This paper proposes an optical fiber sensing system that can be used for the continuous and real-time monitoring of four brain biomarkers simultaneously with high sensing performance. The sensing system is an AI-integrated system that offers robust and precise biomarker prediction. Ex vivo brain models have been used to fully validate the sensing system and promising results have been achieved, indicating a promising potential for the monitoring of patients with brain injury.
Multiplexed optical fiber sensors for dynamic brain monitoring

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SUMMARY
Dynamic brain monitoring can mitigate traumatic brain injury (TBI) deterioration and enable precise treatment. Despite many studies on brain monitoring systems for neuroscience applications, current technologies are limited in providing a continuous and real-time readout of multiple brain biomarkers simultaneously because of limited sensor performance and signal interference. Regression modeling, being a subfield of machine-learning (ML) algorithm, offers great advantages in signal enhancement and prediction. However, studies on ML-integrated optical fiber sensors for precise brain monitoring have been rarely reported. Here, a multiplexed optical fiber sensor regulated by regression algorithms is developed for the dynamic monitoring of brain pH, temperature, dissolved oxygen, and glucose levels. The proposed sensor has demonstrated excellent sensing abilities and can perform dynamic monitoring of TBI stages in ex vivo brain models. The results indicate the capability of the multiplexed optical fiber sensor for continuous brain physiology reflection, suggesting a promising prospect for clinical applications.

INTRODUCTION
Traumatic brain injury (TBI) is defined as “an alteration in brain function, or other evidence of brain pathology, caused by an external force.”1 According to the report by the Centers for Disease Control and Prevention, TBI is a leading cause of mortality and disability worldwide that is responsible for an estimated 2.8 million accidents and emergency department visits annually in the United States and 1 million in the United Kingdom.2 TBI often involves a primary and a secondary injury. After the primary insult of brain injury, secondary brain injury occurs gradually and may involve an array of cellular processes.3 Dynamic monitoring of the brain metabolism, which involves continuous and real-time monitoring of various biomarkers, will thus be able to identify the deterioration of TBI and prevent complications such as hypoxia from happening.3 Given the increasing demand for precise TBI disease diagnosis and treatment, significant improvements have been made to brain monitoring techniques.4,5 Existing TBI monitoring approaches are primarily based on analyzing basic metabolic panels in cerebrospinal fluid (CSF) and brain interstitial fluid (BIF) using benchtop devices to follow the course of disease in clinical settings.6–7 Licox oxygenation probes and intraparenchymal intracranial pressure (ICP) probes are commonly used to measure brain tissue oxygenation and ICP for TBI patients.6–11 However, these probes can only be used for single biomarker monitoring, and multiple tubes need to be inserted in the brain tissue for multi-biomarker monitoring, which leads to further tissue damage and increases the risk of inflammation.

PROGRESS AND POTENTIAL
Real-time assessment of brain biomarkers via biosensors will allow for the expansion of the diagnostic armamentarium in traumatic brain injury (TBI). Although biomarkers in cerebrospinal fluid can be analyzed in benchtop devices in intensive care units (ICUs), no real-time and multiplexed biomarker sensor exists to assess the brain physiology continuously. The proposed sensor allows for the dynamic monitoring of four brain biomarkers simultaneously to reflect the real-time brain physiology status and the response to TBI and treatments. The research aims to establish world-leading research in continuous biosensors allowing for unique and multiplexed monitoring capabilities for patients with TBIs. The research would have potential impacts on the biosensing and optical sensing field by providing experimental and computational methods for miniaturized and multiplexed optical fiber sensors. The sensing platform would also have potential impacts on TBI patients’ diagnosis and treatment.
Microdialysis (MD) is another commonly used method for the monitoring of multiple brain biomarkers, including glucose, glutamate, electrolytes, and proteins.\textsuperscript{12,13} Despite its advances in multimodality monitoring, MD is a sampling-based method that requires routinely withdrawing the brain fluid to an external benchtop for subsequent in vitro analysis, and therefore can scarcely provide continuous monitoring.\textsuperscript{14} Developing a diagnostic armamentarium for dynamic monitoring of multiple brain biomarkers in CSF and BIF remains an unmet need for precise diagnosis and treatment of TBI.

Various electrochemical (EC) sensors and optical sensors\textsuperscript{14–16} have been recently developed for clinical brain monitoring. EC sensors use external circuits that can generate current to drive the redox reactions of the target biomarkers for quantitative analysis. Brain biomarkers, such as glucose,\textsuperscript{17,18} dopamine,\textsuperscript{15,17} and glutamate,\textsuperscript{19} have been monitored continuously and precisely using EC sensors. Monitoring of multiple brain biomarkers has been achieved by bundling several EC electrodes together or fabricating various biomarker electrodes on a similar substrate such as a platinum electrode or carbon fibers.\textsuperscript{18,20} However, the design of the EC sensors involves at least three stiff metal electrodes and an external circuit, which could lead to high cost, foreign-body response, and poor magnetic resonance (MR) compatibility, impeding their utility in clinical settings.\textsuperscript{21} Electrical signal drift, which might lead to false readout, is another concern for dynamic monitoring. In addition, different biomarkers may have the same redox current signals that could cause poor selectivity.\textsuperscript{15} Optical sensors offer several advantages over EC sensors. Optical sensors detect biomarkers based on the optical property changes (fluorescence and color shifts) of the sensors upon binding with the target biomarkers. Through the detection of fluorescence and colorimetric signals, the recognition of biomarkers would lead to changes in optical intensity or peak shifts in reflection spectra.\textsuperscript{15,22–24} Thus, optical sensors are MR compatible, immune to electromagnetic interference, and have lower signal drifts in continuous monitoring. When the optical sensors are combined with optical fibers, remote and deep brain sensing can also be achieved.\textsuperscript{15} Although studies have demonstrated promising results for single brain biomarker monitoring using optical fiber sensors, multiplexed and continuous monitoring of multiple biomarkers has yet to be fully explored.\textsuperscript{25–27} The reason might be attributable to the optical signal interferences between various optical sensors and the background noise during measurement.

Regression analysis, being a subfield of artificial intelligence (AI) and supervised machine-learning (ML) algorithm, is a promising tool in tailoring precision individualized medical therapies when used with novel biosensing techniques.\textsuperscript{28} In the past decades, numerous studies have demonstrated the advantages of AI in precise biomarker detection. For example, AI techniques, such as support vector machine (SVM) and regression models, have been used with glucose and albumin sensors to provide accurate readouts of biomarker concentrations for health references.\textsuperscript{29,30} In another study, SVM has resolved the fluorescence spectral overlap issue in multiplexed sensing and has achieved simultaneous detection of lysozyme and adenosine triphosphate.\textsuperscript{31} It has also proved the functions of AI in data extraction and data dimension reduction.\textsuperscript{28,22} Despite the benefits that AI possesses for biosensing applications, its integration with optical biosensors for dynamic monitoring in brain has yet to be explored.

Here, a multiplexed optical fiber sensor integrated with a programmed AI prediction platform was designed for dynamic monitoring of pH, temperature, dissolved oxygen (DO), and glucose levels in brain artificial CSF (aCSF) simultaneously. The choice
of these four biomarkers is based on their importance in brain physiology and the fact that to date, the dynamic monitoring of these biomarkers simultaneously in TBI patients has yet to be realized owing to the lack of long-term and real-time multiplexed monitoring systems in clinical settings. Highly sensitive and selective indicators were physically encapsulated in silicate and transparent sol-gel films to reduce leaching and improve stability. The sensing films of the four biomarkers were then fixed onto the tip of a flexible optical fiber through a home-made sheath for in situ sensing. Reflection spectra of the optical fiber sensors were obtained and analyzed based on ML regression models, which were able to eliminate the cross-interferences and provide a quantitative and accurate readout of the biomarker concentrations dynamically. To validate the developed sensing platform for clinical applications, in situ and ex vivo tests using lamb brains as simulated TBI models with four stages were conducted. The designed multiplexed optical fiber sensor demonstrated high sensitivity, selectivity, and stability in monitoring the four brain biomarkers simultaneously, with low time latency. The developed sensor can reflect dynamic changes of the biomarker and identify the transition of TBI stages, indicating its ability in real-time and continuous monitoring of multiple physiological biomarkers for applications in clinical settings.

RESULTS AND DISCUSSION

A flexible optical fiber sensor was designed for multiplexed dynamic monitoring in human brain (Figure 1A). The optical fiber sensor is composed of a flexible optical fiber that is designed to be inserted into brain tissue. On the tip of the fiber, four sensing films are attached through a black sheath for sensing four biomarkers simultaneously. The black sheath was designed to reduce the background noise. A flexible Y-type optical fiber was used to transmit the broadband light to excite the sensing films and detect the reflected light continuously via a spectrometer for dynamic brain biomarker monitoring (Figures 1A and 1B). A microcontroller was used to analyze the reflection spectra and calculate the measured biomarker concentrations. To eliminate the interferences between the four optical sensors and provide a highly accurate and quantitative readout of the biomarker concentrations, ML-based regression models were developed and optimized based on the features of the reflection spectra. The working mechanisms of the four optical sensors were based on the changes in their photophysical properties upon the interactions between the sensing films and the target biomarkers (Figure 1Ci). For stable, robust, and biocompatible sensing, the indicators were encapsulated in transparent silicate films (Figure 1Cii). The sensing film was fabricated using an organic-modified sol-gel process, which avoided the indicators leaching from the films and allowed high permeability of biomarkers into the films. Four sensing films were cut into equivalent quarter circles and multiplexed at the tip of a reflection fiber via a silicone sheath to form a round shape. The sensing film was placed 4 mm above the fiber tip by adjusting the sheath to allow for signal detection of all sensing films via the optical fiber. A reflection isolation film made with glass microfibers was coated on the surface of the four sensing films to enhance reflection optical signals and block the background noise (Figure S1). A photograph of the designed optical fiber sensor is shown in Figure 1Cii, where a black silicone sheath with four sensing films on the top is fixed on the distal tip of an optical fiber. The overall size of the sensing area is 2 mm in diameter. Figure 1D shows the optical setup for in vitro measurement, where one end of the Y-type optical fiber with the sensing films on the tip was inserted into aCSF in a cuvette. One end of the Y-type optical fiber was connected to a light-emitting diode (LED) light driven by an LED controller, and the other end was connected to the spectrometer for reflection spectra analysis. The spectrometer was connected
to a microcomputer for optical signal post-processing and visualization. Several optical features were extracted upon obtaining the captured reflection spectra. By learning the extracted features, regression models were built and implemented on the microcomputer to predict the biomarker concentrations and provide a dynamic quantitative readout of the target biomarkers. The prediction accuracy of the model was also used to evaluate the feasibility of the developed sensing platform. Although the proposed sensing system was only tested for the monitoring of pH, temperature, DO, and glucose, the system can be easily expanded for the sensing of other biomarkers by changing the indicators. Considering the importance of ICP in brain monitoring, it is worth mentioning that the proposed system can also be expanded for ICP monitoring by placing a highly reflective thin film on the sheath, whereby the alterations in ICP would lead to changes in the reflectance intensity.

Figure 1. A multiplexed optical fiber-based sensing platform for dynamic monitoring of biomarkers in brain CSF

(A) Schematic of the designed optical fiber sensor for brain CSF dynamic monitoring in clinical settings. Four sensing films are attached to the optical fiber tip and inserted into brain tissue for in situ measurement.

