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Generation of oxygen gradients with arbitrary shapes in a microfluidic device

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Abstract

We present a system consisting of a microfluidic device made of gas-permeable polydimethylsiloxane (PDMS) with two layers of microchannels and a computer-controlled multi-channel gas mixer. Concentrations of oxygen in the liquid-filled flow channels of the device are imposed by flowing gas mixtures with desired oxygen concentrations through gas channels directly above the flow channels. Oxygen gradients with different linear, exponential, and non-monotonic shapes are generated in the same liquid-filled microchannel and reconfigured in real time. The system can be used to study directed migration of cells and the development of cell and tissue cultures under gradients of oxygen.

Introduction

The concentration of oxygen in the biosphere varies from ~21% in the atmosphere to nearly zero in deep sea and soil. Some living creatures are adapted to significant variations in the oxygen concentration, [O₂], whereas others, such as anaerobic organisms or microaerobic soil bacteria populate niches with well-defined levels of oxygen. Many bacterial and some unicellular protists² and multicellular organisms³ are oxygen-tactic (aerotactic). They can detect spatial gradients of oxygen and move towards higher or lower [O₂]. Gradients of oxygen also form in the human body, especially during development of tissues and tumors and when the normal blood supply network is disrupted due to tissue injury. The oxygen gradients may play a role in directing angiogenesis and guiding the migration of immune cells towards oxygen-deprived (hypoxic) regions formed in developing tissues, tumors, and lesions.^{4–7} Studies of all these phenomena would benefit from a reliable instrument generating robust gradients of oxygen with desired shapes.

A traditional method of setting and controlling [O₂] in a liquid medium is by saturating it with a gas mixture with the desired level of oxygen (e.g., by bubbling the gas mixture through the medium). The high gas permeability of polydimethylsiloxane (PDMS), a common material for microfluidic devices, offers an alternative method for controlling [O₂] in microchannels. In a two-layer microfluidic device, [O₂] in channels filled with a liquid medium can be set by flowing a gas mixture with the desired [O₂] through gas channels directly above the flow channels.⁸ As we previously demonstrated,⁹ with this method, [O₂] in a PDMS microchannel can be controlled down to 0.1%, where 100% corresponds to a medium saturated with pure oxygen. Moreover, we and other researchers^{9, 10} showed that a set of gas mixtures with linear or exponential series of [O₂] can be generated from two source gases by on-chip gas mixing in a specialized microchannel network.

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Microfluidics also makes it possible to create O_2 gradients in the gas phase,³ and in several publications, generation of gas gradients in liquid media was described.¹¹⁻¹⁵ The gradients were formed by diffusion from a source (or to a sink) or by O_2 consumption due to cellular respiration, but the control of the gradient shapes was limited. Maharbiz and co-workers¹⁶ built and characterized a series of microfluidic devices, in which a variety of 1- and 2-dimensional O_2 gradients were generated using controlled local release of O_2 by electrolysis with the aid of various arrays of microelectrodes. However, the construction of the devices was relatively complicated, the generation of the gradients depended on a balance between the rates of O_2 production and its diffusion through the device to the atmosphere, and achieving $[O_2] < 21\%$ required an atmosphere with reduced $[O_2]$ around the device.

Here we present a microfluidic device and a method of generating oxygen concentration profiles with arbitrary shapes in liquid-filled microchannels. The device (Fig. 1) is made of a monolith PDMS chip sealed with a cover glass and has two layers of microchannels, a flow layer and a gas layer. As in the previously reported devices,^{9, 10} the device has a set of gas channels with different $[O_2]$. However, in contrast to the previous devices, the gas channels are made relatively narrow and positioned close to each other, making it possible to convert discrete series of $[O_2]$ in the gas channels into smooth gradient profiles of $[O_2]$ in the flow channels. In addition, whereas the previous devices had two gas inlets and generated gas mixtures with intermediate $[O_2]$ by on-chip mixing,^{9, 10} the present device has 9 gas inlets and is fed by O_2/N_2 mixtures from an off-chip multi-channel gas mixer. The gas mixer is computer-controlled and $[O_2]$ in each of its channels is set individually, making it possible to generate $[O_2]$ gradients with linear, exponential, and non-monotonic profiles in a single device and to reconfigure the profiles and reverse the gradient direction in < 0.5 min.

