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An Optical Fiber Sensor for Simultaneous Measurement of pO_2 and pH

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Abstract

Whether in the monitoring of critically ill patients such as shock, respiratory failure, brain injury, or in major anesthesia surgeries, it is necessary to evaluate the patient's pO_2 and pH. An optical fiber sensor presented is capable of monitoring the presence of oxygen partial pressure (pO_2) and pH in the real-time. The sensor is based on fluorescence sensing of polymer immobilized in the oxygen/pH-sensitive membranes and covalently attached to the optical fiber probe. The design of this sensor uses LED as light source, which is an excitation light source, inducing specific wavelengths of fluorescence on the oxygen/pH-sensitive membrane. The intensity and lifetime of fluorescence are related to the pO_2 and pH. So the pO_2 and pH can be measured by the relationship between the pO_2/pH values and the intensity and lifetime of fluorescence. The signal conditioning system based on DSP and STM32 was used to store and process data, and display test values. The response of the sensor for pO_2 and pH monitoring with nitrogen (N_2) as a balancing gas in the laboratory was performed. Finally, the oxygen/pH sensing scheme presented in this work is intended for using in biological, medical and environmental applications.

Keywords

Optical Fiber Sensor, Oxygen, pH, Fluorescence

1. Introduction

In the current clinical medical diagnosis process, it is usually necessary to monitor many physiological parameters to assess the pathological and physiological health status of patients. The monitoring of pH and pO_2 (oxygen partial pres-

sure) in clinical diagnostics of blood are the two most important physiological parameters. However, most detection of pO_2 and pH are used in Water Quality Monitoring [1] [2], cell culture experiments [3] [4] [5].

The traditional sensor used to detect pH and pO_2 is based on optical sensing techniques [3] [6] [7] and Electrochemical sensors [5] [8] [9]. These electrochemical methods are bulky and invasive, and are susceptible to long-term drift, invasive, requires frequent calibration, and not suitable for simultaneous detection especially for low-oxygen environments.

The optical fiber sensors [10] [11] [12] which consist of glass and ceramic parts are non-invasive, can be used in an exhaust environment properly, and have immunity to electromagnetic interference. So the optical sensing techniques sensors are considered as appropriate method for simultaneous with greater accuracy [6].

Fluorescent sensors have been reported recently [13] [14]. Because of low cost, biocompatibility, high selectivity and excellent optical properties, fluorescent sensors have received widespread attention in biosensing [15]. As representative fluorophores, quantum dots and fluorescein have been reported in glucose and pH detection [16] [17]. As fluorophores can be immobilized on the hydrogel optical fiber, so hydrogel optical fiber combined fluorescence is a promising approach [18].

Therefore, on the basis of the above, this paper proposes a new multifunctional polymer fluorescent sensor material synthesis and preparation, and its application in the study of pO_2/pH dual sensor.

2. Theoretical Background

2.1. Principle of pO_2 Detection

According to fluorescence quenching principle, the oxygen concentration (or pO_2) is inversely proportional to the intensity of the fluorescence exhibited. The calibration curve can be linearized by applying Stern-Volmer Equation (1):

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K\left[\mathrm{pO}_2\right] \tag{1}$$

where I_0 and τ_0 are the respective fluorescence intensity and fluorescence lifetime without oxygen present, and I & τ are the respective fluorescence intensity and fluorescence lifetime with oxygen present, k is the Stern-Volmer constant, which is dependant on the chemical composition of the sensor.

2.2. Principle of pH Detection

Optical pH sensors are based on pH indicator dyes that are immobilised on sensing layers. Analogous to pH indicators applied to aqueous solutions, the basic and acidic forms of these dyes have distinct spectral properties. Hence, a pH change leads to a change in the protonation degree of these molecules, which in turn can be detected as a shift of absorbance or fluorescence signal.

When fluorescein reagent is in acidic condition, its molecule will appear pro-

tonation (obtaining h) in the form of ring opening, and the fluorescence intensity will be weakened. When it is in alkaline condition, its fluorescence molecules will diproton (deprotonation loses h) and exist on lipid form, and the fluorescence intensity will be enhanced.

Therefore, the properties of the above-mentioned carboxy fluorescein under different acid and alkali conditions can be used as fluorescent materials for pH detection.

