A micro surface tension alveolus (MISTA) in a glass microchip

(Electronic Supplementary Information)

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Experimental

Microchip fabrication

Photolithographic and wet chemical etching techniques were used to fabricate the microstructure (Fig.S1) on a 1.6mm thick, 60 mm square glass substrate (type SG2506 glass substrate with chromium and S-1805 photore sist obtained from Shaoguang Microelectronics Corp., Changsha, China). The etching speed of dilute HF/NH4F was 2μm/min and the precise etching depth was controlled by inspection under a light microscope (Fig.S2). The glass bonding temperature was 560°C. We developed a reusable bonding method: detergent and water washed the cover glass and the etched substrate glass, and a steel clamp was used to keep them tightly together during the experiment (Fig. S3). After the experiment, the cover glass was removed and washed ready for the next experiment.

Gas pressure control

Cylinders of N2, O2, and CO2 & Air were used in the experiments. The pressure was adjusted precisely at 0~10 ± 0.1kPa (Anthone Elec. Ltd., Xiamen, China). The gases were connected to a gas switch (BD Connecta, ref 394601, Becton Dickinson, Sweden) and a 1mm diameter plastic tube connected the switch to the gas inlet of the microchip.

Extraction and de-oxygen of chicken Hb

Newly collected chicken blood was centrifuged (900g) for the collection of red cells. An equal volume of water was added for effective ultrasonic cell crushing. To preserve the chicken Hb, the temperature was kept below 40 ºC by stopping the ultrasonic treatment and keeping the preparation on ice. The mixture of chicken Hb and cell debris was centrifuged (6000g) to clarify the solution for experiments. Water was de-oxygenated by a N2 stream for 2 days at room temperature (N2-water). We added 200μl Hb (blood volume) to 40ml N2-water and continued the N2 stream for another day. The purpose of the N2 stream is to remove O2 or other gases from the water and Hb. The de-oxygenated Hb solution was now ready for spectrum scan. We used the high concentration de-oxygenated Hb-solution (50% blood red cell) as an on-chip O2 and CO2 probe.

Image processing

The curvature radius of the MISTA front was measured as follows (Fig.S4): we drew the outline of the MISTA front; measured the chord length (L) of the arc; measured the height of the arc from the chord (H). The radius (R) is given as:

\[ R = \frac{L^2 + 4H^2}{8H} \]  

Microscopic images were recorded automatically at intervals of 0.2~60 s by a colour CCD (DH-HV3103UC, China Daheng...
Group, Inc. China) (Fig.S5)). To enhance the image, background was subtracted (Fig.S8d) from the original image (the background was the image before the experiment), phase reversed and adjusted to high contrast. Software was developed to extract the diffusion profile from time-lapse images. This software picked RGB colour on each image at different distances from the MISTA following the diffusion direction and combined all the RGB data into one image, with the x-axis as time and the y-axis as distance (Fig.3b & Fig.S10).

![Fig.S4](image)

**Fig.S4** The measurement and calculation of curvature radius.

![Fig.S5](image)

**Fig.S5** The RGB quantum efficiencies of the CCD used in the experiments.

**Results and discussion**

**The influence of pressure on the curvature radius of MISTA**

Fig.S6 is a graph data of overlapping outlines extracted from experiments. It shows how the pressure difference bends the surface of MISTA. When a series of MISTA are embedded along a wall, the pressure difference bends all the MISTA, simultaneously (Fig.S7a–c). The to and fro effect because of the friction is depicted in Fig.S7d.

![Fig.S6](image)

**Fig.S6** Overlapping outlines the curvature of a MISTA changed to gas pressure in the process of increasing pressure (blue) and decreasing pressure (red). The curvature calculations were based on these outlines.

