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

Xing Yue (Larry) Peng, *ab Lan-Qin Wu, a Na Zhang, a Li-Dan Hu, a You Li, a Wen-Juan Li, a Dong-Hui Li, ‡ Ping Huang ‡ and Yong-Liang Zhou* a

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We have designed a non-membrane micro surface tension alveolus (MISTA) in a glass microchip for direct gas exchange and micro gradient control. Hemoglobin (Hb) in the liquid phase indicates the rapid gas gradient changes of O2 and CO2 shifted by the difference in pressure between the liquid and the gas.

Advances in microtechnology are providing new opportunities for understanding the microenvironment that consists of an extracellular matrix (ECM), cytokines, growth factors, nutrients, oxygen, etc. Microsystems capable of precise monitoring of O2, CO2 that are most important for cell health need to be developed but are challenging. A multi analyte-sensitive hydrogel microarray sensor constructed via microfluidic patterning of hydrogel structures to monitor pH and dissolved oxygen concentration simultaneously in cell culture media has been reported. Luminescent, dye-based, optical oxygen sensors were integrated into cell biology-based applications has focused on the application of controlled gradients of soluble factors. The lack of reliable dissolved gas (oxygen, nitrogen, carbon dioxide, etc.) gradient micro-devices limited the application of microfluidics. A flat interface surface is apparently not the device to control a micro scale gas gradient (<100 μm), which is essential to single cell level on-chip experiments. In addition, the gradient under a flat interface surface is overlapped from the normal (from the direction of observation), and the dimension of the gas gradient is invisible under a microscope. Therefore, two questions arose. Did we have to use a gas-permeable membrane? How is it possible to generate a micro scale gas gradient? These two questions remain a challenge for microchip design. The goal of this paper was to find a novel design for solving these two questions.

Fig. 1 shows a design for an artificial micro-alveolus on a microchip. Instead of a membrane, the design makes use of the surface tension at a gas–liquid interface. This micro gas–liquid exchanger is convenient for rapid gas exchange and for formation of a dissolved gas gradient control on a micro scale. Fig. 1a shows the principle of the MISTA in the wall separating the liquid channel from the gas channel. The gas pressure (P1) is higher than the liquid pressure (P0) (Fig. 1b) so the difference P1 – P0 = ΔP is positive, and the gas pressure drives gas into the liquid to form a meniscoid bubble. The Laplace pressure (ΔP) within the meniscus is given by the Young–Laplace equation:

\[ \Delta P = \gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right) \]  

(1)

Where ΔP is the pressure difference across the fluid interface, \( \gamma \) is the surface tension, and \( R_1 \) and \( R_2 \) are the principal radii of curvature. The site of the MISTA is also meniscoid, which makes the MISTA bubble stable. For a given gas–liquid system, the surface tension constant is stable, and ΔP controls the curvature of the meniscoid bubble. Gas exchange (Fig. 1c) occurs on the meniscoid surface of the MISTA.

A MISTA with both the liquid channel and the gas channel was fabricated by one-step photomask-etching (Fig. 1a). To form a MISTA structure in the 20 μm thick wall, we designed a 100 μm thick wall and a 25 μm high triangular jag on the photomask, and 20–40 μm depth etching was suitable for the formation of the MISTA structure (see ESI†). A series of triangular jags formed a series of MISTAs (see ESI†).

*Department of Biology, Xiamen University, Xiamen, Fujian, China 361005. E-mail: xypeng@xmu.edu.cn; Fax: +86-592-2181386; Tel: +86-592-2181386


†‡These two authors contributed equally to this work.

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Higher gas pressure forced the MISTA into the liquid phase (Fig. 1b, c) (see ESI†). The pressure difference $\Delta P$ was estimated with eqn (1), above. Considering the etching depth, put

$$\gamma = 0.07192 \text{ N/m (water at 25 } ^\circ\text{C)}$$

$$R_1 = R_2 = 20 \mu\text{m}$$

$$\Delta P = 0.07197 (\frac{1}{0.00002} + \frac{1}{0.00002}) = 7187 \text{ Pa}$$

We observed and measured the horizontal values of $R$ as a function of pressure ($\Delta P$) to prove the MISTA theory. Even though it was not possible to measure the vertical $R$ the measured $\Delta P$ should be close to the calculated value (7187 Pa). In the process of measuring the horizontal $R$ vs. pressure difference (see ESI†), we found the difference of horizontal $R$ when the MISTA stretched to and fro along with the pressure (see ESI†). This was because the friction from the inner wall and the contact angle hysteresis changed the curvature a little in different directions of movement (see ESI†).

To accomplish stable control of MISTA, we measured four different liquid media of different surface tension constant: $5 \text{ mM SDS (CH}_3\text{(CH}_2\text{)}_{10}\text{CH}_2\text{SO}_3\text{Na; DMEM (Dulbecco’s modified Eagle’s medium, Gibco, USA); water; and a saturated sucrose solution, where:}$

$$\gamma_{\text{SDS}} < \gamma_{\text{DMEM}} < \gamma_{\text{water}} < \gamma_{\text{sucrose}}$$

Lower $\gamma$ results in lower pressure and a narrow pressure window (Fig. 2). To ensure the best gas exchange, the pressure was controlled near the high end of the pressure window for deeper MISTA and more exchanging surface area. The data in Fig. 2 provided a very good reference for the controls in all our experiments. For all gases, we adjusted the pressure according to the model in Fig. 2. The curves in Fig. 2 showed flat tops because of the rapid increasing vertical $R$ when a MISTA reached deeper into the liquid, and this was propitious for the formation of a stable and effective MISTA in the liquid.