2.3. Principle of pO_2/pH Detection

The frequency domain method is a technique that allows precise determination of decay time. Depending on its decay time, the luminescence of the indicator has the same waveform but is phase-shifted at a given frequency f_{mod}. The decay time (τ) can be determined via the phase shift Φ according to [19]:

$$\tau = \frac{\tan \Phi}{2 \cdot \pi \cdot f_{\mathrm{mod}}} \tag{2}$$

where f_{mod} is the modulation frequency of the excitation light and τ is the luminescent decay time and Φ is the modulation phase shift of the excitation light. Through the calibration measurement in the air and pure nitrogen environment, the coefficient value K that meets the stern Volmer equation can be determined, and the relationship between the phase shift and the content of the tested substance can be obtained. Finally, the concentration or content of the quencher substance to be tested can be determined through the relevant calculation.

It is proposed a double lifetime reference technology [19] in the frequency domain of the measurement of fluorescent lifetime. When the dual parameter fluorescence indicators meet the requirements of short fluorescence lifetime, the same excitation wavelength and the same emission wavelength as much as possible, the relationship is as follows:

$$\cot \Phi_m = \cot \Phi_{ref} + \frac{1}{\sin \Phi_{ref}} \cdot \frac{A_{ind}}{A_{ref}} \tag{3}$$

where Φ_m is the phase shift of the overall fluorescence, A_{ind} is the amplitude of the measured Fluorescence signal, and Φ_{ref}, A_{ref} is the phase shift and amplitude of the reference Fluorescence signal. When applying this technique to dual sensing, the long-lived fluorescent (such as oxygen) indicator acts as a reference for a short-lived fluorescent indicator (such as pH) so that the observed phase shift depends on the concentration of both analytes. Though the long-lived fluorescent indicator sensitive to oxygen is selected as reference, but keeping its response as oxygen indicator is not used as inert reference, and the phase shift of oxygen sensitive indicator is no longer constant. So the total phase shift (Φ_m) which can be adjusted by changing the ratio of the two parameter fluorescent indicator materials is between 0 and Φ .

Then the phase shift of oxygen indicator was calculated according to formula

(2) as follows:

$$\Phi_{pO_2} = \arctan\left(2\pi f \tau\right) \tag{4}$$

Then the fluorescence intensity of pH was calculated according to formula (1) and (3) as follows:

$$I_{PH} = I_{PO_2} \sin\Phi_{PO_2}\left(\cot\Phi - \cot\Phi_{PO_2}\right) \tag{5}$$

where Φ is the total phase shift. This can be used for designing dual sensors capable of simultaneous detection of two analytes (pO_2 and pH).

3. Experimental Details

3.1. Materials

The membrane for dissolved oxygen is prepared using tris(4,7-diphenyl-1,10-phenanthroline) ruthenium (II) dichloride complex [$Ru(dpp)_3Cl_2$] as an indicator. 3-(Trimethylsilyl)-1-propanesulfonic acid, Poly (styrene) (PS), sodium dodecyl benzene sulfonate(SDS), dichloromethane (CH_2Cl_2) and ethanol are as supportive materials. The pH membrane is prepared using fluoresceinisothiocyanate (FITC) as an indicator and sol-gel film as supportive material with chemicals such as Poly(styrene)-amine (PS-NH_2), N,N-dimethyl-Formamide (DMF), Tetrahydrofuran(THF)and ethanol.

3-(Trimethylsilyl)-1-propanesulfonic acid, fluoresceinisothiocyanate (FITC) and Poly(styrene)-amine (PS-NH_2) are purchased from Sigma-Alorich. sodium dodecyl benzene sulfonate(SDS), N,N-dimethyl-Formamide(DMF), Tetrahydrofuran (THF) and ethanol are purchased from Sinopharm Chemical ReagentCo., Ltd. tris(4,7-diphenyl-1,10-phenanthroline) ruthenium (II) dichloride complex [Ru(dpp) $3Cl_2$] is purchased from Alfa Aesar. Poly (styrene) (PS) is purchased from Huge Biotechnology. Dichloromethane (CH_2Cl_2) is purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. The chemicals purchased are of analytical research grade and used without any further purification.

