Supplement

Quantifying the reaction rate constant of O₂ consumption

The kinetic rate constant of the reaction between sodium sulfite and O₂ was determined by a semi-empirical approach that involves experimental measurements of O₂ by PhLIM. The solutions of sodium sulfite and Oxyphor G4 were mixed to generate final concentrations of sodium sulfite in the range of 0 - 7.5 mM while holding Oxyphor G4 at 20 μM. The solutions were placed in well plates made of polystyrene, which has a very low permeability for O₂, allowing the diffusion of ambient O₂ only from the open liquid surface of the well. The volume of the solution was 100 µL in each well (ID = 6.4 mm) resulting in the liquid-column height of 3.1 mm. The O₂ concentration was measured in a confocal-plane 50 µm away from the bottom surface of the well (or 3.05 mm from the open surface) at t = 30 min. A 3D model of a cylindrical well was created, and transport of diluted species module was solved in COMSOL. The resistance to mass transfer in the air was neglected, and the concentration of O₂ near the top surface was assumed to obey Henry’s law. All other surfaces were insulated from the atmosphere. The following consumption reaction with kinetic equation was used:\n
\[
\text{Na}_2\text{SO}_3 + \frac{1}{2}\text{O}_2 \rightarrow \text{Na}_2\text{SO}_4 \quad (S1)
\]

\[
-r_{O_2} = K C_{O_2}^{0.65} \quad (S2)
\]

where K is the kinetic rate constant. The value of the kinetic rate constant was chosen that produced a minimum root mean square error between the measured and predicted O₂. The reaction rate constant (K) of 3 x 10⁻⁵ mM⁻⁰.³⁵ s⁻¹ matched the experimental data (Fig. S1).

Analysis of oxygen concentration in the syringe

The concentration of O₂ in a solution of sodium sulfite (concentration ≥ 1 mM) placed in an insulated container (i.e., the syringe) reaches zero \(C_{in} = 0\) (Eq. 5) within < 4 hr and remains constant thereafter at zero (Fig. S2). Also, within this period, the concentration of sodium sulfite would be reduced by an amount equivalent to the solubility of O₂ in water (0.27 mM at 25 °C in the air) beyond this time period the concentration of sodium sulfite is constant in the syringe (Fig. S2).

Analysis of back diffusion of oxygen in the syringe needle.

We performed an analysis of whether O₂ can diffuse through the outlet into the syringe, against the direction of flow (back-diffusion). The approximate length a molecule can diffuse in a stagnant solution in time \(t\) is \(4\sqrt{D_t}\), which is found by solving Fick’s 2nd Law of Diffusion for a semi-infinite media. As the sodium sulfite solution is flowing in the present case, the time for diffusion can be approximated by the residence time of the solution in the needle (\(\tau_n\)). When \(Q > 1\) nL s⁻¹ (typical low range for nanoliter syringe pumps) and the volume of needle = 1570 nL (ID = 0.4 mm and length = 12.5 mm), then \(\tau_n < 1570\) s, and the back-diffusion length (< 8 mm) is smaller than the length of the needle (12.5 mm). Therefore, the syringe continuously pumping aqueous sodium sulfite solution can be considered an insulated container.

Analysis of sodium sulfite concentration in the tube

It is convenient to assume that the sodium sulfite concentration in the tubing is constant. This can be the case if the sodium sulfite concentration is high or if the flow is high (small residence time). The mean residence time, \(\tau\), is convenient term that captures the flow and volume of the tubing; it is defined as simply the volume of the tubing
divided by the volumetric flow. It can be easily shown that $C_{\text{ss, out}}$ remains relatively unchanged (> 0.9 $C_{\text{ss, in}}$) for $C_{\text{ss, in}} \geq 1\text{mM}$ (Fig. S3). Thus, the maximum $\tau$ below which the concentration of sodium sulfite does not appreciably change through its flow path is $\tau < 2000\text{s}$ (Fig. S3); thus, for our experiment, $\tau < 2000\text{s}$ if $Q > 145\text{ nL s}^{-1}$ ($1.514\text{ m long tube with ID} = 0.5\text{ mm}$).