Experimental

The design and operation of the gas mixer are described in the Supplementary Information. Each of the mixer's 9 channels has a 3-way solenoid valve with two inlets and one outlet. The valve produces mixtures with different fractions of the two gases fed to its inlets by periodic switching with different duty cycles. O_2/N_2 mixtures with $[O_2]$ between 0 and 100% and with an absolute accuracy of $\sim 0.5\%$ in $[O_2]$ (with 100% corresponding to pure oxygen) are generated by feeding the gas mixer with N_2 and O_2 ; O_2/N_2 mixtures with $[O_2]$ between 0 and 21% and with an absolute accuracy of $\sim 0.1\%$ in $[O_2]$ are generated by feeding the gas mixer with N_2 and air.

The fabrication of the microfluidic device followed the same general protocol as in Ref. 9. The device has a single gas outlet and 9 separate gas channels with a depth of $150\ \mu\text{m}$, each connected to an individual inlet (Fig. 1a). The flow layer network consists of channels with a depth $d = 30\ \mu\text{m}$ and has a simple layout, with one inlet, one outlet, and three parallel test channels with widths of 30, 60, and $90\ \mu\text{m}$, which are connected to the inlet through a serpentine-shaped resistance channel. The gradients of $[O_2]$ are produced in the functional region of the device, where the test channels overlap with the gas channels (Fig. 1a). In the functional region, the gas channels are separated by $50\ \mu\text{m}$ wide partitions and are $150\ \mu\text{m}$ wide (except for the two marginal channels, which are both $800\ \mu\text{m}$ wide), forming a linear array with a period $L = 200\ \mu\text{m}$.

The thickness of PDMS between the flow and gas channels, $h = 120\ \mu\text{m}$ (Fig. 1b), is chosen to ensure the conversion of a discrete set of $[O_2]$ in the gas channels into a smooth profile of $[O_2]$ in the test channels. The characteristic time of O_2 exchange is estimated as $\tau = dh/(6D) \approx 0.46\ \text{s}$,⁹ where $D = 1.3 \cdot 10^{-5}\ \text{cm}^2/\text{s}$ is the diffusion coefficient for O_2 in PDMS measured in our previous work,⁹ and the factor of 6 is added because the solubility of O_2 in PDMS is ~ 6 times higher than in water.¹⁷ (The time of equilibration of $[O_2]$ inside the flow channels, $\tau_f =$

$d^2/(2D_w)$, where $D_w = 2 \cdot 10^{-5} \text{ cm}^2/\text{s}$ is the diffusion coefficient of O_2 in water, is 0.23 s, which is shorter than τ .) Flow through the device was driven by hydrostatically generated differential pressure between the inlet and outlet,⁹ which was normally set at 1 kPa, resulting in a mean flow velocity of 8.5 $\mu\text{m}/\text{s}$ in the 90 μm wide test channel, in which the $[\text{O}_2]$ profiles reported below were measured. At this flow velocity, the O_2 exchange time of 0.46 s corresponded to a distance of $\sim 4 \mu\text{m}$ along the test channel, which was less than d and much shorter than L . The gas flow rate, Q , was normally at $\sim 0.2 \text{ mL}/\text{s}$ for each gas inlet.

