxygen profile validation.

Oxygen profile		Low concentration		High concentration	
Gas side		Left	Right	Left	Right
O ₂ (%)	0–3 h	0	0	0	0
	3–6 h	5	5	20	20
	6–9 h	0	5	0	20
	9–12 h	5	0	20	0

whereas under high oxygen tension,

$$K_q = \frac{I_0 - I_{20}}{I_{20}[20\%]} - 1$$

is used. Hence, we calculate the local oxygen tension at every migration channel with the following formula:

$$[O_2] = \frac{1}{K_q} \left(\frac{I_0}{I} - 1 \right).$$

Simulation of chemical gradients and oxygen profiles in the microfluidic device

To better predict the theoretical chemical gradient and oxygen profile in the microfluidic device, we employed the COMSOL Multiphysics 4.2 software to model and simulate the conditions. Using a model of the transport of diluted species, we calculated the chemical gradient and oxygen profile as a function of time after changing the chemical solution or gas flow. For changes in the chemical gradient, we only considered diffusion due to the static medium in main channel (diffusion constant of fluorescein = $500 \mu\text{m}^2 \text{s}^{-1}$).⁴² To establish a timeline of the oxygen profile, we set the ambient environment and the initial oxygen tension in the chip to 20% O₂, with the exception of the gas channels. The oxygen concentration in the gas channels was set to 0%, 5%, or 20%. Water containing 20% O₂ was supplied from two inlets. The parameters used in the simulation are shown in Table II. For the water flow test, we changed the water flow rate from 0 $\mu\text{l/h}$ to 80 $\mu\text{l/h}$ to characterize its influence on the steady-state oxygen profile in the chip.

RESULTS AND DISCUSSION

Chemical gradient characteristics in a cell migration channel

The chemical gradient in migration channels was validated using fluorescein, and fluorescence images were captured every 15 min after the gradient was established (Fig. 2(a)). The ImageJ software was used to measure the fluorescence intensity along the central axis of the migration channel. Changes to the gradient within the first 4 h are described by a solid line in Fig. 2(b). We also simulated gradient change over time; these data are plotted as dashed lines in Fig. 2(b). This analysis shows that a chemical gradient can be established in a zero-flow migration channel within 1 hour and that it lasts for at least 4 h before decreasing gradually over time (supplementary material).⁴³ The results of the simulation and our experiment data match well. The diffusion constant of fluorescein is $500 \mu\text{m}^2 \text{s}^{-1}$, whereas that of the chemoattractant CXCL12 (8.0 kDa) is approximately $100 \mu\text{m}^2 \text{s}^{-1}$.⁴⁴ Because the diffusion time is inversely proportional to the diffusion constant, we could approximate the CXCL12 gradient based on our fluorescein data over 20 h, which is a sufficient amount of time to assay mammalian cell migration under different oxygen conditions.

TABLE II. Parameters for oxygen profile simulation.

Flux_water	40 ($\mu\text{l/h}$)
Flux_gas	3 (ml/min)
Height_channel	100 (μm)
Height_PDMS	1000 (μm)
Height_PVDC	10 (μm)
c_air	20%
c_O ₂ low	5%
c_N ₂	0%
Width_water channel	300 (μm)
Width_gas channel	1500 (μm)
Velocity_water	Flux_water/(height_channel $\times$ width_water)
Velocity_gas	Flux_gas/(height_channel $\times$ width_gas)
O ₂ diffusion in PDMS	3.4×10^{-9} (m ² /s)
O ₂ diffusion in water	3.0×10^{-9} (m ² /s)
O ₂ diffusion in PVDC	3.5×10^{-14} (m ² /s)

Oxygen profiles in the microfluidic device

The oxygen tension distribution in the microfluidic device plays an important role in the analysis. We focused our analysis on the time needed to reach gas equilibrium, the variability of the gas profile in the chip, and the absolute oxygen tension at every observation point.

In a COMSOL simulation, when we set the oxygen environment of the chip to 5% or 20% and flowed a 0% oxygen mixture through one of the gas channels, a new stable oxygen tension profile was established at approximately 3 h (dashed lines in Figs. 3(a) and 3(b)). This result implies that the time required to establish a steady oxygen profile in this device is

FIG. 2. Validation of chemical gradients in a migration channel. (a) Fluorescence images of a cell migration channel and part of the main medium channel 15 min after adding fluorescein. The white dashed line represents the central axis of the migration channel, where we measured fluorescence intensity. (b) Comparison of the simulated (dashed lines) and experimental (solid lines) fluorescence concentration profiling in a cell migration channel at 1 h, 2 h, 3 h, and 4 h after adding fluorescein.