**Measurement of the permeability of the Tygon tubing**

To find the permeability, $P_m$, of the commercially available medical grade Tygon tubing, we used a simple experimental approach. The Tygon tube (ID = 0.508 mm and OD = 1.524 mm) was attached to a syringe via a 23G needle and the tube was looped and secured on the microscope stage at two points which were 5 and 7 ft from the inlet. Thus, two points of measurement separated by 2 ft length of the tubing were in the same field of view of the microscope. Water mixed with oxyphor G4 dye equilibrated with pure nitrogen was perfused through the tube at defined flow rates ($Q = 50, 25,$ and $13\text{ µl min}^{-1}$) and the $O_2$ concentration was measured at the two measurement points using PhLIM. The experimental data were fit to the following equation, which was formulated adapting Eq. 3 for no reaction ($D_a=0$, i.e., in the absence of sodium sulfite), by regression analysis to find $P_m$,

$$\ln \frac{C_1 - Sw_1 y_A}{C_2 - Sw_1 y_A} = P_m \left( \frac{2mL}{Q} \right)$$

(S3)

where $C_1$ and $C_2$ are $O_2$ concentrations measured at the two measurement points and $L = 2\text{ ft}$ (Fig. S3). The permeability of the Tygon tubing was $6.30 \pm 0.03 \times 10^{-8}\text{ m s}^{-1}$.

**References**

Figure S1. Determination of kinetic rate constant of sodium sulfite reaction with oxygen. The $O_2$ concentration in aqueous sodium sulfite solution was experimentally measured (indicated by *) after 30 min of the solution preparation using PhLIM. A COMSOL model was used to find the reaction rate constant by matching the calculated (line plots) and measured $O_2$ concentrations. The reaction rate constant at which the calculated concentrations matched with the measured concentrations was $K = 3 \times 10^{-5}\text{mM}^{0.35}\text{s}^{-1}$ and the corresponding $O_2$ profiles are shown. See supplementary methods for details.

Figure S2. Estimating oxygen and sodium sulfite (SS) concentration in a closed container (syringe). Eq. 1, Eq. S2, and the mass balance of oxygen for a closed system were used to calculate the concentrations. See Supplementary methods for details.
Figure S3. Determining the residence time needed to maintain the sodium sulfite concentration constant. The analytical solution (eq. 1) was used to find the sodium sulfite concentration at the outlet of the tubing ($C_{ss, out}$) for various inlet concentrations of sodium sulfite ($C_{ss, in}$) and residence times ($\tau$). See analysis in supplement for more details.

Figure S4. Determining the permeability of Tygon tubing. The permeability of the Tygon tubing was found by measuring the increase in oxygen concentration for variable residence time (or flow rate). The $X = \frac{2\pi r L}{Q}$ and $Y = \ln \frac{C_1 - y_A}{C_2 - y_A}$ from eq. S3 were plotted and regression analysis (Predicted Y) was used to find the permeability. See Supplementary methods for details.
**Figure S5. Regression analysis for \(Da_1 \leq 0.12\).** Multiple linear regression was performed on log-transformation of Eq. 12 and the actual and predicted \(Y = \log{\frac{C_{wall}^0}{y_{A2} - C_{wall}^0}}\) are plotted (left panel). The pearson correlation coefficient (right panel) indicating a strong (dark red or blue), or weak (faint red or blue) dependence are shown.

**Figure S6. The regression analysis for \(Da_1 > 0.12\).** Multiple linear regression was performed on log-transformation of Eq. 12 and the actual and predicted \(Y = \log{\frac{C_{wall}^0}{y_{A2} - C_{wall}^0}}\) are plotted (left panel). The pearson correlation coefficient (right panel) indicating a strong (dark red or blue), or weak (faint red or blue) dependence.
Figure S7. MDA-MB-231 cells at 5% have greater HIF-1α present than at 20% O₂. Cells were grown at 20% (left column) and 5% O₂ (right column). HIF-1α (green), DAPI (blue), or merged images of the immunofluorescence are shown. Ratio of the number of nuclei (DAPI) with overlapping HIF-1α stain to total number of nuclei is shown.
**Figure S8. The impact of interstitial fluid velocity ($v_{IF}$) on the $O_2$ profiles in the device.** A) The interstitial fluid velocity in the tissue chamber was varied at two levels by changing the hydrostatic pressure head applied across the tissue chamber and modeled in COMSOL. The interstitial fluid velocity ($v_{IF}$) averaged over entire tissue chamber is shown at the top each panel. The streamlines (red lines) show convection of media through the side tissue chambers. B) At the two $v_{IF}$, and four different sodium sulfite concentrations while setting $Pe_1 = 2.2$ and $L^0 = 1.5$, $O_2$ profiles were computed a horizontal axis in x-direction (dotted line in 3A). $Da_1$ values corresponding to each sodium sulfite concentration are indicated.
Figure S9. The impact of cell density on the O$_2$ profiles in the device. The O$_2$ profiles were computed using COMSOL at various cell densities and at two sodium sulfite concentrations, 5 mM (top panel) and 1 mM (bottom panel), while setting $Pe_1 = 2.2$ and $L^0 = 1.5$. The O$_2$ profiles along a horizontal axis in x-direction (dotted line in 3A) are shown. The cell density used in our experiments is shown in the yellow box.