3.2. Preparation and Fabrication of Sensing Membrane

3.2.1. Preparation of Oxygen-Sensitive Fluorescent Microspheres

The $Ru(dpp)_3Cl_2$ was used as Fluorescent indicator molecule. Poly (styrene) (PS) microsphere was used as carrier material of oxygen sensitive indicator. 1.3 mg of the $RuCl_2$ was added to 500 uL of CH_2Cl_2 solution. This solution was mixed with the solution of 500 uL of PS microspheres added to 5 mL of 0.25% sodium dodecyl benzene sulfonate(SDS) solution. The mixed solution was fixed on the magneton mixer and stirred vigorously for 3 h. The microsphere solution was evaporated to remove CH_2Cl_2 at room temperature. Finally, the supernatant was centrifuged at 13,000 r for 10 min and washed repeatedly with ethanol and water until the supernatant was colorless. **Figure 1** shows the fluorescence images of fluorescent microspheres under ultra violet lamp illumination.

In order to verify whether the fluorescence performance of oxygen sensitive fluorescent indicator changes pre and post filling to polystyrene, and the stability

of polystyrene microspheres pre and post filling with oxygen sensitive fluorescent indicator. In order to verify the stability of fluorescence emission spectrum, the scanning spectrum pre and post filling was tested on fluorescence spectrophotometer. Field emission electron microscopy (SEM) was used to scan the embedded polystyrene fluorescent microspheres and observe whether the shape of the microspheres changed pre and post swelling. **Figure 2** and **Figure 3** show the emission spectrum is the basically same pro and post the indicator filling when the excitation wavelength is 480 nm. **Figure 4** and **Figure 5** show the shape and size of microspheres pro and post embedding is all 500 nm, and with smooth surface and good conductivity.

3.2.2. Preparation of PH-Sensitive Fluorescent Microspheres

The fluoresceinisothiocyanate (FITC) was used as Fluorescent indicator molecule. 2.2 mg of FITC and 9.0 mg of PS-NH2 was added to 1 mL of ethanol DMF and well-mixed. 3 mL of THF homogeneously was added to the mentioned solution

Figure 1. Fluorescence images of fluorescent microspheres under ultra violet lamp illumination.

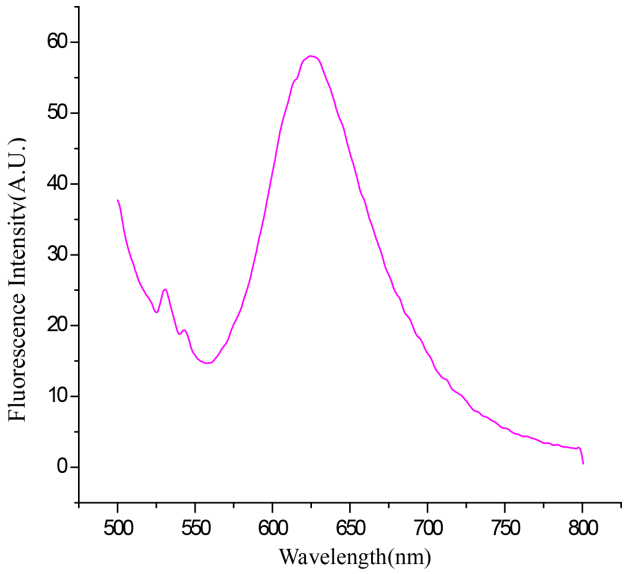

Figure 2. Fluorescence emission spectrum of $Ru(dpp)_3Cl_2$.

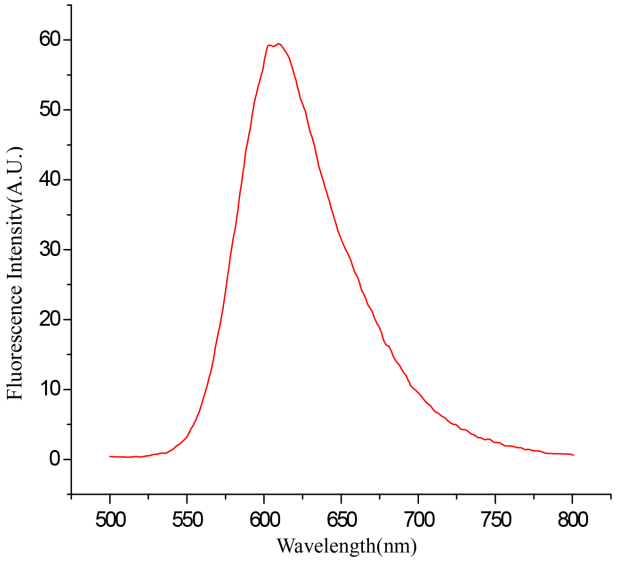

Figure 3. Fluorescence emission spectrum of oxygen-sensitive fluorescent microspheres.