The colours of Hb in air–water, $N_2$–water, $O_2$–water or $CO_2$–water were different (see ESI†). The de-oxygenated Hb solutions exposed to $O_2$ or $CO_2$ (or under PDMS membrane) changed their colours as these gases entered the solutions. Their spectra were measured (see ESI†). The $CO_2$–Hb absorbed more light and showed a dark red. The characteristic $O_2$ peaks between 520 nm and 600 nm (see ESI†) proved that the membrane slowed down the $O_2$ diffusion to $\sim 5\%$ of that without a membrane (see more air–$O_2$ diffusion experiments in ESI†). The spectra of Hb did not indicate the 2-D realtime diffusion of gases in the micro chip channels. Colour CCD images revealed the different spectra. After these images had their background subtracted and were enhanced, we were able to conclude that high concentration de-oxygenated Hb-solution (50% blood red cell) in a microchannel could be a probe for both $O_2$ and $CO_2$ diffusion (see ESI†).

Fig. 3a shows a de-oxygenated Hb-filled micro channel near the end of a MISTA. The time-lapse images were the results of $O_2$ (started at 0 min) and $CO_2$ (from 4.6 min to 14 min) diffusion from the MISTA (see the legend to Fig. 3b). The $O_2$ front penetrated $\sim 100 \mu\text{m}$ in the first minute. The maximum $O_2$–Hb peak (see the blue belt) followed the front and reached $\sim 50 \mu\text{m}$ in 14 minutes (see ESI†). The diffusion detail along the profile A–B (Fig. 3a) was extracted and is depicted in Fig. 3b. The slopes of the $O_2$ and $CO_2$ fronts clearly describe the movement of $O_2$–Hb and $CO_2$–Hb. The $O_2$ front moved at a speed of about $70 \mu\text{m/min}$. The blue peak of maximum $O_2$–Hb moved at $3.6 \mu\text{m/min}$ and the $CO_2$–Hb front moved at the same speed. The succession

![Fig. 1](image1.png)

**Fig. 1** The conceptual design of a MISTA and its fabrication by one photomask. A MISTA was like a tiny air bubble (from gas channel to liquid channel) embedded in the wall (a). Given a differential pressure ($\Delta P$) to balance the surface tension of the curvature (b), a MISTA was controlled for gas exchange (c).

![Fig. 2](image2.png)

**Fig. 2** The pressure ranges of a MISTA in four different liquids (surface tension constants from low to high). The four arrows indicated similar patterns of the curvature changes (see Fig. 1b and ESI†). The hollow symbols and solid symbols represented the processes of increasing pressure and decreasing pressure, respectively.
The concentration of Hb and the diffusive coefficients of O\textsubscript{2} and CO\textsubscript{2} concentration O\textsubscript{2}–Hb (see ESI†). (c) Computer simulations of the speed of moving peaks of O\textsubscript{2}–Hb or CO\textsubscript{2}–Hb but increased results (see ESI†) showed that the adding of Hb slowed down the dissolved gases by diffusion. As the viscosity of Hb solution the gas diffusive fluxes for the dissolved Hb absorbed quickly the 14 minute gas diffusions from the MISTA were depicted as a function of time (b). The blue colour labelled by O\textsubscript{2}–Hb represented high the 14 minute gas diffusions from the MISTA were depicted as a function vs. vs. of colour between blue (O\textsubscript{2}–Hb) and pink (CO\textsubscript{2}–Hb) visualized the substitution processes of O\textsubscript{2}–Hb to CO\textsubscript{2}–Hb in the O\textsubscript{2}–CO\textsubscript{2}

We simulated the convective/diffusive mass transfer from a MISTA into a narrow channel (Fig. 3c). In the simulation based on Fick’s law of diffusion, the gas concentrations at the liquid–gas interface were set to saturation because of the motion of gases. The equilibrium curve of O\textsubscript{2}–Hb vs. O\textsubscript{2} incorporated the Bohr effect\textsuperscript{24} of P\textsubscript{CO\textsubscript{2}}. We employed an equation reported by Michel\textsuperscript{19} to fit the equilibrium curve of O\textsubscript{2}–Hb. The simulation results (see ESI†) showed that the adding of Hb slowed down the speed of moving peaks of O\textsubscript{2}–Hb or CO\textsubscript{2}–Hb but increased the gas diffusive fluxes for the dissolved Hb absorbed quickly the dissolved gases by diffusion. As the viscosity of Hb solution\textsuperscript{26} exceeds 0.1 Pa s, about 100 times of that of water (0.001 Pa s at 20 °C), the diffusion coefficients decrease to about 1/100 to 1/50. The simulation with diffusion coefficients of 1/50 resulted in similar data to the experiments (Fig. 3c).

We also applied the colour of Hb to show a series of rapid switches between N\textsubscript{2} and O\textsubscript{2} (see ESI†). The changing colour, caused by the entering and releasing of O\textsubscript{2} via the MISTA, following the gas switches, demonstrated smooth and effective gas gradient control via a MISTA. The switching of gases took effect on the MISTA in only 20s.

In conclusion, the micro MISTA was easy to fabricate by one-step photomask etching. The MISTA realized micro scale gas gradient control without a gas permeable membrane. The MISTA requires only a special microstructure that makes use of only surface tension, so the function of a MISTA design is independent of the materials used (PDMS, silicon, etc.). Glass was optically and nontoxically the best material for the cell chip. The MISTA technique released the pure glass chip from the limitation of zero gas permeability. Moreover, the micro scale gas gradient control (horizontally and without any membrane) excelled any large area flat interface microchip. The membrane might have significant impact on a cell since the vertical gas gradient was unobservable under a microscope so the gas gradient was not on the micro scale. The pressure control was also an on–off switch for gas diffusion. A series of MISTAs (arranged along a wall) were suitable for a complex on-chip gas gradient environment.

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