Published in final edited form as:

Microfluid Nanofluidics. 2015 November ; 19(5): 1181–1189. doi:10.1007/s10404-015-1636-7.

On-demand in situ generation of oxygen in a nanofluidic embedded planar microband electrochemical reactor

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Abstract

In situ generation of reagents and their subsequent use downstream presents new opportunities to amplify the utility of nanofluidic devices by exploiting the confined geometry to address mass transport limitations on reaction kinetics and efficiency. Oxygen, an inherently valuable reactant, can be produced from electrolysis of water, a process that can be conveniently integrated within a nanofluidic system. Here, we construct and characterize a nanofluidic device consisting of a planar microband electrode embedded within a nanochannel for in situ electrochemical generation and optical monitoring of O₂. Fluorescein, a dye with a pH-sensitive emission intensity, was used to monitor the spatiotemporal characteristics of the oxidation of H₂O, using the co-produced H⁺. Application of anodic potentials at the nanochannel-embedded electrode results in a decrease in fluorescence intensity, which reflects the decreasing solution pH. A combination of fluorescence intensity and chronoamperometric response was used to quantitatively determine proton generation, and the H⁺/O₂ stoichiometry was then used to determine the concentration of the O₂ in the channel. Comparison of the experimental results to finite element simulations validates the use of fluorescein emission intensity to spectroscopically determine the local oxygen concentration in the nanochannel. By varying the applied potential, spatially averaged O₂ concentrations ranging from 0.13 to 0.41 mM were generated. The results demonstrate a convenient route to in situ modulation of the dissolved O₂ level in a nanofluidic device and the use of an optical probe to monitor its spatial and temporal distribution under flow conditions.

1 Introduction

By integrating multiple processing steps onto a single substrate, lab-on-a-chip (LOC) devices provide a platform for rapid chemical and biochemical sensing applications at low cost and small sample volumes (Mir et al. 2009; Piruska et al. 2010). Microchannels are generally used in LOC devices for sample injection, preprocessing, and separation (Bocquet and Charlaix 2010; Dittrich and Manz 2006; Mouradian 2002; Srinivasan et al. 2004; Weigl

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Electronic supplementary material The online version of this article (doi:10.1007/s10404-015-1636-7) contains supplementary material, which is available to authorized users.

et al. 2003), because they combine the advantages of small form factor with convenient fabrication protocols. Decreasing the size of the channel to the nanoscale allows the fabrication of very compact devices with extremely low sample volume requirements and high efficiency, which can exploit the special properties of nanofluidics (Hong et al. 2009; Prakash et al. 2012). As an example, enhanced reaction rates can be achieved in nanofluidic systems when there is at least one surface reaction partner, due to increased molecular collision frequencies that result from the higher surface-to-volume ratio in nanoscale channels (Contento et al. 2011; Schoch et al. 2007), a characteristic that is especially useful in coupling nanofluidics with heterogeneous electron transfer reactions (Gencoglu and Minerick 2014; Rassaei et al. 2012; Webster and Goluch 2012; Zevenbergen et al. 2009). In addition, as the characteristic length of the channel is reduced to be commensurate with the electrical double layer, unique behaviors such as ion permselectivity and ion concentration polarization are observed (Eijkel and Berg 2005; Fa et al. 2005; Piruska et al. 2010), which can be utilized to control molecular transport.

Despite these obvious benefits, sample injection to nanofluidic systems remains a challenge, especially when multiple reactants or gaseous reagents are involved. We have previously shown that on-chip electrodes can be used for in situ generation of reactive species within confined geometries based on solvent electrolysis (Contento et al. 2011), thereby avoiding the need for a Henry's law sorption step. With on-chip generation, the amount of reactant inside the device can be precisely controlled for manipulation of downstream reaction processes, thus avoiding contamination and sample loss during injection. This is especially important when toxic, sensitive, or explosive reactants are used in microfluidic devices (Haswell and Skelton 2000).

For example, Maurya et al. (2011) reported in situ generation of diazomethane on demand and used it for the downstream reaction in a microreactor. In a microflow system, Fuse et al. (2011) realized continuous in situ formation of phosgene, which was used to produce an acid chloride intermediate for amidation. These studies demonstrate the benefit of using in situ generation for minimizing exposure to hazardous substances.

