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Real-time and indicator-free detection of aqueous nitric oxide with hydrogel film

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A sensing hydrogel film is demonstrated for real-time and indicator-free detection of nitric oxide (NO) in aqueous solution. The film composed of NO probe 11,16-bisphenyl-6,6,21,21-tetramethyl-*m*-benzi-6,21-porphodimetheno-chloro-zinc(II) and host polymer poly(2-hydroxyethyl methacrylate). The water-containing nature of this sensing hydrogel film makes the surface area high. The response time is bellow 10 s. This sensing hydrogel film also shows high selectivity, sensitivity, and stability in various *p*H values. © *2010 American Institute of Physics*. [doi:10.1063/1.3425895]

Nitric oxide (NO) is a free radical playing important roles in the human body. NO produced endogenously by one cell can transmit through cell membranes and regulates the function of another cell. NO relaxes the smooth muscle in the walls of the arterioles, regulates the blood pressure, and inhibits the aggregation of platelets.[1] It also serves as a signaling molecule in the nervous system.[2] Direct and real-time detection of NO outside the cell helps to unveil how NO relates to certain physiological function. Conventionally, a fluorescence microscopy is utilized for imaging NO in living cells.[3] Slices of cells are prepared sequentially in order to inspect NO distribution in the cells at different time. However, for real-time detection of NO with lifetime around 5 s, such an inspection method including a long sample preparation time is not suitable. Besides, since NO does not fluoresces itself, a fluorescent indicator that selectively interacts with NO is needed to be loaded into the cells before imaging. The cell functions may be affected by the fluorescent indicator. Therefore, a semiconductor electronic device for real-time and indicator-free detection of NO in liquid environment is needed. An integrated semiconductor device[4] consist of a organic light-emitting diode (OLED), a photodetector (PD), and a sensing unit have been proposed for detecting oxygen, glucose, and ethanol as shown in Fig. 1. The OLED is used to excite the sensing unit, and the PD is used to detect the photoluminescence (PL) from the sensing unit. Since the sensing unit will contact with physiological environment, it must have characteristics of stable PL, rapid response, as well as selectivity. So far an integrated device specific to NO does not exist mainly due to a solid sensing unit specific to NO is lacking. In this paper, a sensing hydrogel film specific to NO is developed to serve as the sensing unit. The sensing hydrogel film is made from a blend of host polymer poly(2-hydroxyethyl methacrylate) (pHEMA) and fluorescent probe. The fluorescent probe must be carefully chosen in order to develop a selective sensing film. Metalloporphyrin and its derivatives are known to have strong affinity to NO and selectivity can be tailored by molecular design. Metalloporphyrin is a transition metal complex with a metal ion at the center of a porphine ligand. The major problem of existing porphyrin derivatives is however that they are not luminescent. It is crucial to develop a porphyrin which combines the selective binding with NO and the PL which is sensitive to NO binding.

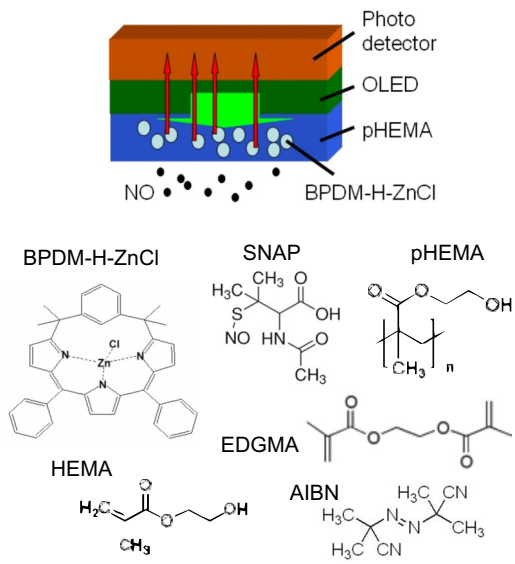

FIG. 1. (Color online) The schematic structure of a biosensor integrated with a polymer light-emitting diode, a photodiode, and a sensing unit. The sensing unit can be the sensing hydrogel film composed of BPDM–H–ZnCl and pHEMA. The molecule structures of the BPDM–H–ZnCl, SNAP, pHEMA, HEMA, EDGMA, and AIBN are also shown.