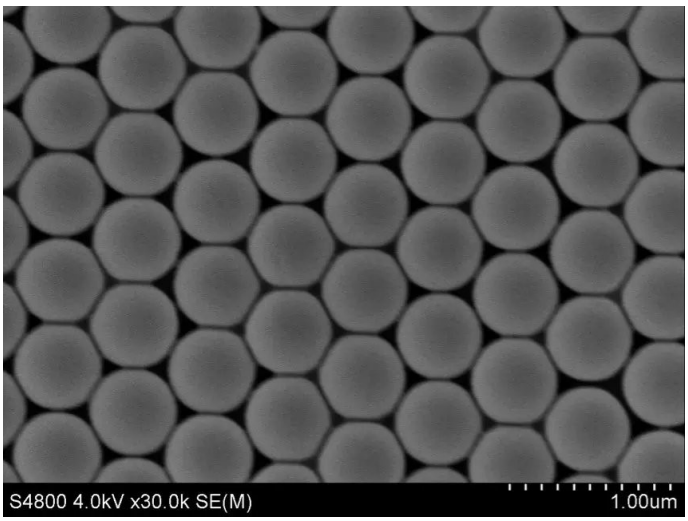

Figure 4. Pre-embedding polystyrene microspheres.

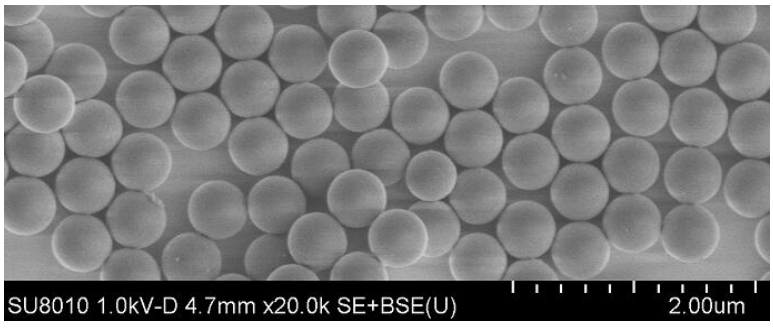

Figure 5. Post-embedding polystyrene microspheres.

by vigorous stirring for 4 h. After the mixture was stirred, 4 mL of ultrapure water stirred while dropping was added to the solution. The mixed solution was centrifugally washed with ethanol and water until the supernatant was colorless,

and pH sensitive fluorescent microsphere was prepared completely.

Both fluoresceinisothiocyanate (FITC) dissolved in ultrapure water and PS-NH-FITC dissolved in ultrapure water were detected by fluorescence scanning. Contrast diagrams were shown in Figure 6 and Figure 7. From the figure, it can be seen that the fluorescence intensity was changed, but the emission peak was basically the same. It can be clearly observed that the PH-sensitive fluorescent microspheres are regular globules, and the particle size is about 200 nm by electron microscope scanning. As it is shown in Figure 8.

3.3. Preparation of Fluorescence Optical Fiber Probe

3.3.1. Preparation of Polyurethane Hydrogel

Polyurethane hydrogel was used as carrier material of Fluorescent microsphere. For the preparation of the sensor membranes, 25 mg of polyurethane hydrogel