(B) Schematic illustration of the analytical equipment and data analysis for a multiplexed sensing Y-type optical fiber. (Left) The Y-type optical fiber was used to deliver broadband light to the sensor and transmit the reflected light to a spectrometer. (Right) AI regression models for biomarker concentrations prediction. The prediction accuracy is evaluated with the index R-squared (right).

(C) (i) Sensing mechanisms of pH, temperature (T), DO, and glucose (Gluc) sensors. (ii) Schematic and fabrication methods of the multiplexed optical fiber sensor. Indicators of the four biomarkers were encapsulated in sol-gel networks and integrated into the fiber tips. An isolation film was used to reduce the background noise and enhance the reflection signals. (iii) Photograph of pH, temperature, DO, and sensing films being fixed at the distal tip of the fiber through a silicone sheath. Scale bar, 1.0 mm. Inset: photograph of the isolation film. Scale bar, 2 mm.

(D) Photograph of the integrated AI-based multiplexed sensing platform, which includes a flexible reflection fiber, an LED source, a spectrometer, a Y-type fiber, and a microcomputer to visualize the spectra and process the signals for quantitative analysis. Scale bar, 1.5 cm.
Figure 2. Characterizations of the optical fiber pH sensor in PBS solutions (10 mmol L\(^{-1}\), 25°C)

(A) 3,3',5,5'-Tetramethoxybenzyl-2,2'-dimethoxyanilino-5,5'-dicyanodiamine was used as the reversible pH indicator, which shows different colors at different pH values.

(B) Reflection spectra of the optical fiber pH sensor when sensing in pH 6.0, 6.4, 6.8, 7.2, 7.6, and 8.0 buffer.

(C) Reflectance intensity of the pH sensing film at 623 nm under pH 6.0–8.0 buffer solutions. Shadows represent the physiological range of pH in human CSF. Insets show the color changes of the sensing film.

(D) 1931 CIE chromaticity diagram. Color changes from pH 6.0 to pH 8.0 were denoted.

(E) Reversibility test of the pH sensor in repeated sensing of pH 6.0 and pH 8.0 PBS solutions.
Performances of the optical fiber pH sensor

pH regulates various cellular processes and influences the activity of ion channels and enzymes in the brain system.\(^{33,34}\) pH also serves as an indicator of TBI, whereby a reduction of pH is observed because of disrupted anaerobic metabolism and increased acetic acid.\(^{35}\) To date, several approaches have been developed to follow the progress of brain diseases for brain pH monitoring, including fluorescence spectroscopy,\(^{36,37}\) electrochemical sensing,\(^{38}\) and colorimetric sensing.\(^{39}\) In this study, the determination of pH in aCSF is based on the colorimetric property of a pH sensing film at the fiber tip.\(^{3,39}\)

0-Tetrabromo-m-cresolsulfonphthalein was selected as the pH indicator, since it has a p\(K_a\) value in a physiological range when encapsulated in a sol-gel film that is suitable for brain pH measurement for both TBI patients and healthy people (Figure 2A).\(^{40,41}\) Tetraethyl orthosilicate (TEOS) is one of the most widely used precursors for the sol-gel process. However, pure TEOS fabricated sol-gel films suffer from severe film cracks, which may lead to low film uniformity and high leaching. Glycidoxypropyltrimethoxysilane (GLYMO) is an organic precursor and has long carbon chains in the chemical structure. Using TEOS and GLYMO together as precursors can prevent cracking and improve the morphology and uniformity of the sensing film.\(^{42}\) Therefore, an organic-inorganic hybrid sensing film was fabricated by encapsulating the pH indicator within the silicate matrix formed by TEOS and GLYMO through the sol-gel process. Surfactant Triton X-100 was applied to reduce the surface tension of the sensing film to prevent cracking and indicator leaching and also improve the uniformity of the sensing film. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) images indicated a very smooth surface of the sensing film (Figure S2). In addition, the porosity of the sensing film allows for biomarkers to interact with the encapsulated indicators. The pH sensing film showed a green color at neutral pH and changed to red or blue color in an acidic or basic environment due to protonation or deprotonation, respectively. The sensing film was optimized by using different fabrication formulas to achieve high uniformity, stability, and sensitivity (Table S1; Figures S3 and S4). It was found that an increased concentration of GLYMO could improve the uniformity of the sensing film but increase the response time owing to the lower porosity in the organic-inorganic sensing film. Moreover, the increase in GLYMO resulted in reduced sensitivity, since GLYMO could decrease the p\(K_a\) of the sensing film.\(^{43}\) Therefore, a concentration molar ratio of TEOS/GLYMO at 1:0.1 was used to ensure the sensing film had high surface uniformity, high sensitivity, and short response time. As shown in Figure 2B, the pH sensing film was tested in phosphate-buffered saline (PBS) solutions under pH ranging from 6.0 to 8.0 at room temperature (25 °C). The reflected spectra of the pH sensing film in PBS with different pH values were obtained by the spectrometer and normalized to the light source. The maximum absorbance of the pH indicator appears at 623 nm in the reflection spectra. After being encapsulated in the silicate gel, the reflection intensity of the pH sensing film increased linearly as the pH value increased from 6.0 to 8.0 (Figure 2C). According to the calibration curve, a sensitivity of 0.12 per pH unit was achieved for the sensing of pH 6.0–8.0, which covers the physiological range of brain CSF. Photographs of the optical pH sensors showed distinct color changes from yellow...
to blue as the pH value increases from 6.0 to 8.0 (Figure 2C, inset). 1931 CIE chromaticity diagrams were analyzed using the RGB (red, green, blue) value of the optical pH sensor (Figure 2D). Reversibility, repeatability, and response time were further determined by dipping the optical fiber sensor in pH 6.0 and pH 8.0 buffer solutions (Figure 2E). High reversibility and repeatability of the sensor with a standard error of 2.4% were observed after ten cycles. The response time of the pH sensor was measured as less than 100 s as the pH increased from 6.0 to 8.0, while response time of 350 s was observed for the change from pH 8.0 to pH 6.0. The “asymmetry” of the response time is in line with the previously reported sol-gel sensor.44,45 No obvious variation in the reflection intensity of the optical pH sensor (pH 7.4) was measured after exposure to a broadband light (20 mW) for 24 h, indicating high stability of the pH sensor for long-term use (Figure 2F). Leaching of the indicators from the sensing film is another index that matters for long-term clinical application of the sensor, since the excessive leached indicators from the film increase cytotoxicity to biological tissues. The leaching results display a relatively high leaching rate of 3.09% at pH 8.0 and a lower rate of leaching of 1.13% at pH 6.0 after 3 days (Figure 2G). The differences in leaching behavior might be attributed to the larger sol-gel pore size in basic solutions. The temperature dependence of the fabricated optical pH sensor was tested from 25°C to 40°C, and no significant temperature interference was observed (Figure 2H). The sensor also demonstrated high robustness when used under different physiological pressures (Figure S5A). Two groups of selectivity tests were conducted to investigate the sensing performance of the optical fiber pH sensor in the presence of potential interferents in brain CSF such as electrolytes, metabolites, and neurotransmitters (Table S2). In the first group, the pH of the selectivity-testing PBS solution was readjusted to 7.4 after adding the interferents before sensing. In the second group, the pH was not readjusted to 7.4 before sensing (Figures 2I and 2J). The results of the selectivity tests show that no major interferences in the two groups were observed, demonstrating high selectivity and an excellent capability of tracking pH changes using the designed multiplexed fiber sensor.

Performances of the optical fiber temperature sensor
Cerebral processes are extensively affected by temperature fluctuations.46,47 Owing to the high temperature-dependent nature of the brain’s energy expenditure, the brain’s thermal regulatory capability can be an indicator of its anatomical and physiological architecture, and can reflect its processing capacity.48 To date, clinical studies have revealed a strong relationship between brain temperature elevation and brain injuries.49 Therefore, brain temperature monitoring has become routine for TBI treatment in clinics. One of the potential methods for brain temperature measurement is using a thermochromic liquid crystalline (TLC), which exhibits good reversibility, quick response, and high resolution. In this study, TLCs were obtained by mixing cholesteryl nonanoate (CN), cholesteryl oleyl carbonate (COC), and cholesteryl benzoate (CB) at a weight ratio of 0.35:0.55:0.10 (Figure 3A). When melted together, the three esters retain their chemical properties while experiencing weak inter- and intramolecular interactions. Chiral molecules are rigid and rod-shaped molecules that can form a layered structure with distinct molecular orientations along the helix axis under temperature changes (Figure 3A). The different twisting arrangements can lead to variations in the reflected light of the TLC. The temperature detection range can be varied by mixing them at different ratios.

The temperature sensor was tested in 0.1 mmol L⁻¹ PBS solution (pH 7.4), ranging from 34°C to 42°C using the same reflection sensing setup as for the pH sensor. The obtained reflection spectra and their color changes in correlation with various
buffer temperatures were analyzed (Figure 3B). When the temperature is raised from 34°C to 42°C, the reflectance peak shifts from 525 to 470 nm linearly with a sensitivity of 6.77 nm/°C ($R^2 = 0.99$) (Figure 3C). The optical temperature sensors were imaged by a smartphone camera, and an evident color change from green to violet was recorded as the temperature increased (Figure 3C, inset). A 1931 CIE chromaticity diagram was analyzed based on the RGB value of the temperature sensor and

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**Figure 3.** Characterizations of the optical fiber temperature sensor in PBS solutions (10 mmol L$^{-1}$, pH 7.4, 25°C)

(A) Cholesteryl esteric molecules were used for temperature sensing based on the thermochromic property of liquid crystals.

(B) Reflectance spectra of the temperature sensor for sensing in PBS solutions at 34°C–42°C.

(C) Calibration curve of the temperature sensor for sensing in PBS at 34°C–42°C. Shading represents the physiological range of human CSF temperature.

(D) 1931 CIE chromaticity diagram. Color changes from 34°C to 42°C were noted.

(E) Repeatability of the temperature sensor for temperature measurement in PBS solutions between 36.0°C and 40.0°C.

(F) Stability of the temperature sensor in sensing PBS solution at 37°C for 24 h. Inset circles show the corresponding color changes of the sensing film in the first 3 h.

(G) Performance of the temperature sensor under different pressure ranges. Error bars ($n = 3$) represent the standard deviation of the mean of three samples. Scale bars, 1 mm.
demonstrated distinct color changes as the temperature shifted from 34°C to 42°C (experimental procedures and Figure 3D). Repeatability of 3.7% of standard error and an average response time of less than 2 s after 20 cycles were observed upon temperature changes between 36°C and 40°C (Figure 3E), indicating the potential of the optical fiber temperature sensor for real-time and continuous brain temperature monitoring. No obvious variations in the reflectance of the temperature sensor (pH 7.4, 37°C) were measured after exposure to the broadband light (20 mW) for 24 h, showing high stability of the temperature sensor for long-term use (Figure 3F). Since the liquid crystal is also sensitive to pressure, the reflection spectra of the temperature sensor were measured under physiological pressure ranges at different temperatures (Figure 3G). The pressure was varied by dipping the optical fiber temperature sensor into the PBS solutions at different depths. The pressure can be calculated as the product of the gravity constant, the solution density, and the depth of the sensor in the solution. The results indicate that no obvious pressure influences the sensor output, suggesting the ability of the sensor for in vivo and deep brain CSF temperature monitoring. The leaching test of the temperature indicator from the sensing film in pH 7.4 PBS solution was also conducted. No leaching was observed in the PBS solution after 3 days. Therefore, the fabricated optical fiber temperature sensor is capable of in situ and continuous monitoring of temperature in aCSF.