In this paper, we describe the fabrication of an electrochemical reactor used for on-demand in situ generation of precise amounts of oxygen in a nanofluidic system. The device consists of a nanofluidic channel with an embedded microelectrode, which is used to generate O₂ from electrochemical oxidation of water,

Because oxygen is a valuable reagent that is difficult to inject into nanoscale environments, methods for delivering quantitative amounts on-demand have value in chemical sensing and processing applications. Oxygen impacts a variety of vital biological processes and is used as an important factor for constructing physiologically realistic microenvironments in in vitro systems (Brennan et al. 2014). For example, a small change in oxygen level in microbial and eukaryotic cell cultures may irretrievably alter metabolic pathways of cells (Maharbiz et al. 2003). Oxygen level is also a critical factor that affects glucose sensing via

enzymatic oxidation in LOC devices (Wang 2008). In addition, when used as an oxidant, O₂ level governs the performance of microfluidic fuel cells (Jayashree et al. 2005; Kjeang et al. 2008, 2009; Mitrovski et al. 2004), and molecular oxygen is an effective oxidant that can be used in other value-added chemical processes, such as remediation of environmental pollutants (Pelaiez et al. 2012; Shannon et al. 2008; Sires and Brillas 2012).

In situ electrochemical generation of oxygen in a nanochannel electrochemical reactor eliminates the need for external O₂ injection, and the oxygen generation rate can be controlled quantitatively by simply controlling the current galvanostatically. Here, we demonstrate the controlled, quantitative generation of O₂ in a nanofluidic device by modulating the applied potential between inactive and oxidative potentials. However, the capacity to use in situ generated O₂ effectively supposes knowledge of its spatial and temporal distribution, especially when the reagent is generated in flowing conditions for delivery to a downstream reaction site. In order to quantitatively determine the amount and spatiotemporal distribution of O₂ generated in the nanochannel, fluorescence microscopy (Miomandre et al. 2011a, b) is used to optically monitor the reaction using pH-sensitive fluorescein emission (Loete et al. 2006). Finite element modeling is used to calculate the concentrations and distributions of H⁺ and O₂ in the nanochannel by simultaneously accounting for water oxidation, transport phenomena, and acid–base reactions. The change in fluorescence intensity resulting from electrolysis is compared with the numerical model to establish a direct relationship between fluorescence intensity and the H⁺ generation rate which can then be converted to the O₂ generation rate. The resulting quantitative understanding of electrochemical O₂ generation in nanochannel environments will prove useful to its ultimate application in circumstances where precise amounts O₂ must be reliably generated and delivered on demand.

2 Experimental

2.1 Chemicals and materials

Fluorescein (Fluka), hexamethyldisilazane (Sigma-Aldrich), sodium phosphate monobasic monohydrate and sodium phosphate dibasic heptahydrate (Sigma-Aldrich) were used as received. Nanochannel and microchannel masters were fabricated on *p*-type <100> silicon wafers (Montco Silicon Technology). SU-8 photoresist and developer were obtained from Microchips Inc. Poly(dimethylsiloxane) (PDMS) (Sylgard 184, Dow Corning) was used to fabricate microchannels for the device. The nanochannel structure was fabricated with hard poly(dimethylsiloxane) (*h*-PDMS), which contains VDT-731, HMS-301, SIP-6831.2, and SIT-7900, obtained from Gelest Inc. AZ5214E and AZ917MIF (AZ Electronic Materials) were used as photoresists and developer for electrode patterning on microscope slides (Propper Manufacturing). All reagents were analytical grade.