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In this work, a fluorescent probe 11,16-bisphenyl-6,6,21,21 - tetramethyl-m-benzi - 6,21 - porphodimethenochloro-zinc(II) (BPDM–H–ZnCl) is synthesized from *m*-benziporphodimethene based molecule.[5] The molecular structure of BPDM–H–ZnCl is shown in Fig. 1. Since pHEMA is water permeable, molecules are likely to permeate into pHEMA. The binding between BPDM–H–ZnCl and NO can happen not only near the surface but also deep inside the pHEMA film. This water-permeable property of pHEMA makes this sensing film to have a high surface area without the need to form the material into fiber.[6] The response time of the sensing film to NO is lower than 10 s. The sensing film also shows selectivity and stability in physiological *p*H values.

Except NO bubbled water, S-nitroso-N-acetylpenicillamine (SNAP) is also utilized in this work to control the amount of NO released. An estimation method is developed to obtain the NO concentration based on NO generation and NO decay. As one of the nitrosothiol derivatives, SNAP undergoes a homolytic cleavage of the S–N bond and liberates NO to the environment. The amount of NO generated over time is equal to the amount of SNAP decomposed. The SNAP concentrations at time t is expressed by $n_{\mathrm{SNAP}}(t) = n_{\mathrm{SNAP}}(0)e^{-K_1 t}$. $n_{\mathrm{SNAP}}(0)$ is the SNAP concentration at the beginning, and K_1 is the rate constant. The changing NO concentration per unit time can be expressed as $-dn_{\mathrm{NO}}(t)/dt = K_2 n_{\mathrm{NO}}(t) - K_1 n_{\mathrm{SNAP}}(0)e^{-K_1 t}$. Here $K_2 n_{\mathrm{NO}}(t)$ represents the decay on NO, while $K_1 n_{\mathrm{SNAP}}(0)e^{-K_1 t}$ represents the NO generation. The rate constants $K_1 = 4.813 \times 10^{-5}$ and $K_2 = 0.139$ are determined from the information that the half-life of SNAP is 4 h and the half-life of the NO is 5 s.[7] The NO concentration at time t can be derived from above equation and expressed as $n_{\mathrm{NO}}(t) = K_1/K_2 - K_1 n_{\mathrm{SNAP}}(0)(e^{-K_1 t} - e^{-K_2 t})$. Since the duration of experiment in this work is relatively shorter than the half-time of SNAP, the NO concentration can be assumed to keep same value during the experiment. This assumption is also consistent with the literature[8] which demonstrated that the NO concentration remains at the same value for 45 min then decreases with time. Here, the NO concentration is estimated to be the one after preparing the solution for 1 min. For many SNAP concentrations utilized in this work, SNAP solutions with 1×10^{-4}, 1×10^{-3}, and 5×10^{-3} molar concentration (M) corresponding to 3.46×10^{-8}, 3.46×10^{-7}, and 1.73×10^{-6} M of NO in the solution. The molar concentration is defined as the number of moles of solute per liter of solution.

The sensing film is prepared from a mixture of BPDM–H–ZnCl, 2-hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), and azobisisobutyronitrile (AIBN). pHEMA is formed from HEMA, EGDMA, and AIBN. HEMA is blended with EGDMA in the weight ratio 12:1. After adding 1% AIBN and BPDM–H–ZnCl, the blending solution is poured into a cell and annealed at 80 °C for 10 min to form a film of 180 μm. The PL of sensing film under various NO concentrations are monitored by Hitachi F-4500 fluorescence spectrophotometer. NO is introduced into water by bubbling NO gas or adding SNAP solution into the cuvette. The real-time detection of NO is carried out with a polydimethylsiloxane microfluidic channel.

The PL spectra of BPDM–H–ZnCl in methanol solution are measured after bubbling N_2 and NO as shown in Fig. 2(a). The PL intensity shows no decrease after N_2 bubbling.