**Performances of the optical fiber DO sensor**

The brain consumes 20% of the whole body’s oxygen even though it accounts for only 2% of body weight. Brain oxygen metabolism plays an important role in whole-brain metabolism and even whole-body metabolism. Sufficient brain oxygen supply is the first step to ensure a normal brain function and is critical for the treatment of brain injury. Electrochemical oxygen sensors are now commonly seen in intensive care units (ICUs) and operating rooms for bedside monitoring of brain oxygen saturation levels.

Here, a fluorescence detection method was developed to monitor the brain oxygenation by encapsulating fluorophore tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride complex (Ru(dpp)) as the DO indicator within a sol-gel film, which was integrated at the fiber tip (Figure 4A). Based on the fluorescence quenching effect of oxygen, the emission intensity of Ru(dpp) is negatively correlated with the level of DO in brain CSF. When being excited with a 450 nm LED light, an emission light at 600 nm was detected. The DO calibration buffer solutions were prepared by mixing 100% DO buffer with 0% DO at a certain ratio to achieve buffers with different DO concentrations. A DO benchtop meter was used as a gold standard for the preparation of the buffers and the calibration of the sensing films. This buffer preparation method was tested three times to obtain gradient DO buffers, and good repeatability was achieved (Figure S6B). As depicted in Figure 4B, when the DO level increased from 1 to 7 mg L⁻¹, the emission intensity of the DO sensor decreased to 76% of the original intensity. The maximum emission intensity of sensing film at different DO levels was calibrated according to the Stern-Volmer equation (Equation 1 and Figure 4C). The Stern-Volmer coefficient (Ksv) was determined as 0.08. Similar to the “asymmetry” response time observed in pH sensing films, when the DO level increased from 1 to 7.5 mg L⁻¹ a response time of 200 s was recorded, while a response time of 100 s was observed for the reverse process (Figure 4D). The sensing film can be used reversibly for the measurement of the DO level, although a slightly decreasing trend in the film emission intensity was observed because of the photobleaching effect (Figure 4E). In the photostability test, the emission intensity was found to decrease during the 24-h exposure under a stable 7.5 mg L⁻¹ of the DO level (Figure 4F). The DO sensor became stable after the 6 h exposure. The
Figure 4. Characterizations of the optical fiber DO sensor in PBS solutions (10 mmol L\(^{-1}\), pH 7.4, 25°C)

(A) Schematic of sensing mechanism. DO sensing is based on the oxygen-quenching effect of the fluorophore Ru(dpp). The indicator was encapsulated within a sol-gel network and integrated on the tip of the optical fiber.

(B) Fluorescence spectra of the sensor under different DO levels.

(C) Calibration curve of the DO sensor using gradient DO PBS solutions. Shading represents the physiological range of DO concentration in human CSF.

(D) Response time of the DO sensor under the alternated 7.5 mg mL\(^{-1}\) and 0 mg L\(^{-1}\) DO buffers.

(E) Reversibility of the DO sensor.

(F) Photostability of the sensing film for 24 h sensing.
performance of the DO sensor under different environmental conditions was also tested. When the temperature increased from 25°C to 40°C, an up to 30% decrease was observed in the emission intensity (Figure 4G), indicating a strong temperature dependence of the sensing film. No evidence has shown the influence of pH or pressure changes on the emission intensity of the Ru(dpp) sensor (Figures 4H and 55B). To further investigate its potential performance when being implanted in brain CSF, the selectivity of the sensor over the interferences of various neurochemicals was tested (Figure 4I). The major interferences in aCSF showed negligible influence (<5%) on the emission intensity compared with the intensity measured in pure PBS, suggesting a high selectivity toward DO. Similar to the pH study, the DO sensing film also has a relatively high leaching percentage of 3.67% in PBS at pH 8.0 after 3 days and relatively lower leaching of 1.72% in PBS solution at pH 6.0 (Figure 4J). Taken together, the fabricated optical fiber DO sensor demonstrated a promising result for real-time and continuous monitoring of brain DO in aCSF.

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\frac{I_o}{I} = 1 + K_v \cdot \text{Concentration (O}_2\text{)}.
\]  
(Equation 1)

**Performances of the optical fiber glucose sensor**

Glucose is a metabolite that serves as the primary energy supply to the brain. In TBI patients, dysregulation of glucose metabolism may cause various further brain pathophysiological disorders, such as secondary brain injury\(^{52}\) and neurodegeneration.\(^{53–55}\) The first generation of commercial devices for glucose monitoring was designed based on glucose oxidase catalyzed electrochemical sensors,\(^{56}\) in which the product of the glucose redox reaction hydrogen peroxide (H\(_2\)O\(_2\)) is measured for glucose measurement. Glucose oxidase catalyzed reaction has also been used in optical sensors for glucose monitoring based on oxygen transduction\(^{57}\) and H\(_2\)O\(_2\) transduction.\(^{58,59}\)

The proposed optical fiber glucose sensor was fabricated by integrating the glucose sensing film to the tip of the flexible optical fiber. The glucose sensing film was fabricated by encapsulating 3,3',5,5'-tetramethylbenzidine (TMB) with glucose enzyme for measuring generated H\(_2\)O\(_2\) in the glucose redox reaction. In the first step, glucose is oxidized and H\(_2\)O\(_2\) is produced, which is subsequently interacted with TMB to induce a color change in the sensing film (Figure 5A). The color change of the sensing film is due to the changes in the absorbance of TMB at 660 nm. The glucose sensor was calibrated with its reflection spectra in PBS solutions under glucose concentrations ranging from 0.0 to 6.0 mmol L\(^{-1}\) (Figure 5B). The reflection intensity decreased 2.7 per mmol·L\(^{-1}\) linearly as the glucose level increased from 0.0 to 6.0 mmol L\(^{-1}\) (R\(^2\) = 0.98) (Figure 5C). The colors of the glucose sensing films during the measurement were recorded using a smartphone camera, which showed a color transition from yellow to dark blue as the glucose concentrations increased from 0 to 6 mmol L\(^{-1}\). A 1931 CIE chromaticity diagram was analyzed with algorithms (experimental procedures and Figure 5D) for accurate demonstration of the color shifts of the film, which indicated a good ability in sensing glucose concentrations within 0–6 mmol L\(^{-1}\). A response time within 30 s was obtained, indicating a fast response for real-time monitoring of glucose in aCSF (Figure 5E). No obvious variation in the reflection intensity of the glucose sensor was observed in glucose of 2 mmol L\(^{-1}\) in
Figure 5. Characterizations of the optical fiber glucose sensor PBS solution (10 mmol L$^{-1}$, pH 7.4, 25°C)

(A) Schematic of sensing mechanism.

(B) Reflectance spectra of the sensing film under different glucose concentrations.

(C) Calibration curve of the sensor for sensing 0 mmol L$^{-1}$ to 6 mmol L$^{-1}$ glucose solutions. Shading represents the physiological range of glucose concentration in human CSF.

(D) CIE image of the sensing film color changing from 0 to 6 mmol L$^{-1}$ of glucose.

(E) Response of the sensing film when the glucose concentration increased from 2 to 5 mmol L$^{-1}$.

(F) Stability of the sensing film over 24 h. Inset circles show the corresponding color changes of the sensing film in the first 3 h.
the PBS solution after exposure to broadband light (20 mW) for 24 h, indicating the ability for long-term use (Figure S5F). The temperature dependence of the fabricated optical fiber glucose sensor was tested by using a 96-well microplate from 25°C to 40°C. A slight increase in the absorbance was observed, which might be due to the increase in the activity of glucose oxidase (Figure 5G). In the pH dependence test, the glucose sensing film was tested in PBS solutions with different pH values (6.0–8.0). The results showed a negative relationship between the pH value and film absorbance when pH increased from 6.0 to 6.5 and a positive relationship when pH increased from 6.5 to 8.0 (Figure 5H). This might be due to a higher glucose oxidase activity in slightly acidic solutions. The sensor can also be used under various pressures, indicating high potential for in vivo brain measurement (Figure S5C). The performance of the glucose sensor in brain aCSF in the presence of various interferents such as electrolytes, metabolites, and neurotransmitters was also tested (Figures S1 and S5J). No obvious variations in the reflection intensity were observed when the interferents were added to the standard 2 mmol L⁻¹ glucose PBS solution (Figure 5I). In addition, the glucose sensor did not show a major response to the interferents in glucose-free solutions (Figure 5J), indicating high selectivity of the optical fiber glucose sensor and good capability of real-time tracking of acid or base neurochemicals. Less than 4% of indicator leaching was found in the buffer solution after immersing the sensing film in PBS solution for 3 days (Figure S7).

These characterization studies on the designed optical sensors have demonstrated good sensing performance of the sensors for brain pH, temperature, DO, and glucose sensing. Compared with commercially available devices for brain monitoring (MRI, MD) or the state-of-the-art EC sensors, the proposed multiplexed sensing sensors achieved comparable sensitivity, reversibility, stability, and selectivity. In terms of implantable diagnosis, optical sensors could also provide battery-free and remote sensing, and they can be easily miniaturized. However, the response time of the proposed fiber optical sensors could take several seconds to minutes to respond and might be slower than EC sensors. However, it is worth mentioning that the sensing speeds (100–350 s for the pH sensor, 2 s for the temperature sensor, 100–200 s for the DO sensor, and 30 s for the glucose sensor) are sufficient for TBI monitoring, as the disease usually progresses over several minutes to hours.

Regression modeling-integrated dynamic and multiplexed monitoring in aCSF and an ex vivo brain model

After the pH, temperature, DO, and glucose sensors were tested separately, each of them was cut into a quarter with diameter of 2 mm. The sensing films were placed evenly on a round glass slide and a silicone tube of 2 mm outer diameter to fabricate a multiplexed sensing film, followed by integrating on the tip of the flexible optical fiber. The sensor was then tested for multiplexed sensing in aCSFs (Table S3). Aiming to achieve dynamic monitoring of brain CSF biomarkers simultaneously, AI models were trained using the reflection spectra features, which were obtained in the calibration tests, for quantitative readout of the biomarker concentrations. The
The calibration curves of aCSF measurement using the multiplexed optical fiber sensor are shown in Figure 6. In the measurement, the mixed reflection spectra of four sensing films were captured using the spectrometer, and the reflection intensities at different wavelengths were used for detection of different biomarkers. In each biomarker’s calibration, the other biomarker levels remained constant in the aCSF buffer. The reflection intensity at 623 nm was used for pH calibration. When the pH value changed to 1 pH unit, a 0.11 change in the 623 nm reflection intensity was observed (Figure 6A). Peak shifts between 470 and 540 nm were used for temperature calibration. For every change of 1 °C in the temperature, a 4.77 nm shift was visualized in the reflection spectra (Figure 6B). The wavelength of interest for DO measurement was 600 nm under the excitation of a 450 nm LED. A sensitivity of 0.06 (a.u.) per change of 1 mg L⁻¹ of DO concentration was obtained (Figure 6C). In the calibration of the glucose sensor, the relationship between 660 nm of reflection intensity and glucose concentration was analyzed, and a sensitivity of 0.16 (a.u.) per change of mmol·L⁻¹ was achieved (Figure 6D). Multiplexing the sensors to one channel showed reduced sensitivity of the sensors owing to overlapping in the optical spectra. pH (Figure 6E), temperature (Figure 6F), and DO (Figure 6G) sensors also demonstrated high reversibility in the aCSF measurement, indicating that the...
developed multiplexed optical fiber sensors were able to be applied for brain dynamic monitoring. The multiplexed sensor also maintained high stability in continuous measurement (Figure S8). The color changes of the multiplexed sensing film in the aCSF were visualized and imaged using a smartphone camera (Figure 6H). It can be seen from these images that the color of the sensor was directly related to the biomarker levels of the measured solution. When the biomarker level was changed, the color of the sensor changed accordingly.