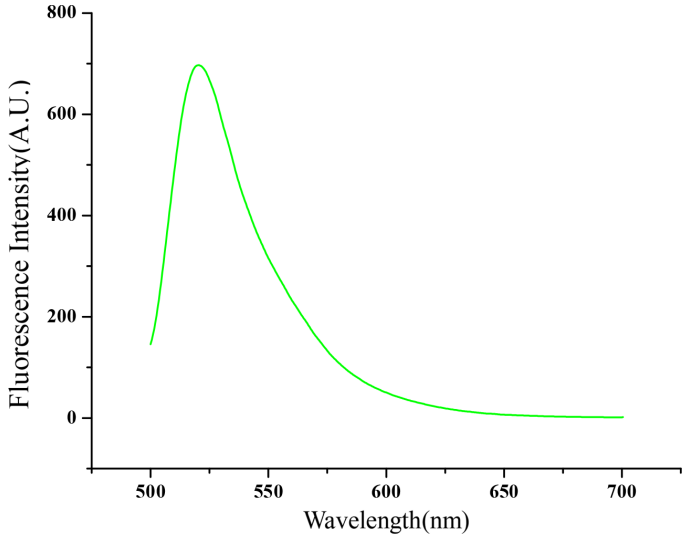

Figure 6. Fluorescence emission spectrum of FITC.

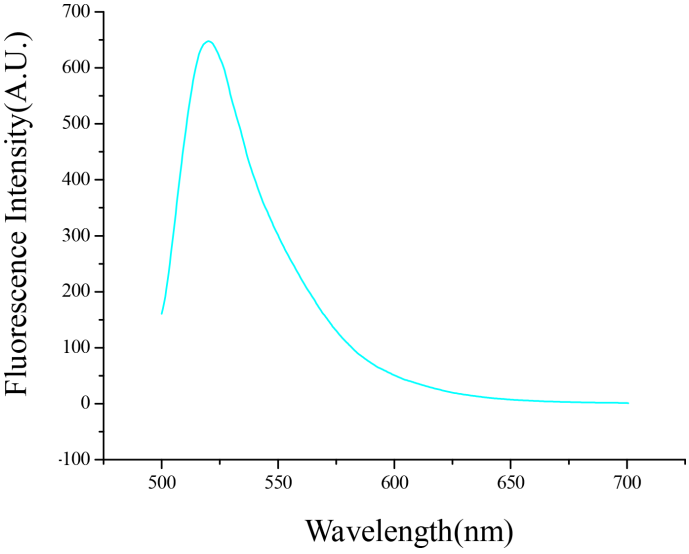

Figure 7. Fluorescence emission spectrum of PS-NH-FITC.

was added to 475 mg of alcohol solution in an ethanol/water (9:1, v:v) mixture. The mixture was vigorously stirred on the magnetic mixer until the polyurethane hydrogel is completely soluble at room temperature, and so a 5% wt/wt solution of Polyurethane hydrogel was obtained.

3.3.2. The Coating Process on Optical Fiber Probe

1 mg of oxygen-sensitive fluorescent microspheres and 2 mg of PH-sensitive fluorescent microspheres were added to 1 mL of ethanol. 2 mL of polyurethane hydrogel solution was added to the fluorescent microspheres solution, and the mixture was vigorously stirred overnight at room temperature. Finally, the front end of the optical fiber probe with a core diameter of 1 mm was immersed in the mixed solution, and then dried for 24 h at room temperature and in ambient air. Then the optical fiber probe detected the pO_2/pH was prepared completely. The photo of fluorescence optical fiber probe excited by excitation light after coating was shown in Figure 9.

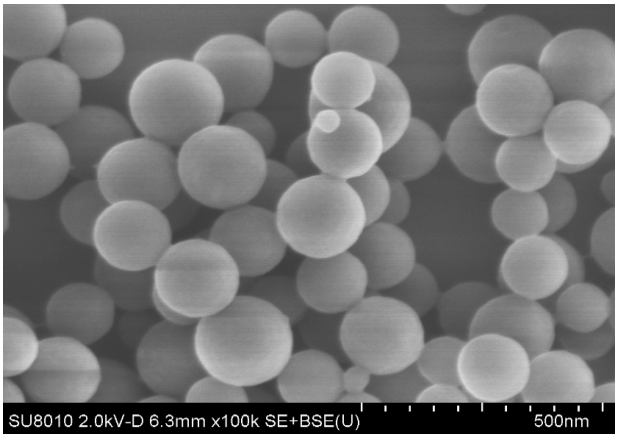

Figure 8. Electron microscope scanning of PH-sensitive fluorescent microspheres.