2.2 Channel fabrication

Both nanochannel and microchannel were fabricated by photolithography and soft lithography with *h*-PDMS/PDMS over a silicon/photoresist master. *p*-type <100> silicon wafers were cleaned in piranha solution (3:1 v:v H₂SO₄/H₂O₂) for at least 3 h. Then, the wafer was rinsed with acetone and isopropanol and dried with nitrogen gas. The

nanochannel master was fabricated on the silicon wafer first. Hexamethyldisilazane (HMDS) was spun on the wafer at 3000 rpm to enhance the attachment between wafer and SU-8 2010 photoresist, which was diluted in cyclopentanone (1:4 v:v SU-8 2010/cyclopentanone) and spun on the wafer at 3000 rpm to make a thin layer of photoresist. After baking, exposure, and developing in accordance with the manufacturer's specifications, the photoresist was rinsed with SU-8 developer diluted in isopropanol (3:1 v:v SU-8 developer/isopropanol). Microchannel masters were fabricated on the same silicon wafer following the nanochannel master fabrication. A wafer was spin coated with SU-8 2010 at 1000 rpm after being coated with a thin layer HMDS. The wafer was developed in SU-8 developer after baking and exposure.

The photolithographic master included two microchannels bridged by a nanochannel. The nanochannel (nanoscale in the depth dimension) was 20 μm wide and 3 mm long. Two microchannels were placed 3 mm apart with a reservoir at each end to supply solution flowing into the nanochannel. On the fabricated channel master, a layer of *h*-PDMS was coated by spinning at 3000 rpm. Then, PDMS was poured over *h*-PDMS to a thickness of 3 mm. The PDMS/*h*-PDMS channel was cured at 65 $^{\circ}\text{C}$ for at least 3 h and then peeled off the silicon wafer. The sizes of the channels were characterized by profilometry (KLA-Tencor).

2.3 Electrode fabrication and device assembly

Electrodes were deposited on glass slides by thermal evaporation after patterning with photolithography. AZ5214, a negative photoresist, was used to pattern electrodes on a glass slide. A 100-nm metal layer (95 nm Au over 5 nm Cr) was deposited by thermal evaporation, and photoresist liftoff was performed in an acetone bath. The substrate was washed by deionized (DI) water, acetone, and isopropanol and dried with nitrogen before use. Both the PDMS/*h*-PDMS channel and glass slide with the working electrode were treated with an oxygen plasma (SPI Supplies) and bonded immediately. The two parts were aligned such that the microchannels were placed on either side of the electrode, while the nanochannel bridged the two microchannels bisecting the electrode, viz. Fig. 1. A syringe pump (Cole-Parmer) was connected to a reservoir to control flow in the device. The connecting tubing was sealed in a reservoir with Epoxy (Loctite). The reservoir on the other end of same microchannel was also connected with tubing to the waste outlet. The solution consisting of 10 mM phosphate buffer and 10 μM fluorescein was sparged with nitrogen for 10 min before use.

2.4 Electrochemical and fluorescence measurements

Electrochemical measurements were taken on a commercial potentiostat (CHI 842C, CH Instruments). A Ag/AgCl reference electrode filled with 3.4 M KCl (ET072, eDAQ) and a Pt wire counter electrode were placed in one of the reservoirs during the measurement. Chronoamperometry was executed over potential steps ranging from +0.8 to +2.0 V versus Ag/AgCl to electrolyze water and generate O_2 . The reaction was monitored simultaneously by an epifluorescence microscope (IX-71, Olympus) equipped with a X-Cite 120 PC illumination system (Exfo, Mississauga) and a fluorescein filter (Chroma Technology Inc.). The fluorescence data were collected by an electron-multiplied CCD camera (PhotonMax512, Princeton Instruments) at rate of 6 frames per second.

2.5 Numerical simulations

The fluorescence intensity as a function of potential was calculated using a 2D simulation in COMSOL Multiphysics finite element modeling (FEM) software. A simplified graphic of the model geometry and boundaries and the corresponding descriptions of the boundary conditions are summarized in Fig. S1 and Table S1 of the Supplemental Information (SI). The electrode reactions were encompassed in the model by including a time-dependent flux at the electrode surface for H_2O , O_2 , and H^+ . In order to accommodate the acid–base reactions in a time-dependent simulation, the Henderson–Hasselbalch equilibrium expressions were further split into the contributing forward and backward elementary reaction steps. The forward rate constants for the acid–base reactions were initially set to an arbitrary large value, and the reverse rate constants were calculated, with the constraint that their ratio produced the known equilibrium constant according to Eq. S1, similar to an approach used previously (Bottenus et al. 2009).