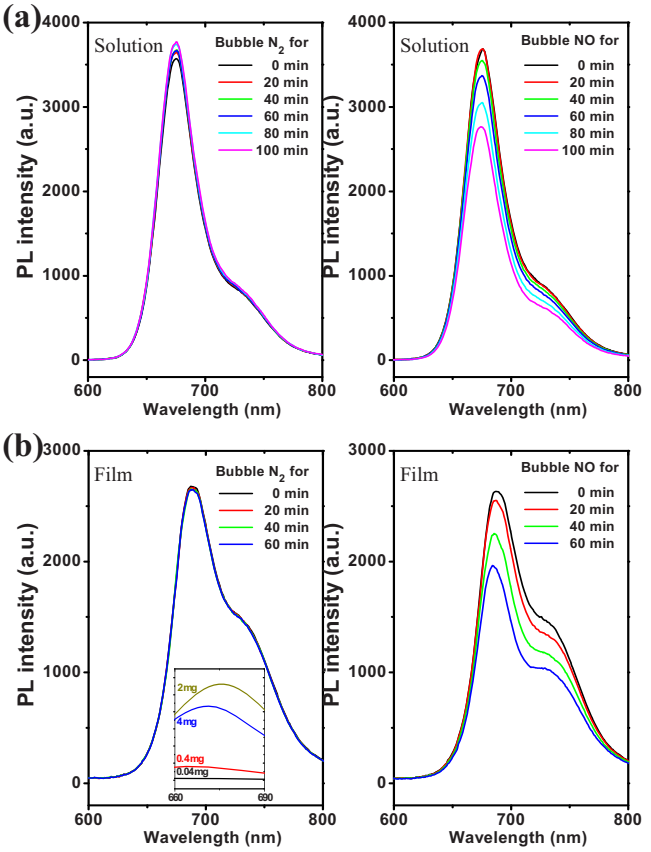

FIG. 2. (Color online) (a) PL spectra of the BPDM–H–ZnCl solution under N_2 and NO bubbling. (b) PL spectra of the sensing hydrogel film under N_2 and NO bubbling. The inset shows the PL spectra of the sensing hydrogel films contain different amount of BPDM–H–ZnCl.

However, after bubbling NO, the PL intensity decreases with time. The variation in PL can be explained as follows. Without NO binding the radiative decay of the exciton is determined by the optical transition matrix element between the metal d_{z^2} orbital and the porphine LUMO. The intense PL suggests a dipole-allowed transition with large wave function overlap.[9] With NO binding the radiative decay of the lowest exciton is determined by the transition matrix element between the NO π^* orbital and porphine HOMO. It is small because the two orbitals are separated in space and have little overlap. Other than NO, nitrate (NO^{3-}), nitrite (NO^{2-}), hydrogen peroxide, oxygen, carbon monoxide, and carbon dioxide are also tested. No PL decrease can be observed after adding these molecules. The BPDM–H–ZnCl is highly selective to NO.

The optimum PL intensity of the sensing film is first obtained by adding 2 mg BPDM–H–ZnCl into 10 ml blending solution as shown in the inset of Fig. 2(b). The PL intensity keeps at a stationary value even the N_2 is bubbled for 1 h as shown in Fig. 2(b). However, the PL intensity decreases with time after NO bubbling. The PL spectrum of the sensing hydrogel film changes in the same way as BPDM–H–ZnCl solution which means BPDM–H–ZnCl probe functions well even after being trapped in pHEMA.

To further understand the real-time detection ability of the sensing film, a microfluidic delivery channel is fabricated to produce NO pulses. The 405 nm laser is used to excite the hydrogel film, and the PL spectrum contributed from the sensing film is monitored with charge-coupled device (CCD) as shown in the inset of Fig. 3(b). The PL intensity of the