Figure 9. The photo of the fluorescence optical fiber probe.

4. Results and Discussions

4.1. Assembly of the Optical Fiber Sensing System

Figure 10 shows the optical fiber sensing system as assembled in the laboratory. The LED (the excitation wavelength is 480 nm) was attached to the Y type optical fiber using an APC connector, and so as the light was transmitted to the optical fiber probe. pH/Oxygen sensitive fluorescence indicators are immobilized in a highly crosslinked film and covalently attached to the optical fiber probe. When it meets with oxygen and pH, fluorescence was to be excited. The fluorescence is transmitted to the photoelectric conversion and processing system through Y-type optical fiber. The fluorescence signal is converted into current signal through photodiode, processed into voltage signal, and then transmitted to DSP signal conditioning system through I/O interface. Finally, the amplitude and phase of fluorescence signal and the values of pO_2/pH are calculated and displayed in the STM32 control system.

4.2. Results and Analysis

The performance of dual fluorescence optical fiber sensing system was validated with test solutions of pH 7.53, 6.50 and 4.46, respectively. They were equilibrated with a mixture of nitrogen and oxygen of different proportions to give a pO_2 of 5 and 15 kPa, respectively. Then the driving frequency of LED is 30 kHz and 60 KHZ respectively, the measured values of pH and pO_2 of solution were processed and displayed in the DSP & STM32 system. Table 1 compares the tested and real values of pH and pO_2.

It can be seen from Table 1 that the dual sensor can simultaneously determine pH and pO_2, and the error is small. The deviations between real and tested pO_2 do not exceed 14%, while the highest error in pH determination is 0.27 pH units. In addition, the pO_2 measured in different pH solutions is relatively stable, and the pH measured in different solutions does not change greatly with the change of pO_2. Therefore, it can be concluded from the measurement data that there is no obvious cross-interference between the two parameters while monitoring the two parameters at the same time.

5. Conclusion

In this work, we have developed an optical fiber sensing system which is capable

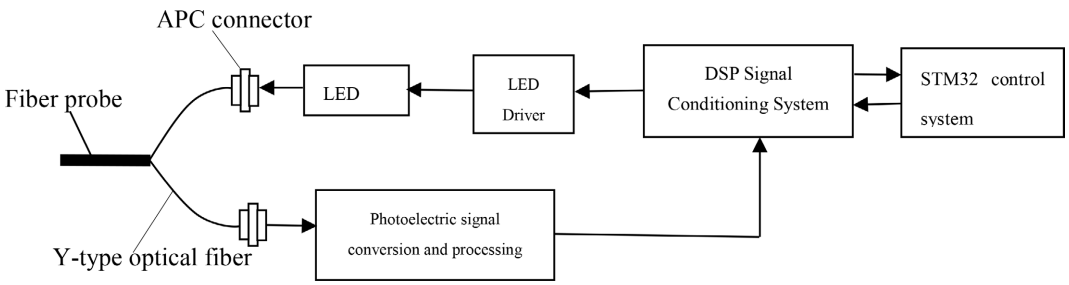

Figure 10. The optical fiber sensing system assembled.

Table 1. Determination of the pH and pO_2 in kPa by the dual sensor in test solutions.

Solution number	Analyte	Actual value	Test value	Absolute error
1	pO_2	5	5.5	0.5
	pH	7.53	7.36	0.17
2	pO_2	15	16.2	1.2
	pH	7.53	7.68	0.15
3	pO_2	5	5.6	0.6
	pH	6.86	6.96	0.1
4	pO_2	15	15.9	0.9
	pH	6.86	6.8	0.06
5	pO_2	5	5.7	0.7
	pH	4	4.07	0.07
6	pO_2	15	16.4	1.4
	pH	4	3.73	0.27

of simultaneous measurement of two important parameters such as pH and pO_2. Comparing the measurement results of the system with the actual values, the dual pO_2/pH sensing system designed and manufactured by the combination of nano fluorescent microsphere sensing material and DSP & STM32 fluorescent signal conditioning system show good stability, anti-electromagnetic interference and high-precision. The new technique can be used for minimal-invasive measurements of high spatial resolution and high-throughput bioprocess optimization.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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