approximately 3 h. Thus, we carried out an oxygen concentration profile validation protocol (Table I) that lasted for 3 h for every oxygen distribution scenario. Figs. 3(a) and 3(b) show that 3 h is sufficient for the establishment of a linear oxygen profile in the chip, in agreement with numerical simulation results. To test the variability of gas composition in the chip, we switched the gas mixture at the 9th hour and recorded data at several observation points (Fig. 3(c)). We plotted the calculated oxygen tension of the ROI as a function of time (solid line in Figs. 3(d) and 3(e)). As expected, the oxygen tension distribution changed immediately and the oxygen tension in every channel gradually approached its respective theoretical value. Under low oxygen tension, the steady oxygen tension values at individual channels were 1.4%, 1.8%, 2.1%, 2.5%, 2.9%, 3.3%, and 3.6%, whereas under high oxygen tension, they were 5.5%, 7%, 8.5%, 10%, 11.5%, 13%, and 14.5%.

MCF-7 cell migration in different oxygen environments

After establishing that this microfluidic platform equilibrates to a stable gaseous profile within 3 h and that the chemoattractant gradients in different gaseous environments can last for over 20 h in the absence of shear force, we performed an MCF-7 breast cancer cell migration experiment to demonstrate the utility of the platform for the study of mammalian cell chemotaxis in different oxygen environments.

FIG. 3. Validation of the oxygen profile within the device. (a) and (b) Comparison of the simulated (dashed lines) and experimental (solid lines) oxygen tension at every point at 0 h, 1 h, 2 h, and 3 h after supplying two gas channels with a 0% and 5% oxygen gas mixture or with 0% and 20% oxygen gas mixture. (c) RTDP fluorescence images at point1 and point7 of the reservoir 3 h after a gas switch at time points 9 h and 12 h. (d) and (e) The oxygen profile change in the low concentration and high concentration ranges after gas switch in Table I from 9 to 12 h. Dashed lines present theoretical stationary values calculated using the COMSOL Multiphysics software.

Fig. 4(a) shows the micrograph of motile MCF-7 cells in a migration channel taken with a large image combination strategy to capture the entire channel. Colored lines indicate cell trajectories plotted using the ImageJ manual tracking function, and a cell chemotaxis movie is provided in the supplementary material.⁴³ We define the velocity of single cell as the average velocity of its steps. The amount of steps used for calculating the average depends on the total time of cell motion. Before division, a moving cell will slow down and stop; we ignore these steps to prevent interference. After division, daughter cells begin to move, and we count these as new cells. By including the migration velocities of motile cells in parallel migration channels under the same oxygen tension from at least three experiments, we can obtain a cell migration velocity distribution for a specific oxygen tension. In the absence of a CXCL12 gradient in the migration channel, MCF-7 cells extend pseudopodia randomly (supplementary material).⁴³ Fig. 4(b) shows the cell migration velocity distribution at 1.4% and 7% oxygen tension (over 30 cells analyzed). Their mean velocities towards high CXCL12 concentration along the migration channel (X axis) were approximately $0.76 \mu\text{m}/\text{min}$ (1.4% O_2) and $0.47 \mu\text{m}/\text{min}$ (7% O_2), whereas their movement velocities perpendicular to the chemical gradient (Y axis) were distributed nearly equally on each side of the central line. This result indicates that the cell movement in our platform is directional migration. For each oxygen tension, we analyzed the migration velocity, as shown in Fig. 4(b), and plotted V_x under different oxygen tensions in a box chart (Fig. 4(c)). We find that the cell chemotaxis rates are not equivalent, even under two similar oxygen tensions. Thus, it is crucial that the effect of different oxygen tensions on a cell be investigated to determine how the gaseous microenvironment regulates cell behaviors. When the oxygen tension fell below 3% (hypoxia), the cell migration velocity increased significantly compared with high oxygen conditions. This finding is consistent with previous research

FIG. 4. Mammalian cell migration under different oxygen conditions. (a) Manual tracking trajectories of motile cells in a migration channel. (b) Velocity distribution of migratory cells under 1.4% and 7% oxygen tension. (c) Box chart depiction of V_x under different oxygen tensions. (d) Comparison of the mean V_x of migrating cells under different oxygen tensions (<3%, 3%–10%, and 10%–20%). Error bars represent standard deviation, *** $p < 0.005$.

demonstrating the important role played by hypoxia in the development of metastatic disease.⁵ Our ability to take precise measurements will allow for the clear determination of mammalian cell migration rates under different oxygen tensions. Given that most mammalian tissues exist at 3%–9% O_2 and *in vitro* biological experiments are performed in ambient air with an oxygen tension of approximately 20%, we divided the oxygen tension range into three groups <3%, 3%–10%, and 10%–20% to better compare the difference in cell migration under hypoxic and non-hypoxic conditions. We find that there is a significant difference between <3% and

FIG. 5. Mode of cellular chemotaxis in hypoxic and non-hypoxic environments. (a) and (b) 15 min steps V_x as a function of time, calculated from four cells under 1.4% and 7% oxygen tension. (c) and (d) Representative images of cell chemotaxis under 1.4% and 7% oxygen tension. The interval between the two photographs is 15 min. Bar: 50 μm . (e) 15 min-step V_x distribution for all cells under 1.4% and 7% oxygen tension, respectively.