![Fig.S7](image)

**Fig.S7** The changing shape of a MISTA and its curvature measurements to differential pressures. (a–c) Increasing pressure from gas pushed two MISTAs into the liquid. (d) The background image showed a MISTA. The two blue arrows indicated the increasing pressure and the increasing curvature. The two red arrows indicated the decreasing pressure and the decreasing curvature.
The colour of hemoglobin (Hb) as an indicator to dissolved O2 and CO2. (a) The Hb (5% blood) showed different colours in Air-water, N2-water, O2-water and CO2-water. (b) The diffusions of O2 and CO2 via gas-liquid interface (a) or PDMS membrane (b) were measured by a spectrophotometer (c). The spectra of N2-Hb (5% blood, O2 or CO2 was removed from Hb by N2), O2-Hb, CO2-Hb were different. The spectrum of O2-Hb under PDMS membrane during 2h O2 diffusion (O2-HbP in b) was similar to that of O2-Hb under no membrane gas-liquid interface during five-minute O2 diffusion (the grey lines between red and black lines representing measurements every three minutes). (d) These spectrum differences in microchannel on chip were inspected by colour CCD camera under microscope and the CCD images were enhanced by a RGB (red, green and blue value) colour process. BK: background colour.

Absorbency ratio (A542nm+A577nm)/A562nm) of Hb was increasing because of oxygen diffusion from the air. Circles represented oxygen entering the Hb solution (0.5% blood red cell) directly and crosses represented oxygen in the air entered the Hb solution via a PDMS membrane (600μm thick).

Fick's first law predicts how diffusion causes the concentration field to change with time:

\[ J = -D \frac{\partial \Phi}{\partial x} \]  

where \( J \) is the diffusion flux, \( D \) is the diffusion coefficient, \( \Phi \) is the concentration and \( x \) is the position.

Fick's first law states that the mass flow rate of a substance across a surface is proportional to the concentration gradient across the surface.

The diffusion of O2 and CO2 through a PDMS membrane

Results about the diffusion of O2 and CO2 through a PDMS membrane can be found in Fig.S8c and Fig.S9.

Rapid switching of gases

Fig.S10 shows the image records inside a MISTA with rapid N2-O2 gas shifting.

The simulation of convective/diffusive mass transfer from a MISTA

To evaluate the convective or diffusive mass transfer, we simulated the O2 and CO2 diffusion in the Hb solution from a MISTA into the microchannel. The design of the MISTA diffusion channel in the experiments was narrow and the diffusion in this channel acts like one dimensional diffusion. The liquid in the microchannel is stationary and the model includes only diffusive mass transfer. The gas phase is mobile and the convective mass transfer of gas enables a constant saturated boundary at the MISTA gas-liquid surface. So the simulation set a saturated O2 or CO2 in the process of calculation. The calculations are based on Fick's first law.28, 29

\[ \frac{\partial \Phi}{\partial t} = D \frac{\partial^2 \Phi}{\partial x^2} \]  

where \( t \) is time.

Eqn (4) is a solution of eqn (3) in one dimension:

\[ n(x, t) = n(0) \text{erfc} \left( \frac{x}{2 \sqrt{D t}} \right) \]  

where \( \text{erfc} \) is the complementary error function, which is defined in term of the error function:

\[ \text{erfc} (x) = 1 - \text{erf} (x) = \frac{2}{\sqrt{\pi}} \int_x^\infty e^{-t^2} dt \]
The comparisons of the Fick’s first law simulation model in this paper by a solution of Fick’s second law.

One approximation of the \( \text{erf}(x) \) is given by

\[
\text{erf}^2(x) = 1 - \exp\left( -x^2 \frac{4}{\pi} + ax^2 \right)
\]

where

\[
a = -\frac{8(\pi - 3)}{3\pi(\pi - 4)}
\]

Eqn (4), (6) and (7) are the approximate solution to predicts how diffusion causes the concentration field to change with time. They are used to validate the simulation. Only when the model calculations are confirmed, can Hb be incorporated into the model.