Ex vivo measurement was conducted using lamb brains in an artificial skull to further validate performances of the developed multiplexed optical fiber sensor (Figure 7A). In the clinic, brain catheters were inserted in either brain superior sagittal sinus to withdraw brain CSF or brain tissue for BIF and CSF measurement. In this study, lamb brains were immersed in standard buffer solutions and the fiber sensor was inserted 4 mm into the brain tissue during sensing calibration. The calibration curves of each sensor were measured, and linear relationships were obtained (Figure S9). However, unavoidable crosstalk between the sensing film signals and background noise were observed throughout the ex vivo calibration experiment. To reduce the crosstalk interferences on the multiple biomarker measurement and compensate the environmental effects for accurate and robust readout of multiple biomarker concentrations, four ML models using different regression methods were trained and compared for the prediction of the concentrations of four biomarkers simultaneously and accurately (Figure 7B). Bayesian regression showed the best accuracy toward the prediction of biomarker concentrations (Table S5). In training the models, ten features were extracted from the obtained reflection spectra and fed into the ML models (Figure S10 and Table S4). The features included the intensities at 623 nm, 600 nm, and 660 nm, and spectra peaks for pH, DO, glucose, and temperature level calculation. Other features including reference intensity at 450 nm, valley location, and valley height were also extracted from the spectra to eliminate the crosstalk between the four sensing films. The temperature and the excitation time length were also extracted and utilized as two additional features to compensate the temperature influence and photobleaching effect on the DO sensor. A Bayesian model was selected and fed with the features, because of its advantage in taking account of prior knowledge when building the prediction models. The models were trained with 70% of the dataset and tested with 30% of the dataset (Figures 7C–7F), and 4-fold validation was applied for the evaluation of the model performance. $R^2$ values of 0.84, 0.93, 0.94, and 0.94 were obtained for the pH, temperature, DO, and glucose prediction, respectively, indicating high prediction accuracy of the ML model in predicting biomarker concentrations. By means of the ML algorithms, the temperature influences and photobleaching of the sensors were successfully compensated for the sensing of aCSF DO level. The models also provided quantitative readouts of all biomarkers within a short computational time (<1 ms). The AI-integrated multiplexed optical fiber sensor demonstrates a promising possibility for accurate and multiplexed brain biomarker monitoring.

To investigate dynamic monitoring for TBI diagnosis and treatment, the developed multiplexed optical fiber sensor was examined using an ex vivo model to perform real-time and continuous monitoring of multiple biomarkers in brain aCSF. The pH, temperature, DO, and glucose levels and other biological CSF constituents of the aCSF buffer solution were tuned to mimic the chemical compositions in four stages of TBI patients including pre-TBI (healthy), mild TBI, severe TBI, and back to mild TBI stages (experimental procedures). The lamb brain was washed to remove the existing chemicals. Before ex vivo measurement, the brain was immersed in the prepared healthy-stage aCSF solution to change the chemical composition of the
lamb brain to mimic a patient’s brain pre-TBI. During the dynamic measurement, the designed optical sensing fiber was inserted into the brain at a depth of 4 mm, and the reflection spectra were obtained every 6 s using a self-designed Python program. After the signal stabilized for 4 min, the aCSF of the next stage of TBI was injected to replace the previous TBI buffer. The acquired reflection spectra were first smoothed to remove noises, and the same ten features were extracted (Table S4). The ML models built with standard calibration spectra were directly used here in Python algorithms to predict the biomarker concentrations using the extracted features for continuous and precise readouts. The results of dynamic monitoring are

Figure 7. AI-integrated multiplexed optical fiber sensor for dynamic brain monitoring of multiple biomarkers in an ex vivo brain model

(A) Photographs of the ex vivo experimental setup. (i) Multiplexed optical fiber sensor used in a brain model. Scale bar, 4 cm. (ii) Sensor end inserted in the brain tissue through the human skull. Scale bar, 1 cm. (iii) Detail of how the sensor interacts with the brain tissue. Scale bar, 1 cm.

(B) Algorithm flowchart of the AI model for biomarker level prediction.

(C) pH value prediction using feature set 1.

(D) Temperature prediction using feature set 1.

(E) DO prediction using feature set 2.

(F) Glucose concentration prediction using feature set 1.

(G) Multiplexed and dynamic monitoring for simulated TBI disease using an ex vivo lamb brain. Three disease stages were simulated, and four biomarkers were monitored in continuous mode. Images of the sensing film in four stages are also presented. Scale bar, 1 mm.
shown in Figure 7G. During the transition from healthy to mild TBI, and mild TBI to severe TBI, a decrease in pH from 7.4 to 7.0 to 6.5, an increase in temperature from 37°C to 38°C to 39°C, a decrease in DO from 8 to 4 mg L⁻¹ and to 3 mg L⁻¹, and an increase in glucose from 2 to 4 mmol L⁻¹ and to 5 mmol L⁻¹, were detected using the multiplexed optical fiber sensor. The biomarker levels were dynamically predicted by the ML model with a resolution of 6 s. The non-reversible glucose sensing film can only monitor the one-way increasing trend continuously until film saturation at a glucose concentration of 50 mmol L⁻¹ is reached. When the glucose was decreased back to a mild stage, the saturated sensor film was replaced by new glucose sensing film, resulting in signal drop at 720 s. All signals stabilized after 120 s, and the biomarker prediction values exhibited high accuracy. The proposed multiplexed optical fiber sensor demonstrated excellent ability in dynamic monitoring of TBI brain biomarkers and successfully detected the fluctuations in the biomarkers for the indication of disease stages. Medical interventions can be applied to the patients when the disease develops into a worse stage. For example, insulin therapy can be performed when glucose increases to a high level and oxygen supply can be offered to avoid TBI complications, such as brain anoxia. The scenario of a severe TBI patient being properly treated and the condition being improved back to mild stage was also mimicked to examine the reversibility of the multiplexed optical fiber sensor in dynamic monitoring. Using the multiplexed optical fiber sensor, improvements in pH, temperature, DO, and glucose levels were observed in the TBI ex vivo model during the transition from severe to mild stages, indicating an excellent ability for dynamic monitoring for real-case scenarios.

To investigate whether the developed multiplexed optical fiber sensors are safe for long-term in vivo applications, further biocompatibility studies were conducted by evaluating the growth and viability of cells. L929 fibroblasts were cultured in multi-well plates in the presence of sol-gel sensing film (sol-gel film, pH sensing film) (diameter = 9 mm) for 1, 3, 5, and 7 days. The ability of cell proliferation was assessed using a Cell Counting Kit-8 (CCK-8) assay to investigate the effect of the used sensing film on cellular growth and function (Figure S12A). It was observed that cell proliferation ability increased rapidly during the first 3 days and became stable from day 3 to day 7 in all samples. In addition, no significant differences were observed between the growth of cells in the presence of the sensing film, pure sol-gel film, and the control group (pure cells) during the 7-day observation. Cellular viability was also assessed using a Live/Dead staining assay over a 7-day incubation (Figures S12B and S12C). It was clearly observed that most of the cells showed up as green (live cells) and only a few cells were red (dead cells), demonstrating the high cellular viability (>95%) of the developed sensors.

**Conclusions**

A multiplexed optical fiber sensor was developed for dynamic brain pH, temperature, DO, and glucose monitoring simultaneously. The pH, temperature, and DO sensors exhibited high reversibility; however, the developed glucose sensor showed low reversibility. A high reversible detection of glucose could be achieved by using synthetic reversible receptors. 23,64 The developed optical sensor also demonstrated high sensitivity, high selectivity, fast response, good stability, and high accuracy for the measurement of the four target biomarkers. In this research, only one fiber channel was utilized to provide multiplexed monitoring, which largely reduced the complexity of the optical system and the costs. However, the alternation of light sources (broadband and 450 nm) for excitation of four sensors was manually controlled, which can be improved by adding wavelength division multiplexers or a programmed light source switcher. In the study, AI-based models were first
proposed and optimized to provide the brain biomarker concentration readout precisely in real time. Temperature and photobleaching were successfully compensated using AI models. However, only basic regression models were used in the study, and more complex AI techniques such as recurrent neural networks can be explored to offer a more accurate readout. In the validation tests, the AI models were able to provide an accurate and robust prediction of the biomarker concentrations in a short computational time. In the ex vivo test using the simulated TBI brain model, the optical fiber sensor successfully identified the deterioration of TBI disease and vice versa between various disease stages in a short time. The applications of the multiplexed optical sensor can also be extended to the detection of other biomarkers, including metabolites or neurotransmitters, by selecting different indicators. The biocompatibility and cytotoxicity studies of the proposed sensing probe demonstrated good biocompatibility and high potential for long-term insertion. For better \textit{in vivo} applications, the silica-based optical fiber used in this study can be further replaced by more biocompatible and softer materials such as hydrogel fibers for better \textit{in vivo} applications.\textsuperscript{65} In addition, \textit{in vivo} tests using living animals are needed to further assess the immunogenicity and foreign-body response and to test the ability of the fiber sensor for real-case applications. At the end of the study, a prototype and fabrication methods for probe miniaturization were introduced (see supplemental information), which has reduced the sensing probe from 8 to 1.6 mm and can be potentially used with a brain microdialysis catheter for continuous clinical monitoring (“miniaturization of the multiplexed fiber sensor” and Figure S13).

This study demonstrated good ability in dynamic monitoring of multiple physiological biomarkers using flexible MRI-compatible intracranial optical fiber sensors to assess abrupt metabolic changes, which can continuously reflect the pathophysiological status and help doctors to follow the disease courses precisely and arrive at proper clinical decisions and treatment. The developed multiplexed optical fiber sensor can be potentially applied in medical diagnostics to offer new pathways for precise brain diagnosis and treatment.

**EXPERIMENTAL PROCEDURES**

**Resource availability**

**Lead contact**

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**Materials availability**

GLYMO (98%), TEOS (98%), Ru(dpp), D-(+)-glucose (99.5%), TMB, peroxidase (POD), CN, COC, CB, 3,3',5,5'-tetabromo-m-cresolsulfonphthalein (95%), Triton X-100, PBS, sodium chloride (NaCl) (99.5%), calcium chloride (CaCl\textsubscript{2}) (93%), potassium chloride (KCl) (99%), magnesium chloride (MgCl\textsubscript{2}) (98%), zinc chloride (ZnCl\textsubscript{2}) (98%), sodium phosphate dibasic (Na\textsubscript{2}HPO\textsubscript{4}) (99%), sodium phosphate monobasic (NaH\textsubscript{2}PO\textsubscript{4}) (99%), copper(II) chloride (93%), D-(+)-glucose (99.5%), L-ascorbic acid (99%), uric acid (99%), sodium nitrite (NaNO\textsubscript{2}) (97%), albumin from porcine serum (98%), hydrochloric acid (HCl) (37%), nitric acid (HNO\textsubscript{3}) (70%), sodium hydroxide (NaOH) (97%), 5-hydroxyindole-3-acetic acid (98%), and ethanol (99%) were purchased from Sigma-Aldrich. Sodium L-lactate (98%), sodium pyruvate (99%), and dopamine hydrochloride (99%) were purchased from Alfa Aesar. Dulbecco’s modified Eagle’s medium (DMEM) and penicillin-streptomycin were purchased from Gibco. Fetal bovine serum (FBS) was purchased from PAN-Biotech (Germany). Trypsin was purchased from BioSharp. The CCK-8 was purchased from APEXBio. LIVE/DEAD Viability/Cytotoxicity Kit was purchased from Beyotime. Microglass
fibers were purchased from Whatman. All chemicals were reagent grade and used as received without further purification.