3 Results and discussion

3.1 Device characterization and flow rate

As shown in the schematic layout of the device in Fig. 1, the nanofluidic electrochemical reactor is based on a pair of microchannels bridged by a single nanochannel in contact with a planar embedded working electrode. The PDMS microchannels were measured by profilometry and found to be 6 μm in depth and 105 μm in width. Similar measurements of the nanochannel yielded a trapezoidal cross section with a base width of 10 μm , top width of 20 μm , and height of 850 nm. Oxygen plasma treatment and epoxy sealing were adequate to ensure that no leakage occurred during flow injection. The counter electrode was placed in the downstream reservoir to avoid undesired electrolysis products (e.g., H_2 and OH^-) that could interfere with the electrochemical generation of O_2 and its electrochemical and spectroscopic characterization. The fluid velocity in the nanochannel was controlled by syringe pump in the range 0–13 pL s^{-1} , and its magnitude was determined by measuring the movement of quenched fluorescein solution (dark band).

It is well established that pressure-driven flow is difficult to achieve in nanofluidic architectures due to the d^{-3} dependence of required pressure on the nanochannel height, d (Conlisk et al. 2002; Jin et al. 2007). This presents problems by either requiring extraordinarily long times to fill the nanofluidic device or requiring large pressures to attain appreciable flow rates. Many commercially available syringe pumps do not have a volume delivery rate small enough to match the volumetric flow rates characteristic of nanofluidic devices, typically in the range of pL s^{-1} or less. Furthermore, leakage is a problem at all flow rates, when a syringe pump is directly connected to a nanochannel, due to the large pressure that develops. Others have designed systems of pressure regulators and HPLC pumps to monitor pressures and accomplish controllable pressure-driven flow in nanofluidic systems (Tamaki et al. 2006; Tsukahara et al. 2008). However, in this study, a split-flow fluidic network was designed as an integral part of the micro/nanofluidic device, so that a syringe pump could be used at a high flow rate to quickly exchange fluid in the nanofluidic device, while producing a relatively small pressure drop that did not compromise any of the material seals.

3.2 Oxygen generation

Electrochemical generation of O_2 in the nanofluidic channel was monitored by fluorescence microscopy. In contrast to conventional techniques for liquid-phase oxygen measurement, such as Winkler titration (Bryan et al. 1976) and the use of Clark-type electrodes (Clark et al. 1953), this approach does not require the introduction of O_2 reactive chemicals or consume O_2 (Li et al. 1993). Fluorescein, a pH-sensitive dye, was employed to monitor the change in solution pH, resulting from the co-production of H^+ with O_2 , as given in Eq. (1). Although O_2 can have deleterious effects on fluorescence emission generally (Hauser and Tan 1993; Song et al. 1996), Arik et al. (2005) showed that small amount of oxygen have a limited effect on the fluorescence intensity of fluorescein. Thus, since O_2 and H^+ are produced in strict 1:4 stoichiometry, we set about to determine whether changes in solution pH accompanying H_2O electrolysis could be used to quantitate production of O_2 , using the pH-dependent luminescence of fluorescein.

The electrochemical generation of O_2 was first evaluated by cyclic voltammetry in a buffer solution of 10 mM phosphate buffer containing 10 μ M fluorescein. Buffer solution was employed in this study to control the final solution pH within the most sensitive range of the fluorescein, thus permitting the determination of a larger range of O_2 generated. Figure 2a shows a heat map corresponding to the spatiotemporal fluorescence intensity, as the applied potential is swept (vertical axis), and Fig. 2b represents a vertical slice through the heat map, illustrating the fluorescence behavior as an explicit function of applied potential. The behavior of the fluorescein emission intensity, I_f , agrees qualitatively with a reduction in pH, decreasing significantly at positive potentials versus Ag/AgCl, simultaneous with an increase in anodic current (not shown). Fluorescence intensity recovers to the initial baseline level, as the potential is scanned negative, which is due to replacement of the more acidic electrolyzed solution by fresh solution as a result of left-to-right fluid flow. In addition, no other significant oxidation peaks are observed in the potential sweep fluorescence response, indicating that fluorescein is electrochemically stable, i.e., I_f does not change significantly as a direct result of changing the applied potential. We note that the decrease in fluorescence intensity is consistent with the change that might be expected from oxygen quenching; however, the detailed simulations (vide infra) reproduce the emission data quantitatively without a quenching contribution. Finally, the interplay between product generation, fluorescence emission, and mass transport can be clearly seen in the low emission regions of Fig. 2a, which shows the effects of diffusion as distance from the working electrode increases. These results demonstrate that fluorescence microscopy using fluorescein is a suitable technique to monitor electrochemical oxidation of water in situ.