To measure $[\text{O}_2]$ in the test channel, we used a 250 ppm (by weight) solution of an oxygen-sensitive fluorescent dye, Ruthenium tris(2,2'-dipyridyl) dichloride hexahydrate (RTDP; obtained from Sigma),^{9, 14} in a 10 mM phosphate buffer with pH = 7.5. Fluorescence of RTDP was measured on an inverted fluorescence microscope (Nikon TE300), using a 10 $\times$ /0.50 Nikon Planfluor objective, 0.42 $\times$ video relay lens, and a 2/3" CCD camera (Basler A102F). The field of view of the setup was $\sim 2.0 \text{ mm}$ wide, covering nearly the entire length of the functional region. The fluorescence light source was a blue LED with the illumination intensity varying by $< 0.1\%$ in a 20 min period.⁹

Results and Discussion

Fluorescence of RTDP is quenched by oxygen, and its intensity in the presence of oxygen, I , is reduced compared to the intensity without oxygen, I_0 , according to the Stern-Volmer equation, $I_0/I = 1 + K_q[\text{O}_2]$, where K_q is a quenching constant. To evaluate K_q (which is temperature- and solution-dependent⁹), we measured the intensities of fluorescence in the 90 μm wide test channel, I_0 and I_{100} , when pure N_2 ($[\text{O}_2] = 0$) and pure O_2 ($[\text{O}_2] = 100\%$), respectively, were fed to all gas inlets, and used the equation $K_q = I_0/I_{100} - 1$.⁹ (A background correction was performed by imaging a region without fluorescent dye in it.) The value of the ratio I_0/I_{100} remained unchanged at 3.20 within an experimental error of 0.03 when the gas flow rate was varied between $Q = 0.1$ and $0.25 \text{ mL}/\text{s}$, indicating that no significant gas contamination occurred on the way to the device and the measured values of I_0 and I_{100} indeed corresponded to $[\text{O}_2] = 0$ and 100% , respectively.⁹ The measured value of I_0/I_{100} was constant in a 2.25 mm long segment of the test channel, which corresponded to the overlap of the test channel with the gas channel array, excluding two 400 μm wide marginal regions under the external sides of gas channels 1 and 9 (region between two arrows in Fig. 1b). Hence, in the entire 2.25 mm long segment of the test channel, the local value of $[\text{O}_2]$ at given conditions was calculated from the local fluorescence intensity, I , as $[\text{O}_2] = (I_0/I - 1)/K_q$, using the previously obtained values of I_0 and K_q (see Ref. 9 for a detailed discussion).

The system was tested in three sets of experiments, in which we produced $[\text{O}_2]$ profiles of various linear, exponential, and non-monotonic shapes (Fig. 2) by feeding the gas channels with O_2/N_2 mixtures with $[\text{O}_2]$ listed in Table 1. To generate linear profiles of $[\text{O}_2]$ in the test channels (Fig. 2a), $[\text{O}_2]$ fed to the gas inlets followed linear series (Table 1). The first two profiles (curve 1 and 2) had $[\text{O}_2]$ varying between 0 and 100% with gradients of 12.5% per $L = 200 \mu\text{m}$ and with opposite directions of the gradients. We also generated an $[\text{O}_2]$ profile with a doubled gradient (curve 3) and with the gradient reduced to half (curve 4). All four $[\text{O}_2]$ profiles (Fig. 2a) had extended linear regions in the middle and plateaus on their margins (under gas channels 1 and 9).

To generate exponential profiles of $[\text{O}_2]$ in the test channels (Fig. 2b), we fed gas inlets with mixtures in which $[\text{O}_2]$ followed geometrical series with the common ratios of 2, 3, and 4 (curves 1 – 3, respectively). All three $[\text{O}_2]$ curves (Fig. 2b) had exponential shapes in their internal regions (straight lines in the semi-logarithmic coordinates). Exponential fits to the curves in these internal regions resulted in exponents of 3.6/mm, 5.3/mm, and 6.2/mm, which were all within 4% of the values obtained from numerical simulations with the same

computational domain as in Fig. 1b. Two non-monotonic profiles with wavy shapes (Fig. 2c) were produced by feeding the gas inlets 1 – 9 with gas mixtures that had $[O_2] = 0\%$, 50%, 100%, 100%, 50%, 0%, 0%, 50%, and 100% (curve 1) and with the same series of gas mixtures in a reversed order (curve 2). The two profiles were symmetric with respect to the line $[O_2] = 50\%$ and their shapes were in good agreement with the results of our numerical simulations (black curve in Fig. 2c). The last two $[O_2]$ profiles (Fig. 2d) had shapes similar to the profiles in Fig. 2c, but had $[O_2]$ varying between 0% and 21% (physiological range). They were produced by supplying the gas mixer with N_2 and air (instead of O_2).