Data and code availability
The data and code are available upon reasonable request.

Instrument and experimental setup
A fiber-coupled LED (MCWHF2; Thorlabs) and a 450 nm collimated laser diode (CPS450; Thorlabs) were used as a broadband light source and a 450 nm excitation light source, respectively. A T-cube LED driver (LEDD1B; Thorlabs) was used to control the light intensity. The reflection light was guided by a 400 µm premium bifurcated fiber (QBIF400-VIS-NIR; Ocean Insight) and measured using a FLAME-S-VIS-NIR-ES spectrometer assembly (350–1,000 nm) (Ocean Insight). The absorbance was measured using a microplate reader (Molecular Devices, USA). The micrographs were obtained by an upright microscope for polarization (DM2700 P; Leica). A fluorescence microscope (MSHOT; Guangzhou Micro-shot Technology) was used to capture fluorescence micrographs for cell experiments. A hotplate stirrer (Fisher-brand Isotemp) was used to homogenize the sol-gel precursor solutions and control the temperature of the buffer solutions during experiments. A pH meter (FiveEasy F20; Mettler Toledo) was used to measure the pH value. An oxygen benchtop meter (HI-2004 Edge; Hanna Instruments) was used as a golden standard to prepare DO buffer solutions. The spectrometer was connected with Jetson Xavier NX Developer Kit (NVidia) to visualize and capture the spectra. The reflection spectra obtained during the experiment were analyzed offline using a Python code. Ocean View 2.0 and Thermo Scientific Skanlt were used as the software to analyze data from the spectrometer and microplate reader, respectively. The camera of a smartphone iPhone 11 was used to capture the photographs. An Apreo S scanning electron microscope was used to examine the film morphologies. The accelerating voltage was maintained at 15 kV. Images were taken with an atomic force microscope (MultiMode 8 SPM; Bruker) to examine the roughness of the sensing film.

pH sensor characterization
Fabrication of sol-gel sensing film
Sol-gel films were used to encapsulate the indicators to prevent leaching and improve stability. TEOS and GLYMO were mixed and used together to encapsulate the pH indicator and fabricate uniform pH sensing films. Triton X-100 was used to reduce the film tensions. The ratios of the chemicals used for the fabrication of the sensing films were optimized as described below to achieve the optimal morphology and sensing performance.

Sol-gel precursor solutions (1 mL) containing TEOS and GLYMO (single precursor or mixture), 1.2 mL of ethanol, 0.4 mL of HCl, 16 mg of BCG, and surfactant (Triton X-100) were mixed according to Table S1 to obtain sol-gel coating solutions. The mixture was homogenized for 3 h using a hotplate stirrer that allowed for subsequent aging at a room temperature for at least 72 h.

Silica sol was obtained by using 500 µL of TEOS and 600 µL of ethanol. This solution was added into a mixture of HCl (25 µL, 1 mol L\(^{-1}\)) and deionized (DI) water (125 µL) with constant stirring under 60 °C to accelerate the hydrolysis and condensation reactions. 1.6 mg of the pH indicator was added to the solution under stirring for 60 min. The sol was then spin coated on a transparent plastic film to form a uniform layer (500 rpm, 1 min). The film was air dried for a thorough gelation and indicator stabilization, followed by rinsing with DI water to remove weakly entrapped dyes.
SEM, AFM, and optical microscope images were taken to examine the morphology of the sensing film. Sensitivity (characterization methods mentioned in "characterization of pH sensing film") and film morphology were studied to optimize the sensing film fabrication formula. The optimal fabrication formula was also applied to DO and glucose sensing film fabrication. An isolation film made from glass microfiber (Whatman, grade GF/C), which has high reflection properties and high water permeability, was placed above the sensing films, and the edges of both layers were glued together using epoxy glue. The reflection spectrum and photograph of the film are shown in Figure S1.

Fabrication comparisons
Based on the morphology and the calibration curve of each sensing film (Figures S3 and S4), the optimal fabrication method was determined to be TG (1:0.1). Therefore, this film was used for the following film characterization experiment, and the same fabrication formula was applied to the fabrication of other sensing films.

Characterization of pH sensing film

Buffer preparation. PBS solutions were prepared by dissolving PBS tablets in DI water (one tablet in 200 mL of DI water), which yields 0.01 mol L$^{-1}$ of PBS, 0.0027 mol L$^{-1}$ of potassium chloride, and 0.137 mol L$^{-1}$ of sodium chloride. The original buffer pH is 7.45 at 24°C, and PBS solutions with pH values at 6.0, 6.4, 6.8, 7.2, 7.6, and 8.0 were adjusted by adding HCl or NaOH. The ionic strength of the buffer was adjusted to 150 mmol L$^{-1}$.

$pH$ sensitivity. The fabricated optical fiber sensor was characterized using the prepared standard PBS solutions. The optical fiber sensor was immersed in the PBS solutions, and the reflection intensity was recorded in each buffer solution when the signal was stabilized. The correlation between various pH buffers and the reflection intensities was evaluated. The sensitivity of the sensor was calculated by the slope of the calibration curve.

The sensing of biomarkers is based on the principle that the absorption abilities the pH indicator possesses vary under different pH levels. To indicate the absorption abilities, the normalized reflectance spectrum was calculated for the calibration of the sensing films, which is reversely correlated with the absorbance spectrum. The normalized reflectance spectra were calculated using

$$\frac{I}{I_0} = \frac{\text{Spectrum (with sensing film)} - \text{Spectrum (background noise)}}{\text{Spectrum (without sensing film)} - \text{Spectrum (background noise)}},$$

(Equation 2)

where $I$ denotes the intensity of the reflectance spectrum with sensing film and $I_0$ denotes the intensity of the reflectance spectrum without sensing film (Figure S14). The higher the absorption ability, the lower the $I/I_0$ in the normalized reflection spectrum.

Response time and reversibility. Response time was measured using pH 6.0 and pH 8.0 PBS solutions. The optical fiber sensor was first immersed in pH 6.0 buffer. When the signal stabilized, the optical fiber sensor was immersed in the pH 8.0 buffer. After the signal stabilized, the optical fiber sensor was moved back to pH 6 PBS solution. This procedure was repeated ten times to evaluate the reversibility of the sensor. The reflection intensity at 625 nm was measured in a time-dependence mode during the experiment. The collected data were then used to calculate the response time from pH 6.0 to pH 8.0 and pH 8.0 to pH 6.0 offline. The response
time was calculated as the time required for the sensor output to stabilize from the moment it was inserted to a new buffer solution. Repeatability was measured by calculating the standard deviation of the measured intensity at pH 6.0 and pH 8.0 during the ten repeats.

*Temperature dependence.* The fabricated pH sensing films were placed in microplate wells and immersed in 5 mL of pH standard solutions (pH 6.0, 6.4, 6.8, 7.2, 7.6, and 8.0) in the well of a microplate. The microplate reader with an incubator was used to control the temperature and measure the absorbance at 623 nm of the soaked sensors under temperatures of 25°C, 30°C, 35°C, and 40°C to study the temperature effects.

*Stability.* To measure the stability for continuous monitoring, the reflection spectra of the proposed optical fiber sensing setup in pH 7.4 PBS solution were taken every 2 h for a continuous 24 h monitoring.

*Selectivity.* In the first selectivity test, the interference solutions were prepared by adding interference compounds into PBS solutions (Table S2). The pH values of the solutions were adjusted by 1 mol L⁻¹ HCl and 1 mol L⁻¹ NaOH to pH 7.4. The optical fiber pH sensor was immersed into the interference solutions (pH 7.4) separately. The reflection intensity at 623 nm with different interferences was measured through a spectrometer using the same sensing setup. In the second selectivity test, the interference analytes were added to PBS solutions (pH 7.4), and the reflectance spectra of the sensor were obtained.

*Pressure dependence.* The sensor performance at different brain ICP was also studied. Brain ICP is in the range of 7.2–15.5 mmHg; therefore, the optical fiber sensor was dipped into the buffer solution at different depths (0–30 cm) to simulate human physiological pressure ranges according to equation pressure = ρ · g · h, where ρ is the density of the buffer solution (approximately 1 g/cm³) and g is the gravity constant. The sensing spectra at different depths were obtained and analyzed. This study was also conducted for the other three sensors.

*Leaching test.* The leaching test was performed by immersing the fabricated sensing film in 10 mL of PBS for 3 days. Blank sol-gel films were fabricated using the same process without adding the indicator. Three types of leaching conditions were investigated: (1) pH 6.0; (2) pH 7.0; and (3) pH 8.0. The degree of indicator leaching was assessed by measuring the absorbance of the PBS solution after the sensing film was immersed for 3 days. The PBS solution with blank silicate gels was measured as a reference. Absorbance spectra were acquired by measuring a 180 μL buffer sample using the microplate reader. All measurements were performed in triplicate. Leaching absorbance percentage, which was defined by the ratio of the solution absorbance to the immersed sensing film absorbance at the measured wavelength, was used to indicate the leaching severity over 3 days.

Leaching tests were also conducted for the other three sensing films (temperature sensing film, DO sensing film, and glucose sensing film) using the same protocol after fabrication.

**Temperature sensor fabrication and characterization**

**Temperature sensing film fabrication**

The temperature sensor was fabricated on the basis of thermostats of cholesteric liquid crystals. The cholesteric liquid crystal mixtures were achieved by mixing
COC, CN, and CB (weight ratio 0.35:0.55:0.10). Sixty microliters of the mixture drop was dipped between two transparent round-shaped contact papers (1 mm in diameter) and sealed to prevent leakage. The sensing film was then attached to the fiber tip sheath with medical-grade tapes.

**Temperature sensor characterization**

**Sensor calibration curve.** Temperature sensors were characterized with a pH 7.4 PBS. A hotplate was used to adjust the buffer temperature, which was monitored by a thermometer to precisely control temperature variations. The sensors were immersed in PBS solutions with temperature ranging from 34.0°C to 42.0°C with steps of 1.0°C. Reflection spectra were acquired in a reflection mode, and the spectra peak shift over temperature was calculated.

**Temperature sensor response time and reversibility measurement.** Response time was measured using two pH 7.4 PBS solutions with one at 36.0°C and another at 40.0°C. The optical fiber sensor was first immersed in a 36°C buffer. When the signal was stabilized, the fiber sensor was moved to a 40°C buffer. After the signal stabilized, the fiber sensor was moved back to a 36°C buffer. This procedure was repeated ten times for the evaluation of reversibility. Reflection spectra were measured, and the reflection peak at each measurement was calculated. The response time was defined as the time interval immediately after the sensor was immersed in a 36°C or a 40°C buffer until it reached a stable value. Repeatability was measured by calculating the standard deviation of the measured intensity at 36.0°C and 40.0°C during the ten repeats.

**Temperature sensor stability for long-term monitoring.** The stability for continuous temperature monitoring was also measured. The reflection spectra of the proposed optical fiber sensing setup in 37.0°C pH 7.4 PBS solution were taken every 2 h for a continuous 24 h monitoring.

**DO sensor fabrication and characterization**

**DO sensing film fabrication**
The fabrication procedures were same as for fabrication of the pH sensor, except here 22 mg of oxygen fluorophore Ru(dpp) was used instead of the pH indicator.