Fig.S11 compares the simulation results by Fick’s first law and the solutions by Fick’s second law. These coincident lines proved the correct of the simulation program and the Hb was hereafter incorporated into the model.

Fig.S11 The comparisons of the Fick’s first law simulation model in this paper by a solution of Fick’s second law.

Table 1 parameters in the simulation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length from MISTA</td>
<td>0–1000 (μm)</td>
</tr>
<tr>
<td>Precision</td>
<td>1 (μm)</td>
</tr>
<tr>
<td>Time step</td>
<td>10^{-4} (s)</td>
</tr>
<tr>
<td>Diffusion coefficient of O(_2) in water</td>
<td>1.77x10(^{-3}) (μm/μs)</td>
</tr>
<tr>
<td>Saturated dissolved O(_2) in water</td>
<td>1.34x10(^{-18}) (mol/μm(^2))</td>
</tr>
<tr>
<td>Saturated dissolved CO(_2) in water</td>
<td>3.92x10(^{-17}) (mol/μm(^2))</td>
</tr>
<tr>
<td>Saturated dissolved O(_2)-Hb</td>
<td>6.06x10(^{-18}) (mol/μm(^2))</td>
</tr>
<tr>
<td>Saturated dissolved CO(_2)-Hb</td>
<td>2.24x10(^{-18}) (mol/μm(^2))</td>
</tr>
</tbody>
</table>

In the process of simulation, the flux of O\(_2\) and CO\(_2\) are firstly figured out by Fick’s first law for the total mass of O\(_2\) and CO\(_2\) at each position. With the equilibrium curve of CO\(_2\)-Hb (Fig.S12), we have the concentrations of dissolved CO\(_2\) and CO\(_2\)-Hb. With the equilibrium curve of O\(_2\)-Hb (Fig.S13) and the concentration of CO\(_2\), we have the concentrations of dissolved O\(_2\) and O\(_2\)-Hb. We now go to the next step for the new flux of O\(_2\) and CO\(_2\) with the new concentrations O\(_2\) and CO\(_2\).

The equilibrium curve of CO\(_2\)-Hb is defined by eqn (8):

\[
y(x) = -1.3151x^4 + 4.1554x^3 - 5.0529x^2 + 3.1997x
\]

where \( n=2 \) and \( a \), the parameter of Bohr Effect, fitting eqn (9), is defined by eqn (10):

\[
a = \frac{76 \times 3.5}{P_{CO_2}^{\text{O},Hb} + 5}
\]

where \( P_{CO_2}^{\text{O},Hb} \) is partial pressure of CO\(_2\) defined by eqn (11)

\[
P_{CO_2} = \frac{76C_{CO_2}}{C_{CO_2}^s}
\]

where \( C_{CO_2} \) is the concentration of dissolved CO\(_2\) and \( C_{CO_2}^s \) is the saturated dissolved CO\(_2\).

Fig.S14 and Fig.S15 (next pages) show the main result of the simulation (see also ESI movies about the simulation).
Fig. S14 A Computer simulation of convective/diffusive mass transfer from a MISTA. A-F Concentrations of dissolved oxygen and O\textsubscript{2}-Hb at 100s. D, D/10 and D/50 represent diffusion coefficient of O\textsubscript{2} in water (D), one tenth of D and fiftieth of D, respectively. 1x blood, 0.5x blood and water represent the concentration of Hb of one time, 0.5 time of blood concentration and no Hb, respectively.
Fig. S15 A Computer simulation of convective/diffusive mass transfer from a MISTA. A-F Concentrations of dissolved oxygen, Hb-O₂, dissolved CO₂ and CO₂-Hb at 300s, just 30s after CO₂ fillings the MISTA (at 270s). D, D/10 and D/50 represent diffusion coefficient of O₂ in water (D), one tenth of D and fiftieth of D, respectively. 1x blood, 0.5x blood and water represent the concentration of Hb of one time, 0.5 time of blood concentration and no Hb, respectively.
References