3.3 Potential-modulated oxygen generation

Having shown that oxygen generation can be optically monitored, chronoamperometry was employed to quantitatively control O_2 production. Potential steps ranging from +0.8 to +2.0 V versus Ag/AgCl were applied sequentially while I_f was monitored. Similar to the results shown in Fig. 2, a repeatable decrease in I_f was observed with each positive potential step, and recovery of the fluorescence was seen after return to the resting potential, 0.0 V versus Ag/AgCl, Fig. 3. In addition, the application of increasingly more positive potential steps produces successively larger decreases in I_f , consistent with a lower resulting solution pH.

The change in I_f can, thus, be used to determine the amount of H^+ generated from the potential step by using the pH dependence of fluorescein emission intensity and by integrating over the volume affected. The change in the solution pH is determined by (1) the amount of the electrochemically generated H^+ , (2) the initial solution pH, (3) the concentration of the buffer solution, and (4) the flow rate. For example, for a given amount of generated H^+ , a higher buffer concentration leads to a smaller change in solution pH and I_f . In order to allow the H^+ produced from the electrochemical reaction to be optically monitored with sufficient sensitivity, a 10 mM buffer with an initial pH 7 was used. Under these conditions and with a flow rate of 2.5 pL s^{-1} , the fluorescence intensity decreases by over 80 % at +2.0 V, while there is only ~10 % decrease at +0.8 V, viz. Fig. 3. With applied potential steps smaller than +0.8 V, the change in fluorescence intensity was too small to accurately determine the generation of O_2 through fluorescence emission.

In order to quantitate the generation of oxygen from the electrochemical reaction, a quantitative relationship between I_f and pH is needed. To establish this, a $10 \text{ }\mu\text{M}$ fluorescein solution in 10 mM phosphate buffer was titrated between pH 5 and pH 9, and the resulting I_f measured at different pH values was fit to the Henderson–Hasselbalch equation, as shown in Fig. 4. In terms of the fluorescent species and measured emission intensities, the Henderson–Hasselbalch equation is,

$$\text{pH} = \text{pK}_a + \log \frac{[\text{Fl}^{2-}]}{[\text{HFl}^-]} = \text{pK}_a + \log \frac{I_1 - I_f}{I_f - I_2} \quad (2)$$

where I_1 is the fluorescence intensity of the highly fluorescent dianion, Fl^{2-} , and I_2 is the fluorescence intensity of the less fluorescent monoanion, HFl^- , form of fluorescein. By rearranging the above equation, the fluorescence intensity can be expressed as a function of pH and fit to the data. Performing this fit with I_1 , I_2 , and pK_a as fitting parameters results in a best fit equation from which a pK_a of 7.04 is obtained. Fluorescein is typically found to have a pK_a in the range of 6.3–6.8, and the value determined in this study is in reasonable agreement with these bounds. Furthermore, differences in ionic strength can shift the fluorescein pK_a (Lavis et al. 2007), so this result is reasonable. Using the fit equation, calculated changes in H^+ concentration, and therefore pH, can be obtained directly from the measured fluorescence intensity and compared to finite element simulations.