**DO sensing film characterization**

**Buffer preparation.** DO level is highly sensitive to many factors including temperature and pressure. A buffer syringe method was used to prepare various DO level solutions for sensor characterization. A low-oxygen (0% DO) solution was syringed to decrease DO level while 100% DO saturated DI water was added to increase DO. Both methods showed a corrected trend in DO buffer preparation. All characterization tests were conducted under a stable room temperature (25°C) and standard atmosphere using a 10 mmol L⁻¹ pH 7.4 PBS solution.

**Excitation and emission wavelength determination.** The fabricated sensing films were first measured using a 96-well microplate reader for the determination of excitation and emission wavelengths. The Ru(dpp) indicator has an excitation wavelength at around 470 nm and an emission wavelength at around 600 nm. Therefore, for excitation spectrum measurement, 600 nm was used as an emission point. For emission spectrum measurement, 470 nm was used as an excitation wavelength.

**Sensor calibration.** The optical system for the measurement of DO is as shown in Figure S6. A commercially available oxygen probe was used as the gold standard.
for the determination of DO levels. Both the DO probe and designed DO fiber sensor were inserted into the buffer solution using a three-neck flask. The sensing film was fixed to the sheath tip and dipped into the buffer solutions. Reflectance spectrum was utilized for the measurement of the fluorescence. A 450 nm LED source was used as the excitation of the sensing film. Reflectance spectra were measured by using a spectrometer (from 450 to 780 nm). The emission intensity was used to calculate the relationship with the DO levels.

**Response time and reversibility.** Response time was measured using 0% (nitrogen-saturated PBS) and 100% (air-saturated PBS) DO PBS solutions. The optical fiber sensor was first immersed in a 0% DO buffer. When the signal was stable, the fiber sensor was moved to 100% DO buffer. After the signal stabilized, the fiber sensor was moved back to 0% DO buffer. This procedure was repeated ten times to study the reversibility of the sensor. The collected data were then used to calculate the response time from 0% DO to 100% DO and 100% O₂ to 0% O₂ offline. Repeatability was measured by calculating the standard deviation of the measured intensity at 0% DO and 100% DO during the ten repeats. The results were presented in red/blue to eliminate inaccurate intensity readouts owing to drift in excitation intensity for robust measurement. Red/blue value was calculated as the integration of the fluorescence intensity in the red spectral range (560–650 nm) and normalizing it to the excitation light intensity in the blue spectral range (420–500 nm).

**Photostability.** The sensor was exposed in water at room temperature under an excitation light for 24 h. The emission spectra were measured every 2 h.

**pH and temperature dependence.** For pH dependence, PBS solutions with a pH value of 6.0 were prepared to make 100% DO by pumping compressed air into the solution for several minutes until the oxygen meter stabilized at around 8 mg L⁻¹ of DO value. The DO concentration was kept constant. The pH value of the buffer solution was adjusted by pipetting the NaOH solution under the monitoring of the pH meter. When the pH value was adjusted to 6.0, 6.5, 7.0, 7.5, and 8.0, the reflection spectra of the DO sensing film were measured. The experiment was repeated to study the pH dependence when the DO values were equal to 4 mg L⁻¹ and 8.25 mg L⁻¹.

A temperature dependence test was operated using a hotplate. The sensors were excited at different temperatures and the emission intensities were recorded. The temperature (2°C at a time) was slightly increased from room temperature (25°C) to 40°C (human fever).

**Selectivity.** The interference solutions prepared were the same as the ones used in the pH selectivity test. Compressed air was then bubbled into the solutions to prepare air-saturated PBS solutions for the DO measurement.

**Glucose sensor fabrication and characterization**

**Glucose sensing film fabrication**

The glucose biosensor was prepared by encapsulating 310.5 μg of TMB, 18 μg of GOD, and 105 μg of POD in the sol-gel film.

**Glucose sensing film characterization**

**Glucose buffer preparation.** A glucose buffer was prepared by dissolving glucose into PBS solutions. In total, 1 mmol L⁻¹, 2 mmol L⁻¹, 3 mmol L⁻¹, 4 mmol L⁻¹,
5 mmol L\(^{-1}\), and 6 mmol L\(^{-1}\) of glucose buffers were prepared for the test. The ionic strength of the buffer was adjusted to 150 mmol L\(^{-1}\).

**Glucose sensing sensitivity.** The optical fiber sensor was immersed in the buffers, and the reflection intensity was recorded in each buffer when the signal stabilized. The correlation between various glucose concentrations and the reflection intensities was evaluated. The sensitivity of the sensor was calculated by the slope of the calibration curve.

**Response time.** Response time was measured using 2 mmol L\(^{-1}\) and 5 mmol L\(^{-1}\) glucose PBS solutions. The optical fiber sensor was first immersed in a 2 mmol L\(^{-1}\) buffer. When the signal was stable, the fiber sensor was moved to 5 mmol L\(^{-1}\) buffer. The reflection intensity at 660 nm was measured in a time-dependence mode during the experiment to indicate the response time.

**Selectivity.** The interference buffer solutions prepared were the same as those described in the pH selectivity test. Two types of selectivity tests were conducted. In the first test, interference solutions without glucose were used, and the reflectance spectra were measured. In this test, whether the interference analytes could be falsely detected as glucose was studied. In the second selectivity test, 2 mmol L\(^{-1}\) of glucose solution was added to each interference solution, and the reflection spectra obtained. This test explored whether the interference analyte could affect the glucose measurement.

**Temperature and pH dependence.** The sensor responses when immersed in pH 6.0 to pH 8.0 buffer solutions under temperatures from 25 °C to 40 °C were measured using a microplate reader and a reflectance measurement to evaluate the temperature influence and optimal storage temperature.

**Stability.** Stability in continuous measurement and shelf-life stability were conducted. To measure the film stability for dynamic measurement, the reflection spectra of the sensor in glucose buffer at 2 mmol L\(^{-1}\) were taken every 2 h for a 24 h continuous monitoring using the proposed optical fiber sensing setup.

**aCSF and ex vivo measurement**

Standard aCSF buffer solutions with different chemical concentrations were used for further evaluation.

**aCSF buffer preparation.** aCSF buffer was prepared by mixing 0.0075 mol L\(^{-1}\) Na\(_2\)HPO\(_4\), 0.0025 mol L\(^{-1}\) NaH\(_2\)PO\(_4\), 0.0027 mol L\(^{-1}\) KCl, 0.137 mol L\(^{-1}\) NaCl, 0.0024 mol L\(^{-1}\) CaCl\(_2\), and 0.0013 mol L\(^{-1}\) MgCl\(_2\) in DI water. The resulting buffer solutions were prepared for pH, temperature, DO, and glucose sensing film characterization using the multiplexed optical fiber sensor (Table S3).

**Multiple sensing film fabrication.** The fabricated sensing films for pH, temperature, DO, and glucose were fabricated together on the tip of the sheath using a medical-grade glue. The sheath was then fixed on the tip of the fiber for real-time measurement.

**Multiplexed optical fiber sensor characterization**

Using the aforementioned buffer solutions, the fabricated multiplexed optical fiber sensor was characterized. Calibration curve, sensitivity, reversibility and stability
were measured using the same method as described in “temperature sensor fabrication and characterization.”

Algorithm design for signal post-processing

2D (x, y) CIE 1931 chromaticity diagram

The average RGB pixel values of the captured images were first collected. Subsequently, the non-linear values were linearized by the following equations:

\[
R_l = \left( \frac{0.055 \times R_0}{1.055} \right)^{2.4}, \quad G_l = \left( \frac{0.055 \times G_0}{1.055} \right)^{2.4}, \quad B_l = \left( \frac{0.055 \times B_0}{1.055} \right)^{2.4},
\]

(Equation 3)

where \(R_0, G_0, B_0\) represent the non-linear RGB values, and \(R_l, G_l, B_l\) correspond to the values of linearized \(R_0, G_0, B_0\), with a gamma correction of 2.4 being used here. Thereafter, the tristimulus values \(X, Y, Z\) were calculated using a base transformation:

\[
\begin{bmatrix}
X \\
Y \\
Z
\end{bmatrix} = \begin{bmatrix}
0.4124564 & 0.3575761 & 0.1804375 \\
0.2126729 & 0.7151522 & 0.0721750 \\
0.019339 & 0.1191920 & 0.9503401
\end{bmatrix} \begin{bmatrix}
R \\
G \\
B
\end{bmatrix}.
\]

(Equation 4)

The tristimulus values \(X, Y, Z\) were transformed to the 2D (x, y) CIE 1931 chromaticity space as follows:

\[
x = \frac{X}{X + Y + Z}, \quad y = \frac{Y}{X + Y + Z};
\]

(Equation 5)

where \(i\) was defined as the number of calibration points.

Regression modeling for quantitative readout

Feature extraction. To eliminate the crosstalks between each optical sensing film, several features were extracted and used for ML model training to predict biomarker concentrations. The following features were extracted (Figure S10 and Table S4).

To build models for concentration prediction, we collected data from 210 spectra as a training dataset, which contains the spectra of the multiplexed sensing film in aCSF with different biomarker concentrations. The data were labeled with the buffer pH, DO, temperature, and glucose values. Four models were trained using 75% of the dataset for the prediction of pH, temperature, DO, and glucose, respectively, and the remaining 25% of the dataset was used as testing dataset. To determine the best model training algorithm, we trained several models using different methods (linear regression, polynomial regression, support vector regression [SVR], decision tree [DT], random forest [RF], and Bayesian regression). Mean absolute error, mean squared error, and R-squared \((R^2)\) were used as evaluation matrix for the comparison between the models, and the best method was determined. The results indicated that Bayesian regression outperformed the other methods for biomarker concentration prediction and, thus, was selected for the ex vivo test (Table S5).

Bayesian regression modeling training. Bayesian ridge regression was used to build the model for analyte concentration prediction based on the features.

Model validation. Repeated 4-fold cross-validation was used to evaluate the regression model performance. To perform the validation, the extracted feature set was split into four subsets, whereby three subsets were used for model training and the remaining subset was used as an independent validation set. This process was repeated four times whereby each time a different and independent validation
set was used. $R^2$ was used to measure the accuracy of the proposed models. It is calculated as

$$R^2 = 1 - \frac{\sum(y_i - \bar{y})^2}{\sum(y_i - \bar{y})^2}$$

(Equation 6)

**Ex vivo measurement on TBI models**

To further evaluate the developed sensing platform for the monitoring of brain physiological status in clinical scenarios, three stages were simulated whereby the platform was used to provide continuous and dynamic monitoring of the TBI disease. It was reported that TBI patients showed decreased pH of 6.59–7.17 in CSF due to the increase of CO$_2$.\(^{40}\) Furthermore, the DO level in TBI patients could be as low as 20% of normal oxygenation value, which equals 1.6 ppm.\(^{68}\) An increased temperature above 38°C and increased glucose of 4.8–5.8 mmol L$^{-1}$ have also been observed in patients with TBI.\(^{69,70}\) Therefore, PBS with pH of 7.0, pO$_2$ of 2 ppm, temperature of 38.5°C, and glucose of 5.0 mmol L$^{-1}$ were prepared. A flow channel for the measurement was then created.

**Stage 1: Healthy stage**

In this stage, an aCSF buffer that has a pH value of 7.4, temperature of 37°C, 100% saturated DO (around 7.8 mg L$^{-1}$), and glucose of 2 mmol L$^{-1}$ was injected into the insulated glassware. Clean lamb brain was immersed in the buffer during the whole measurement.