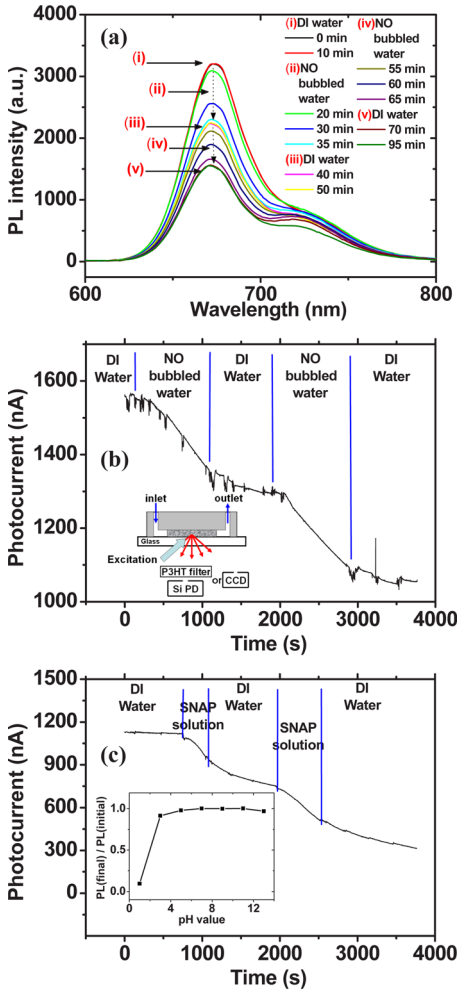

FIG. 3. (Color online) (a) PL spectra of the sensing hydrogel film settled in microfluidic channel. In time periods (i), (iii), and (v), DI water is injected into the microfluidic channel. In time periods (ii) and (iV), NO bubbled water is injected into the microfluidic channel. (b) Real-time response of the photocurrent to the solution injected into the microfluidic channel. NO bubbled water is used as the NO source. (c) Real-time response of the photocurrent to the solution injected into the microfluidic channel. SNAP solution is used as the NO source. The inset shows the normalized PL intensity at 670 nm as the sensing hydrogel film is immersed in solutions with various pH values.

hydrogel film is stable in DI water as shown in Fig. 3(a). DI water or NO bubbled water is injected into the microfluidic channel at different time. The injection of DI water is defined as 0 min. The PL intensity of the film is stable in DI water. Once the fresh NO bubbled water is injected into the microfluidic channel (11 min), the PL intensity decreases with time until the NO bubbled water is replaced by DI water. After refilling the channel with fresh NO bubbled water (51 min), the PL intensity decreases again with time. These phenomena can be seen more clearly after replacing CCD with silicon PD. A thick poly(3-hexylthiophene) (P3HT) film is placed in front of the silicon PD to filter out the light with wavelength below 660 nm. The photocurrent is initially stable in DI water as shown in Fig. 3(b). Each noise in photocurrent results from the replacement of solution. After replacing DI water with NO bubbled water, the photocurrent starts to decrease with time. The decrease in photocurrent ceases once the channel is refilled with DI water. The photocurrent starts to decrease again when the DI water is replaced by NO bubbled water. Figure 3(c) shows the change in photocurrent when the 0.005 M SNAP solution is used as the NO solution. Similar photocurrent variation is observed. The change in the slope of the photocurrent can be seen almost immediately after injecting another solution. The response time is estimated to be bellow 10 s. The slope of the photocurrent is decreased as lower SNAP concentration (1×10^{-3} M) is injected. When the SNAP concentration is further decreased to 1×10^{-4} M, almost no photocurrent variation can be observed. The minimum detectable NO concentration of the sensing film is therefore in the range from 3.46×10^{-8} to 3.46×10^{-7} M. This low concentration detection limit is even lower than the one achieved by electrochemical sensors.[10] The sensitivity of the sensing film is high.

After making sure that the sensing film with the properties of fast response time, great emphasis is put on the stability. The sensing film is examined by solutions with various *p*H values prepared from HCl and KOH. As shown in the inset of Fig. 3(c), the PL intensity keeps almost the same in the range from *p*H 3 to 15. This demonstrates that the sensing film is very stable in the physiological environment. Another important signaling molecule H_2O_2 of 0.01 M is also tested, and no PL decrement can be observed. This result infers again that the sensing film is highly stable and may play a key role in the research area on the NO biosensor.

The amount of NO bubbled into solution is estimated. 1,2-diaminoanthraquinone (DAQ) is dissolved in ethanol for NO bubbling or adding SNAP ethanol solution. Since the decrease in absorption peak of DAQ is proportional to the NO concentration in solution, the NO produced by NO bubbling can be obtained by comparing with the rate of decrease in absorption caused by SNAP. The NO concentration in ethanol produced by NO bubbling is estimated to be 2.9×10^{-7} M.

In summary, a sensing hydrogel film with high surface area is demonstrated for real-time and indicator-free detection of NO. This hydrogel film shows rapid response time, high sensitivity, selectivity, as well as stability. This sensing hydrogel film opens a possibility for a solid-state biosensor specific to NO.

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