3.4 Modeling spatiotemporal proton/oxygen distributions

Due to the small dimensions, x , of the extended nanospace, diffusion times, τ , are very short as determined by the scaling law,

$$\tau \sim \frac{\langle x^2 \rangle}{2D} \quad (3)$$

where D is the diffusion coefficient. The time it takes for a proton to diffuse the height of the nanochannel is on the order of a few hundred microseconds, corresponding to a fluid displacement $\sim 100 \text{ nm}$ at the 2.5 pL s^{-1} flow rate used above. Because of this short diffusion

time, the nanofluidic reactor can be approximated as a well-mixed reactor system with no effective boundary layer. Under this assumption, the concentration of protons leaving the electrochemical reactor can be approximated by,

$$C_{\text{final}} = C_{\text{initial}} + \frac{i}{nFV} \quad (4)$$

where i is the steady-state current, n is the stoichiometric ratio of protons to electrons, F is Faraday's constant, and V is the volumetric flow rate. In this model, there is no diffusional limitation for the analyte to get to the reaction site, since the solvent is the analyte.

Figure S1 and Table S1 show the boundary values used in the model, and Fig. 5 shows a heat map of the calculated proton concentration above and near the end of the working electrode after application of a potential step and given sufficient time for the concentration distribution to reach steady-state. It is evident that the assumption of well-mixed behavior is justified, as no gradients are discernible in the vertical direction after the potential is applied. This is due to the large magnitude of the diffusion coefficient of H^+ in water, $D \sim 10^{-4} \text{ cm}^2 \text{ s}^{-1}$ at 300 K and pH 7 (Bard and Faulkner 2001). Furthermore, Fig. 5 shows that the proton concentration slightly increases going from left to right up to the end of the electrode. This is expected, because downstream volume elements contain H^+ generated directly under them as well as H^+ delivered from upstream volume elements.

Using the fit equation for the fluorescence intensity and simulating the convection, diffusion, and reaction processes in this system allows a quantitative comparison between the simulation and the experimental results to be made, as shown in Fig. 6. It is apparent that the model predicts changes in pH and fluorescence intensity quantitatively at potentials larger than 1.2 V, with difference smaller than 5 %. Somewhat larger deviations are obtained at potentials smaller than 1.2 V, which might contain contributions from the uncertainty in the measured Faradaic current used for the simulation. Nevertheless, the model accurately captures the experimental dynamics with little error at potentials between +1.2 and +2.0 V, and no other reactions or transport processes are needed to account for the spatially integrated intensity of the fluorescent marker. In particular, O_2 -associated quenching effects are not included in the model, an assumption that is justified by the excellent agreement with experiment. This observation confirms that the pH-sensitive fluorescein emission can be used to obtain H^+ production rates, even in the presence of co-produced O_2 . Compared with the results in Fig. 3, smaller changes in I_f observed in Fig. 6 are likely due to the larger flow rate used, as predicted by Eq. (4). Thus, understanding of the processes occurring in the nanofluidic electrochemical system allows for the direct correlation between measured current, or fluorescence intensity, and oxygen production. In this experiment with the given flow rate, spatially integrated oxygen concentrations are 0.41 mM for the 2.0 V step and 0.13 mM for the 1.2 V step, as calculated from Eq. (4). In this concentration range, the generated O_2 would produce <1 % fluorescence quenching for 10 μM fluorescein (Arik et al. 2005), which is negligible compared to the effect of H^+ and agrees with our simulation results.

These O_2 concentrations are well within the solubility limits for O_2 in water at 300 K, consistent with the fact that no bubble nucleation was observed in the nanochannel, as one might expect if the solubility concentration limit had been exceeded. Therefore, total O_2 concentrations produced from electrolysis of water can be obtained from either the spatially integrated fluorescence intensity or the measured current from chronoamperometry. In addition, the optical measurement supplies accurate information concerning the spatiotemporal distribution of pH from which the local concentration of oxygen in solution can be obtained by integration.