**Stage 2: Mild-stage TBI**

After brain injury, a mild TBI status was simulated using an aCSF buffer that has a decreased pH value of 7.0, temperature of 38°C, DO of 50% (around 4 mg L$^{-1}$), and glucose of 4 mmol L$^{-1}$. The old buffer was syringed out and new mild TBI buffer was injected into the glassware.

**Stage 3: Severe-stage TBI**

As the disease was progressing, a severe stage was simulated and transited from a mild stage, with aCSF buffer of pH 6.6, temperature of 39°C, 20% saturated DO (around 2 mg L$^{-1}$), and glucose of 5 mmol L$^{-1}$. The buffer was syringed into the glassware to replace the old buffer.

**Stage 4: Back to mild stage**

The last scenario is the mimic of proper treatment, whereby the severity of brain injury was improved to mild status. In this stage, the mild TBI status was simulated using aCSF buffer with an improved pH value of 7.2, temperature of 38°C, 60% saturated DO (around 5 mg L$^{-1}$), and glucose of 3 mmol L$^{-1}$. The new buffer was syringed into the glassware to replace the old buffer.

During the experiment, a DO meter was also inserted into the brain tissue to ensure a corrected DO level after the buffer solutions were syringed into the glassware. Compressed air and nitrogen gas were bubbled into the system if the DO value became lower or higher, respectively, after injection.

Dynamic monitoring using the multiplexed fiber sensor was conducted during the simulated four stages. During the experiment, the designed multiplexed optical fiber sensor was inserted into the brain dura and fixed through all stages. The reflection spectra of the sensing films were obtained and transmitted to the software in real time and the biomarker levels were predicted with the proposed ML algorithms. The scan of the spectrum was obtained every second, and the biomarker
concentrations were predicted immediately based on the scan. The results were the average value of three predictions of three continuous scans for more robust output. The results can be continuously displayed on a user interface for direct clinical reference (Figure S11).

**Cellular studies of the biocompatibility of the multiplexed fiber sensor**

**Cell culture**

Mouse fibroblast L929 cells were grown in a DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in a 12-well plate under 5% CO₂ atmosphere at 37°C. After confluence the cells were trypsinized with trypsin, then concentrated by centrifugation and resuspended with the extracted cell culture medium to adjust the cell concentration to 1 × 10⁴ cells mL⁻¹, after which cells were seeded with 2 mL of medium per well in a 12-well plate and cultured for 1, 3, 5, and 7 days. The cell culture medium was changed with the extracted solution every 2 days, and at each time point the cytotoxicity and cell viability were examined.

**Biocompatibility test**

The fabricated pH sensing film was immersed in fresh culture medium for 72 h. Subsequently, the film was removed from the culture medium, which was used for cell culture over 7 days.

**Cytotoxicity analysis**

Cell proliferation was measured by CCK-8 assay and quantified by a plate reader. CCK-8 (200 μL) was added to each well, and after incubation at 37°C for 4 h, 100 μL of culture medium was transferred to a 96-well plate, the absorbance of which was then measured at 450 nm using a microplate reader.

**Live/dead cell staining assay**

A LIVE/DEAD Viability/Cytotoxicity Kit was diluted 1,000-fold, and 500 μL of diluent was aspirated to the microplate wells after being washed using PBS. Subsequently, the cells were incubated at 37°C with 5% CO₂ for 30 min. The cells were observed with a fluorescence microscope to determine the cell viability.

**Miniaturization of the multiplexed fiber sensor**

The miniaturization of the sensing fiber was explored. A self-designed Y-shape fiber was fabricated using two pigtailed multimode fibers (RS Pro, 62.5/125 μm; Multimode). The fiber tips were stripped and flat-cut using a fiber cleaver. A silicone tube (0.5 mm inner diameter and 1.5 mm outer diameter) was used as the silicone sheath for film attachment. The fiber tips were glued together and fixed inside the sheath. The sensing film was also attached to the sheath tip using glue (Figure S13A). The gap between the fiber distal ends and the sensing film was 1 mm to allow light transmission and detection. A black sheath and an isolation film was be used to block the background noise (Figures S13B–S13D). The total diameter of the sensing fiber was reduced to 1.6 mm. The miniaturized fiber was further used for the measurement of the four sensing films to evaluate its performance using PBS solutions, and the spectra obtained were the same as the spectra obtained using the original lager fiber (Figures S13E–S13H). In the future, the fiber can be further miniaturized to less than 1 mm using microfabrication devices and methods.

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.matt.2022.07.024.
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AUTHOR CONTRIBUTIONS

Y.Z. and Y.H. designed the experiments and wrote the paper. Y.Z., K.L., S.W., N.Z., and N.J. conducted the experiment on sensor fabrication and characterization. Q.L. conducted the cell experiment. A.K.Y., Y.H., and N.J. offered support and supervision to the whole project.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Menon, D.K., Schwab, K., Wright, D.W., and Maas, A.J. (2008). Demographics and Clinical Assessment Working Group of the International and Interagency Initiative toward Common Data Elements for Research on Traumatic Brain Injury and Psychological Health (2010). Position statement: definition of traumatic brain injury. Arch. Phys. Med. Rehabil. 91, 1637–1640. https://doi.org/10.1016/j.apmr.2010.05.017.

2. Taylor, C.A., Bell, J.M., Breiding, M.J., and Xu, L. (2017). Traumatic brain injury-related emergency department visits, hospitalizations, and deaths—United States, 2007 and 2013. MMWR Surveill. Summ. 66, 1–16. https://doi.org/10.15585/mmwr.ss6609a1.

3. Werner, C., and Engelhard, K. (2007). Pathophysiology of traumatic brain injury. Br. J. Anaesth. 99, 4–9. https://doi.org/10.1093/bja/aem131.

4. Tisdall, M.M., and Smith, M. (2007). Multimodal monitoring in traumatic brain injury: current status and future directions. Br. J. Anaesth. 99, 61–67. https://doi.org/10.1093/bja/aem143.

5. Irimia, A., Wei, S., Lu, N., Moore, C.M., and Kennedy, D.N. (2017). Mobile monitoring of traumatic brain injury in older adults: challenges and opportunities. Neuroinformatics 15, 227–230. https://doi.org/10.1007/s11043-017-9335-z.

6. Sheriff, F.G., and Hinson, H.E. (2015). Pathophysiology and clinical management of moderate and severe traumatic brain injury in the ICU. Semin. Neurol. 35, 42–49. https://doi.org/10.1055/s-0035-1542438.

7. Gupta, A.K. (2002). Monitoring the injured brain in the intensive care unit. J. Postgrad. Med. 48, 218–225.

8. Güzza, F., Depreitere, B., Piper, I., Citerio, G., Chambers, I., Jones, P.A., Lo, T.-Y.M., Enblad, P., Nilsson, P., Feyen, B., et al. (2015). Visualizing the pressure and time burden of intracranial hypertension in adult and paediatric traumatic brain injury. Intensive Care Med. 41, 1067–1076. https://doi.org/10.1007/s00134-015-3806-1.

9. Badri, S., Chen, J., Barber, J., Temkin, N.R., Dikmen, S.S., Chesnut, R.M., Deem, S., Yaenez, N.D., and Treggiari, M.M. (2012). Mortality and long-term functional outcome associated with intracranial pressure after traumatic brain injury. Intensive Care Med. 38, 1800–1809. https://doi.org/10.1007/s00134-012-2655-4.

10. Ngwenya, L.B., Burke, J.F., and Manley, G.T. (2016). Brain tissue oxygen monitoring and the intersection of brain and lung: a comprehensive review. Respir. Care 61, 1232–1244. https://doi.org/10.4187/respcare.04962.

11. Scheeren, T.W.L., Kuizenga, M.H., Maurer, H., Struys, M., and Heringlake, M. (2019). Electroencephalography and brain oxygenation monitoring in the perioperative period. Anesth. Analg. 128, 265–277. https://doi.org/10.1213/ANE.0000000000002812.

12. Lee, W.H., Slaney, T.R., Hower, R.W., and Kennedy, R.T. (2013). Microfabricated sampling probes for in vivo monitoring of neurotransmitters. Anal. Chem. 85, 3828–3831. https://doi.org/10.1021/ac303579x.

13. Ngnensutuvorakul, T., Steyer, D.J., Valenta, A.C., and Kennedy, R.T. (2018). In vivo chemical monitoring at high spatiotemporal resolution using microfabricated sampling probes and droplet-based microfluidics coupled to mass spectrometry. Anal. Chem. 90, 10943–10950. https://doi.org/10.1021/acs.analchem.8b02468.

14. Zhang, Y., Jiang, N., and Yetisen, A.K. (2021). Brain neurochemical monitoring. Biosens. Bioelectron. 189, 113351. https://doi.org/10.1016/j.bios.2021.113351.

15. Schwerdt, H.N., Shimazu, H., Amemori, K.I., Amemori, S., Tiemey, P.L., Gibson, D.J., Hong, S., Yoshida, T., Langer, R., Cima, M.J., and Graybiel, A.M. (2017). Long-term dopamine neurochemical monitoring in primates. Proc. Natl. Acad. Sci. USA 114, 13260–13265. https://doi.org/10.1073/pnas.1713756114.

16. Adelsberger, H., Zanos, A., Alvarez, M., Romo, R., and Konnerth, A. (2014). Local domains of motor cortical activity revealed by fiber-optic calcium recordings in behaving nonhuman primates. Proc. Natl. Acad. Sci. USA 111, 463–468. https://doi.org/10.1073/pnas.1321612111.

17. Smith, S.K., Lee, C.A., Dausch, M.E., Hornam, B.M., Patiasul, H.B., McCarty, G.S., and Sombers, L.A. (2017). Simultaneous volumetric measurements of glucose and dopamine demonstrate the coupling of glucose availability with increased metabolic demand in the rat striatum. ACS Chem. Neurosci. 8, 272–280. https://doi.org/10.1021/acschemneuro.6b00363.

18. Li, S., Zhu, A., Zhu, T., Zhang, J.Z.H., and Tian, Y. (2017). Single biosensor for simultaneous quantification of glucose and pH in a rat brain of diabetic model using both current and potential outputs. Anal. Chem. 89, 6656–6662. https://doi.org/10.1021/acs.analchem.7b00881.

19. Coelho, C.A., Sias, A., Koster, T., Westerink, B.H.C., and Cremers, T.I.F.H. (2018). In vivo “real-time” monitoring of glucose in the brain with an amperometric enzyme-based biosensor based on gold coated tungsten (W-Au) microelectrodes. Sens. Actuators B Chem. 263, 605–613. https://doi.org/10.1016/j.snb.2018.02.116.
Vibrational sensing using infrared nanoantennas toward the noninvasive quantification of physiological levels of glucose and fructose. ACS Sens. 4, 1973–1979. https://doi.org/10.1021/acssensors.9b00486

31. Saberi, Z., Rezaei, B., Rezaei, P., and Ensafi, A.A. (2020). Design a fluorometric aptasensor based on CuO NPs platform with carbon dots for simultaneous detection of lysozyme and adenosine triphosphate. Spectrochim. Acta Mol. Biomol. Spectrosc. 233, 118197. https://doi.org/10.1016/j.saa.2020.118197.

32. Schweder, H.N., Kim, M.J., Amenomi, S., Homma, D., Yoshida, T., Shimazu, H., Yerramreddy, H., Karasan, E., Langer, R., Graybiel, A.M., and Cima, M.J. (2017). Subcellular probes for neurochemical recording from multiple brain sites. Lab Chip 17, 1104–1115. https://doi.org/10.1039/c6lc01398h.