3%–10% or 10% and 20% ($P < 0.005$). Our result indicates that breast cancer cells are sensitive to hypoxia in the presence of a CXCL12 gradient, which may be used as an *in vitro* model to test the specificity of breast cancer metastasis to bone and to quantitatively determine the MCF-7 chemotaxis rate under a range of pathological and physiological oxygen tensions.

Hypoxia as a trigger of tumor migration plasticity

To further understand the difference in chemotaxis velocity between hypoxic and non-hypoxic conditions, we analyzed images taken every 15 min of single cells under hypoxic (1.4%) and non-hypoxic (7%) oxygen tensions, and we compared their migration modes. For each situation, we analyzed four cells whose velocity was close to the overall average for a specific oxygen tension and plotted their 15 min-step V_x as a function of time (Figs. 5(a) and 5(b)). We observed that cells always move forward, with rare stops, when they are in hypoxic environment. Under non-hypoxic conditions, cells stop (V_x is zero) more frequently, although they are not slower during chemotaxis. To determine whether cells employ an alternative mode of migration in hypoxic and non-hypoxic environments, we examined photographs of cell chemotaxis under these two conditions (Figs. 5(c) and 5(d)). Under 1.4% oxygen tension, most cells move forward every 15 min and assume a round shape, which is reminiscent of the amoeboid migration mode. In contrast, under 7% oxygen tension, cells often elongate *in situ* (first two images in Fig. 5(d)) and move forward to the leading anterior position (the last image in Fig. 5(d)), similar to the mesenchymal migration mode.⁴⁵ At the time of elongation, the cell's center of mass barely shifts, and its velocity decreases to zero. We compared the 15 min-step V_x distribution of all cells under 1.4% and 7% oxygen tension (Fig. 5(e)). Because the data along negative V_x axis are scattered and we are more interested in the forward velocity (positive values), we included velocities at and below $-0.2 \mu\text{m}/\text{min}$ to the percentage bar at -0.2 . We found that there seem to be two V_x distributions for each condition: the velocity at which cells move forward, and the $V_x = 0$ distribution. For hypoxic conditions, the average cell velocity was $\sim 0.76 \mu\text{m}/\text{min}$, and its move forward percentage is much higher than the stop portion. For non-hypoxic conditions, the average cell velocity was $\sim 0.47 \mu\text{m}/\text{min}$, and its move forward percentage is much lower than the stop portion. This finding means that cells tend to move more often than stop in hypoxic situations. Research has shown that increased HIF-1 level during hypoxia activates Rho GTPase and that Rho is directly involved in the transition to the amoeboid mode of movement.⁴⁶ Investigating the effect of the gaseous microenvironment on mammalian cell chemotaxis on our microfluidic platform is an important step toward building pathological and physiological models *in vitro*.

CONCLUSION

Because a cell's gaseous environment has a critical effect on properties such as division, metastasis, and differentiation, we developed a novel microfluidic platform for studying mammalian cell chemotaxis under different oxygen conditions. The oxygen profile of the chip can be stabilized within 3 h, and a PDMS-PVDC-PDMS sandwich structure prevents the disturbance of the ambient environment. Zero-flow cell migration channels, in which a chemoattractant gradient can be formed over the course of 20 h, are set at different oxygen tensions. In addition, water channels protect the cell and medium channels from evaporation. Cell trajectories in multiple migration channels can be analyzed in parallel, facilitating the high-throughput study of chemotaxis in different oxygen environments. As an illustration, we demonstrated the rate of MCF-7 migration under a range of pathological and physiological oxygen tensions. Chemotaxis velocities and modes of movement between hypoxic and non-hypoxic microenvironments were assessed quantitatively and compared. We found that migration rate varied between cells, even for two close oxygen tensions, and that cells move faster towards high concentrations of chemoattractant when the oxygen tension is below 3%. This may be due to increased levels of HIF-1 in hypoxic environments, which would promote the transition to the amoeboid from the mesenchymal mode of movement.⁴⁵ In this paper, MCF-7 cells migrating towards CXCL12 in a hypoxic environment serve as a model for studying the specificity of breast cancer metastasis to bone. Broadly, this platform can be used for the quantitative study of cell chemotaxis in

other gaseous environments and could shed light on the effect of the gaseous microenvironment on cellular behavior.

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