We repeated the experiment that resulted in curve 1 in Fig. 2c at mean flow velocities of 1, 2, 4, 17, and 34 $\mu\text{m/s}$. The differences between the $[O_2]$ profiles in the entire velocity range were within experimental errors (not shown), indicating that the flow of the RTDP solution through the test channel was sufficiently slow for equilibration of $[O_2]$ in the solution with $[O_2]$ in PDMS around the channel.⁹ When the gas mixer was reprogrammed (all 9 channels simultaneously) from $[O_2] = 0\%$ to 50%, $[O_2]$ in the test channel reached 45% (90% way through the switching) after ~ 24 s, and the transition time between $[O_2] = 5\%$ and 45% (from 10% to 90% through the switching) was ~ 12 s (Supplementary Fig. S-4). Therefore, $[O_2]$ gradients in the device take ~ 24 s to reconfigure, and the actual transition time is ~ 12 s.

The presented system composed of the microfluidic device and multi-channel gas mixer differs from the previously described devices^{9, 10} in two major respects: conversion of discrete series of $[O_2]$ in the gas channels into continuous $[O_2]$ gradients in flow channels and supply of gas mixtures with adjustable $[O_2]$ from a computer-controlled off-chip mixer. The $[O_2]$ profiles in Fig. 2 showcase the versatility of the system. The shapes of $[O_2]$ profiles it can create are only limited by the finite number of the gas channels, finite period of the gas channel array, and finite thickness of the PDMS layer between the gas and flow channels in the microfluidic device. The system easily generates low levels of $[O_2]$ (Fig. 2d), which are relevant for experiments on microaerobic bacteria and mammalian cells under hypoxia, and can also be used to produce graded profiles of concentration of other biologically relevant gases (e.g., CO_2 and NO) in liquid media. The depth of the flow channels (30 μm) and the velocity of liquid medium used in our tests (1 – 34 $\mu\text{m/s}$) are adequate to support a variety of cell cultures, and the estimated time of O_2 exchange (0.46 s) is expected to be sufficiently short to prevent changes in $[O_2]$ in the medium due to cellular respiration.⁹

In many respects, the presented system is more versatile and robust in generating gas concentration gradients than the devices reported in the literature are in generating gradients of soluble compounds.^{18–22} Most of these devices are specifically designed for a desired gradient shape, whereas with the presented system, a wide variety of concentration profiles can be generated using a single device and the magnitude and direction of the gradient can be modified in <0.5 min. Moreover, the shapes of the $[O_2]$ gradients have little sensitivity to resistances of the gas and flow channels and to the rates of gas and liquid flow.

Possible applications of the presented system include studies of oxygen-taxis of bacteria and other unicellular organisms as well as of the development of mammalian cell cultures (including tissue-emulating co-cultures of different cell types) under gradients of oxygen and other gases. In addition, graded profiles of $[O_2]$ produced by the system can be used as an alternative to the previously reported discrete sets of $[O_2]$ for studies of cells under hypoxia. Graded concentration profiles of other gases (e.g., CO_2 and NO) can be used to study dose responses of cells to various concentrations of these gases. The graded profiles would provide the advantage of continuous variation of concentration with a possibility to increase the resolution in a selected range by reducing the slope of the profile in this range.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Microfluidic device. **(a)** Drawing of microchannels in the device (xy -plane), with the flow channels shown in dark gray and gas channels shown in light gray. Gas inlets are marked by numbers 1 – 9. **(b)** Color-coded concentration of oxygen, $[O_2]$, in the yz -cross-section of the device in the functional area (where the gas and flow channels overlap) from a 2D numerical simulation in FEMLAB. A 4×1.25 mm center bottom fragment of the 10×4 mm computational domain is shown. White rectangles near the bottom are cross-sections of the $150 \mu\text{m}$ deep gas channels, which are numbered according to the numbers of the gas inlets in (a). The boundary conditions of the simulation are $[O_2] = 21\%$ at the upper and side boundaries (atmosphere air; not shown) and insulation at the bottom of the domain (cover glass). The conditions at the walls