4 Conclusion

The work reported here describes: (1) a nanofluidic reactor with an embedded planar microband electrode, which is competent for the generation of precisely quantitated amounts of dissolved molecular oxygen by direct electrolysis, suitable for delivery to downstream sites where it can be immediately employed as a reagent; (2) a fluorescence emission method for the measurement of the spatiotemporal distribution of pH, which through the strict stoichiometry of the electrolysis reaction also gives O_2 concentrations; and (3) the development of a computational model that integrates electrochemical reaction velocities, acid–base buffering reactions, and molecular transport to yield H^+/O_2 concentrations in quantitative agreement with experiment, thus lending great credence to the model. The nanofluidic electrochemical reactor was used to control the oxygen generation process by application of well-defined potential programs. Because oxidative water electrolysis yields H^+ and O_2 in a 4:1 stoichiometry, fluorescence emission from the pH-sensitive dye, fluorescein, could be employed to monitor the reaction. The luminescence imaging experiment yields pH values that are integrated over the thickness of the nanochannel, but are spatially resolved laterally. This is not a limitation practically at the nanoscale, because as shown by the finite element calculations in Fig. 5, the H^+ distributions are uniform in the vertical direction across the entire height of the nanochannel.

However, because the diffusion properties of H^+ and O_2 are different, the results yield the spatially averaged concentration of O_2 rather than its detailed spatial distribution. The experimental results of fluorescence measurements were also compared to H^+ concentrations obtained from finite element simulations and were found to be in quantitative agreement. At the flow rates employed here, the in situ generation of oxygen produced total concentrations ranging from 0.13 to 0.41 mM at potential steps from +1.2 to +2.0 V versus Ag/AgCl, again in excellent agreement with finite element simulations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Department of Energy Office of Basic Energy Sciences through Grant DE FG02 07ER15851 (WX and EF) and by the National Institute of Allergies and Infectious Diseases Grant 1R01AI113219-01 (CM). Fabrication and structural characterization of the devices studied here were accomplished at the Notre Dame Nanofabrication Facility and the Notre Dame Integrated Imaging Facility, respectively, whose generous support is gratefully acknowledged.

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Fig. 1.

a Schematic illustration of the nanofluidic electrochemical reactor. **b** Enlarged schematic of device in operation, showing the region near the working electrode in the nanochannel. Microchannels and nanochannels filled with solution are shown in *green*. *Dark band* represents a region of decreased fluorescence intensity due to electrochemical generation of H^+ . **c** Fluorescence microscopy image of nanochannel (*bright*) and working electrode (*dark*) in operation. Image shows initial fluorescence intensity before application of positive potential pulse. **d** Optical image of channel shows fluorescence intensity downstream of the working electrode in the nanochannel after application of +2.0 V versus Ag/AgCl potential pulse (color figure online)

Fig. 2.

a Spatiotemporal heat map of fluorescence intensity developed during potential sweeps. The working electrode is given by the region of time-independent low emission intensity (*vertical dark blue band*), and the *x*-axis distance is measured downstream from the working electrode. **b** Plot of fluorescence intensity as a function of applied potential in a potential sweep cyclic fluorometry experiment. The fluorescence trace represents a single vertical slice through the heat map in panel (a) (color figure online)

Fig. 3. Fluorescence intensity as a function of time (*top*) during repeated positive potential steps (*bottom*). A representative applied potential program versus time is shown at the *bottom*. Potentials steps range in amplitude from +0.8 to +2.0 V versus Ag/AgCl. The flow rate is $\sim 2.5 \text{ pL s}^{-1}$

Fig. 4.
Fluorescence intensity of 10 μ M fluorescein in 10 mM phosphate buffer as a function of pH.
Solid line is a fit to the Henderson–Hasselbalch equation

Fig. 5.

Results of finite element simulation of proton concentration near ($\sim 1 \mu\text{m}$) the end of the working electrode at steady state after application of a positive potential step, $E_{\text{appl}} = +2.0 \text{ V}$. The heat map gives spatial distribution of proton concentration at a greatly expanded scale (shown below)

Fig. 6.

Comparison between experimental (*blue circles*) and simulated (*red squares*) fluorescence intensities at steady state as a function of the amplitude of the voltage step. The experimental intensities were collected during the steady-state portion of long (150 s) voltage steps to avoid contributions arising from voltage transients. *Error bars* represent one standard deviation. Result obtained at an average fluid velocity is $\sim 7.1 \text{ pL s}^{-1}$ (color figure online)