33. Khan, M.I., Mukherjee, K., Shoukat, R., and Dong, H. (2017). A review on pH sensitive materials for sensors and detection methods. Microsyst. Technol. 23, 4391–4404. https://doi.org/10.1007/s00542-017-4355-3.

34. Wanke, E., Carbone, E., and Testa, P.L. (1980). The sodium channel and intracellular H+ blockage in squid axons. Nature 287, 62–63. https://doi.org/10.1038/287062a0.

35. Zauner, A., Doppenberg, E.M., Woodward, J.J., Choi, S.C., Young, H.F., and Bullock, R. (1997). Continuous monitoring of cerebral substrate delivery and clearance: initial experience in 24 patients with severe acute brain injuries. Neurosurg 41, 1082–1093. https://doi.org/10.1097/00006123-199711000-00011.

36. Linan, A.A., Chebotarev, A.S., Pochechuev, M.S., Kelmanzon, I.V., Kotova, D.A., Bilan, D.S., Ermakova, Y.G., Fedotov, A.B., Ivanov, A.A., Belousov, V.V., and Zheltikov, A.M. (2020). Two- and three-photon absorption cross-section characterization for high-brightness, cell-specific multiphoton fluorescence brain imaging. J. Biophotonics 13, e201900243. https://doi.org/10.1002/jbio.201900243.

37. Tang, X., Zhu, Z., Wang, Y., Han, J., Ni, L., Wang, L., Zhang, H., Li, J., and Gou, Y. (2018). A dual site controlled probe for fluorescent monitoring of intracellular pH and colorimetric monitoring of Cu2+. Sens. Actuators B Chem. 270, 35–44. https://doi.org/10.1016/j.snb.2018.04.173.

38. Dong, H., Zhao, L., Zhou, Y., Wei, X., Xu, C., Zhang, Y., and Xu, M. (2021). Novel self-calibrating amperometric and ratiometric electrochemical nanotip microsensor for pH measurement in rat brain. Anal. Chem. 93, 13815–13822. https://doi.org/10.1021/acs.analchem.1c02436.

39. Promphet, N., Rattanawanedirojn, P., Sirirattmekul, K., Soathiyanon, N., Pothayar, P., Thanawattano, H., Hinroza, J.P., and Rodthongkum, N. (2019). Non-invasive tissue based colorimetric sensor for the simultaneous detection of sweat pH and lactate. Talanta 192, 424–430. https://doi.org/10.1016/j.talanta.2019.09.067.

40. Shigori, T., Nara, I., Saruta, K., Harada, M., and Saito, I. (1999). Continuous monitoring of cerebrospinal fluid acid-base balance and oxygen metabolism in patients with severe head injury: pathophysiology and treatments for cerebral acids and ischemia. In Neuror Monitoring in Brain Injury (Springer), pp. 49–55. https://doi.org/10.1007/978-3-642-46151-1_18.

41. Andrews, R.J., Bringas, J.R., and Alonzo, G. (1994). Cerebrospinal fluid pH and PCO2 rapidly follow arterial blood pH and PCO2 with changes in ventilation. Neurosurgery 34, 466–470. https://doi.org/10.1227/00006123-199403000-00012.

42. Makote, R., and Collinson, M.M. (1999). Organically modified silicate films for stable pH sensors. Anal. Chem. Acta 394, 195–200. https://doi.org/10.1006/saco.1999.0305-0.0.

43. Kassal, P., Šurina, R., Vrsaljko, D., and Steinberg, I.M. (2014). Hybrid sol–gel thin films doped with a pH indicator: effect of organic modification on optical pH response and film surface hydrophilicity. J. Sol. Gel Sci. Technol. 69, 586–595. https://doi.org/10.1007/s10971-013-3261-9.

44. Jurmanovič, S., Kordić, S., Steinberg, M.D., and Steinberg, I.M. (2010). Organically modified silicate thin films doped with colourimetric pH indicators methyl red and bromocresol green as pH responsive sol–gel hybrid materials. Thin Solid Films 518, 2234–2240. https://doi.org/10.1016/j.tsf.2009.07.158.

45. Wu, S., Cheng, W., Qiu, Y., Li, Z., Shuang, S., and Dong, C. (2010). Fiber optic pH sensor based on mode-filtered light detection. Sens. Actuators B Chem. 144, 255–259. https://doi.org/10.1016/j.snb.2010.05.058.

46. Fohlenmeier, J.F., Cohen, E.D., and Newman, E.A. (2010). Mechanisms and distribution of ion channels in retinal ganglion cells: using temperature as an independent variable. J. Neurophysiol. 103, 1357–1374. https://doi.org/10.1152/jn.00123.2009.

47. Wang, H., Wang, B., Normoye, K.P., Jackson, K., Spitterl, K., Sarroock, M.F., Miller, C.M., Best, C., Llano, D., and Du, R. (2014). Brain temperature and its fundamental properties: a review for clinical neuroscientists. Front. Neurosci. 8, 307. https://doi.org/10.3389/fnins.2014.00307.

48. Yu, Y., Hill, A.P., and McCormick, D.A. (2012). Warm body temperature facilitates energy efficient cortical action potentials. PLoS Comput. Biol. 8, e1002436. https://doi.org/10.1371/journal.pcbi.1002436.

49. Dietrich, W.D., Atkins, C.M., and Bramlett, H.M. (2007). Protection in animal models of brain and spinal cord injury with mild to moderate hypothermia. J. Neurotrauma 26, 301–312. https://doi.org/10.1089/neu.2008.0806.

50. Rolfe, D.F., and Brown, G.C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol. Rev. 77, 731–758. https://doi.org/10.1152/physrev.1997.77.3.731.

51. Yang, C., Denno, M.E., Pyakurel, P., and Benton, B.J. (2015). Recent trends in carbon nanomaterial-based electrochemical sensors for biomolecules: a review. Anal. Chim. Acta 887, 12–37. https://doi.org/10.1016/j.aca.2015.05.049.
52. Pascual, J.M., Wang, D., Hinton, V., Engelstad, K., Saxena, C.M., Van Heertum, R.L., and De Vivo, D.C. (2007). Brain glucose supply and the syndrome of infantile neuroglycopenia. Arch. Neurol. 64, 507–513. https://doi.org/10.1001/archneur.64.4.noc60165.

53. Dunn, L., Allen, G.F., Mamais, A., Ling, H., Li, A., Duberley, K.E., Hargreaves, I.P., Pope, S., Holton, J.L., and Lees, A. (2014). Dysregulation of glucose metabolism is an early event in sporadic Parkinson’s disease. Neurobiol. Aging 35, 1111–1115. https://doi.org/10.1016/j.neurobiolaging.2013.11.001.

54. An, Y., Varma, V.R., Varma, S., Casanova, R., Dammer, E., Pletnikova, O., Chia, C.W., Egan, J.M., Ferrucci, L., Troncoso, J., et al. (2018). Evidence for brain glucose dysregulation in Alzheimer’s disease. Alzheimers Dement 14, 318–329. https://doi.org/10.1016/j.jalz.2017.09.011.

55. Blanco, M.M.B., Prashant, G.N., and Vespa, P.M. (2016). Cerebral metabolism and the role of glucose control in acute traumatic brain injury. Neurouog. Clin. 27, 453–463. https://doi.org/10.1016/j.nec.2016.05.003.

56. Steiner, M.S., Duerkop, A., and Wolfbeis, O.S. (2011). Optical methods for sensing glucose. Chem. Soc. Rev. 40, 4805–4839. https://doi.org/10.1039/c1cs15063d.

57. Ramon-Marquez, T., Sesay, A.M., Panjan, P., Medina-Castillo, A.L., Fernandez-Gutierrez, A., and Fernandez-Sanchez, J.F. (2017). A microfluidic device with integrated coaxial nanofibre membranes for optical determination of glucose. Sens. Actuators B Chem. 250, 156–161. https://doi.org/10.1016/j.snb.2017.04.140.

58. Zhou, Z., Qiao, L., Zhang, P., Xiao, D., and Chou, M.M.F. (2005). An optical glucose biosensor based on glucose oxidase immobilized on a swim bladder membrane. Anal. Bioanal. Chem. 383, 673–679. https://doi.org/10.1007/s00216-005-0023-8.

59. Lenczuk, T., Wencel, D., Głąb, S., and Koncki, R. (2001). Prussian blue-based optical glucose biosensor in flow-injection analysis. Anal. Chim. Acta 447, 23–32. https://doi.org/10.1016/S0003-2670(01)01289-2.

60. Briones, A.V., and Sato, T. (2010). Encapsulation of glucose oxidase (GOD) in polyelectrolyte complexes of chitosan–carrageenan. React. Funct. Polym. 70, 19–27. https://doi.org/10.1016/j.reactfunctpolym.2009.09.009.

61. Pavlovic, D., Pekic, S., Stojanovic, M., and Popovic, V. (2019). Traumatic brain injury: neuropathological, neurocognitive and neurobehavioral sequelae. Pituitary 22, 270–282. https://doi.org/10.1007/s11102-019-00957-9.

62. Adrian, H., Marthen, K., Salla, N., and Lasse, V. (2016). Biomarkers of traumatic brain injury: temporal changes in body fluids. Eneuro 3. https://doi.org/10.1523/ENEURO.0294-16.2016.

63. Sakka, L., Coll, G., and Chazal, J. (2011). Anatomy and physiology of cerebrospinal fluid. Eur. Ann. Otorhinolaryngol. Head Neck Dis. 128, 309–316. https://doi.org/10.1016/j.anorl.2011.03.002.

64. Jiang, N., Davies, S., Jiao, Y., Blyth, J., Butt, H., Montelongo, Y., and Yetisen, A.K. (2021). Doubly photopolymerized holographic sensors. ACS Sens. 6, 915–924. https://doi.org/10.1021/acssensors.0c02109.

65. Guo, J., Liu, X., Jiang, N., Yetisen, A.K., Yuk, H., Yang, C., Khademhosseini, A., Zhao, X., and Yun, S.H. (2016). Highly stretchable, strain sensing hydrogel optical fibers. Adv. Mater. 28, 10244–10249. https://doi.org/10.1002/adma.201603160.

66. Parale, V.G., Lee, K.-Y., and Park, H-H. (2017). Flexible and transparent silica aerogels: an overview. J. Korean Ceram. Soc. 54, 184–199.

67. Yetisen, A.K., Moreedu, R., Seifi, S., Jiang, N., Vega, K., Dong, X., Dong, J., Butt, H., Jakobi, M., and Elsner, M. (2019). Dermal tattoo biosensors for colorimetric metabolite detection. Angew. Chem. 131, 10616–10623. https://doi.org/10.1002/anie.201904416.

68. Martini, R.P., Deem, S., and Treggiari, M.M. (2013). Targeting brain tissue oxygenation in traumatic brain injury. Respir. Care 58, 162–172. https://doi.org/10.4187/respcare.01942.

69. Thelin, E.P., Nelson, D.W., Ghatan, P.H., and Bellander, B.M. (2014). Microdialysis monitoring of CSF parameters in severe traumatic brain injury patients: a novel approach. Front. Neurol. 5, 159. https://doi.org/10.3389/fneur.2014.00159.

70. Rossi, S., Zanier, E.R., Mauri, I., Columbo, A., and Stocchetti, N. (2001). Brain temperature, body core temperature, and intracranial pressure in acute cerebral damage. J. Neurol. Neurosurg. Psychiatry 71, 448–454. https://doi.org/10.1136/jnnp.71.4.448.