of the gas channels are $[O_2] = 0\%$ (pure N_2) for channels 1, 6, and 7, $[O_2] = 50\%$ (1:1 $O_2:N_2$) for channels 2, 5, and 8, and $[O_2] = 100\%$ (pure O_2) for channels 3, 4, and 9, as in the experiment shown in Fig. 2c, curve 1. The test channels (not shown) are immediately adjacent to the bottom of the computational domain. Arrows near the bottom indicate lateral boundaries of a 2.25 mm wide internal region (between the centers of the 800 μm wide gas channels 1 and 9), in which $[O_2]$ just above the bottom as obtained from simulations is $>99.95\%$ when all gas channels are filled with O_2 ($[O_2] = 100\%$ at all gas channel walls).

Figure 2.

Concentration of oxygen, $[\text{O}_2]$, as a function of position, x , along the 90 μm wide test channel in the microfluidic device, as evaluated from fluorescence of RTDP, with different gas mixtures flowing through the gas channels. $x = 0$ corresponds to the middle of gas channel 5 (the center of the gas channel array; Fig. 1); $x = -0.725$ and 0.725 mm correspond to the inner edges of gas channels 1 and 9, respectively. **(a)** Four $[\text{O}_2]$ profiles obtained with four different linear series of $[\text{O}_2]$ in the gas channels. **(b)** Three $[\text{O}_2]$ profiles (in semi-logarithmic coordinates) obtained with three different geometrical series of $[\text{O}_2]$ in the gas channels. Curve 1 is shifted by 0.075 mm along the x -axis for better visibility. Some undulations in the curves, with a period of ~ 0.2 mm, are likely due to fluorescence background originating from the scattering of the fluorescent light by the gas channel walls, which is difficult to correct for. **(c)** Two $[\text{O}_2]$ profiles (orange and cyan curves) obtained with two non-monotonic series of $[\text{O}_2]$ in the gas channels. Thin black curve shows the result of a numerical simulation in FEMLAB (with no fitting parameters; cf. Fig. 1b). Some differences in the height of the peaks at $x = -0.3$ and 0.3 mm between the experiment and simulation could be due to the fact that the simulation was 2D and did not account for the presence of the test channel. **(d)** Two nonmonotonic $[\text{O}_2]$ profiles obtained when the gas mixer was fed with N_2 and air and $[\text{O}_2]$ in the gas channel varied between

0 and 21%. The $[O_2]$ profiles appear noisier than the similar profiles in (c) because of the 5-times higher resolution in $[O_2]$.

Table 1

Concentrations of oxygen, [O₂] (in %), in the 9 channels of the device for different curves in Fig. 2a – d.

Figure and curve	1	2	3	4	5	6	7	8	9
2a, 1	0	12.5	25	37.5	50	62.5	75	87.5	100
2a, 2	100	87.5	75	62.5	50	37.5	25	12.5	0
2a, 3	0	25	50	75	100	100	100	100	100
2a, 4	0	6.25	12.5	18.75	25	31.25	37.5	43.75	50
2b, 1	1	1	2	4	8	16	32	64	64
2b, 2	1	1	1	3	9	27	81	81	81
2b, 3	1	1	1	4	16	64	64	64	64
2c, 1	0	50	100	100	50	0	0	50	100
2c, 2	100	50	0	0	50	100	100	50	0
2d, 1	0	10.5	21	21	10.5	0	0	10.5	21
2d, 2	21	10.5	0	0	10.5	21	21	10.5	0