A. Implementation of wireless multi-channel near-infrared spectroscopy (NIRS) system

A wireless multi-channel near infrared spectroscopy system was designed and implemented to continuously monitor the relative concentrations of oxy-hemoglobin (HbO_2) and deoxy-hemoglobin (HbR) under traumatic brain injury. The system architecture of the wireless multi-channel NIRS system, which includes a wireless signal acquisition module, a NIRS probe, and a host system. The NIRS probe consists of light emitting diodes (LEDs) and photodiodes (PDs). LED is used to provide red and infrared light sources, and PD is used to detect and convert the diffusely reflected lights into electrical signals. The wireless signal acquisition module was designed to drive the red and infrared lights of LEDs and receive the signal from PDs. When LED emits the light to pass through the biological tissue, most of light will be diffused rapidly due to scattering, and part of light will be absorbed by the tissue, especially HbO_2 and HbR which are mainly absorbers in the region of near infrared wavelength. In general, the mean path of light is like a banana shaped, and the maximum penetrating depth is about a half of the separating distance between the light source and the detector. Then, the diffusely reflected light detected by photodiodes will be delivered into the wireless signal acquisition module, and be amplified, filtered, digitized and transmitted wirelessly to the host system.

Probe

The LED and photodiode used in the NIRS probe are SMT735/850 (EPITEX, Japan) and PD15-22C/TR8 (EVERLIGHT, Taiwan) respectively. The used LED is a dual-wavelength LED (red and infrared lights), and its light intensity is about 0.3 mW. The distance between LED and PD is about 1.6 cm. In order to contact closely with rat's head, the substrate of the NIRS probe was made of flexible black rubber. And the rubber tape with Velcro hook and loop is used to tie on rat's head to help hold the probe.

Wireless signal acquisition module

The wireless signal acquisition module is mainly consisting of a LED driving circuits, a front-end amplifier circuits, a microcontroller, and a wireless transmission circuit. The LED driving circuits include a multiplexer, operation amplifiers, and NPN transistors. The multiplexer controlled by the microcontroller is used to turn the LED on or off. The operation amplifiers are used to provide the emitter of NPN transistors the stable bias to ensure constant current through these LEDs. The front-end amplifier circuits are made up of trans-impedance amplifiers and low-pass filters. The trans-impedance amplifier with the gain of 2×10^7 V/A, was designed to covert the PD current signal into the voltage signal. And the cut-off frequency of the low-pass filter was set to 0.5 kHz. Then, a 12-bit analog-to-digital converter (ADC) built in the microcontroller will digitize the amplified PD signal with the sampling rate of 25 Hz. The microcontroller is used to control ADC and wireless transmission circuit, and calculate NIRS parameters, such as $\Delta[HbO_2]$, $\Delta[HbR]$, and $\Delta[HbT]$. Finally, the digitized PD signals and the calculated NIRS parameters will be sent to the wireless transmission circuit. The wireless transmission circuit composed of a printed circuit board (PCB) antenna and a Bluetooth module which is fully compliant with the Bluetooth v2.0+ EDR specification.

Host system

In this study, a laptop was used as the platform of the host system, and the operation system of the host system is Windows 7. The NIRS parameter monitoring program built in the host system was developed on Microsoft Visual C#. The program provides the functions of receiving data from the wireless signal acquisition module, real-time signal display and data storage.

B. Theoretical models of tissue optics

The variation in the concentrations of HbO_2 and HbR can be quantified by using modified Beer-lambert law (MBLL) (14,15). MBLL is an empirical equation of optical attenuation in a highly scattering medium. The variation in the concentration of absorber will cause the change of optical intensity, and the relationship between the absorber's concentration and the optical intensity can be expressed as

$$\Delta OD = -\log\frac{I_f}{I_i} = \Delta\mu_a BL \quad [1]$$

where ΔOD is the variation of the optical density, and I_f and I_i are the detected intensities of the diffusely reflected light before and after the concentration change respectively. The parameter B, named the differential path length factor (DPF), is a correction factor related to the average light traveling pathway between light source and detector. L denotes the distance between the light source and the detector. And $\Delta\mu_a$ denotes the change of absorption coefficient of the tissue. In the infrared wavelength (from 700 to 900 nm), HbO_2 and HbR are most significant absorbers in the biological tissue. Thus, $\Delta\mu_a$ can be simplified as

$$\Delta\mu_a = \varepsilon_{HbO_2}\Delta[HbO_2] + \varepsilon_{HbR}\Delta[HbR] \quad [2]$$

Here, ε_{HbO_2} and ε_{HbR} denote the extinction coefficients of HbO_2 and HbR respectively. $\Delta[HbO_2]$ and $\Delta[HbR]$ are the variations in the concentrations of HbO_2 and HbR respectively. Then the Equation [1] corresponding to specific wavelength λ can be rewritten as

$$\Delta OD^{\lambda} = (\varepsilon_{HbO_2}{}^{\lambda}\Delta[HbO_2] + \varepsilon_{HbR}{}^{\lambda}\Delta[HbR])B^{\lambda}L \quad [3]$$

Next, the variations in the concentrations of HbO_2 and HbR can be solved by using the change of the optical intensity

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corresponding to two or more different wavelengths. The solution of $\Delta[HbO_2]$ and $\Delta[HbR]$ can be expressed as

$$\Delta[HbO_2] = (\varepsilon_{HbR}^{\lambda 1}\frac{\Delta OD^{\lambda 2}}{B^{\lambda 2}} - \varepsilon_{HbR}^{\lambda 2}\frac{\Delta OD^{\lambda 1}}{B^{\lambda 1}})/(\varepsilon_{HbR}^{\lambda 1}\varepsilon_{HbO_2}^{\lambda 2} - \varepsilon_{HbR}^{\lambda 2}\varepsilon_{HbO_2}^{\lambda 1})L \quad [4]$$

$$\Delta[HbR] = (\varepsilon_{HbO_2}^{\lambda 2}\frac{\Delta OD^{\lambda 1}}{B^{\lambda 1}} - \varepsilon_{HbO_2}^{\lambda 1}\frac{\Delta OD^{\lambda 2}}{B^{\lambda 2}})/(\varepsilon_{HbR}^{\lambda 1}\varepsilon_{HbO_2}^{\lambda 2} - \varepsilon_{HbR}^{\lambda 2}\varepsilon_{HbO_2}^{\lambda 1})L \quad [5]$$

Here, the wavelengths of $\lambda 1$ and $\lambda 2$ used in this study are 735 and 850 nm respectively. And assuming that the biological tissue is a semi-infinite medium, the parameter B can be given by

$$B = \frac{1}{2}(\frac{3\mu_s'}{\mu_a^{initial}})^{1/2}\left[1 - \frac{1}{1 + L(3\mu_s'^{initial}\mu_a^{initial})^{1/2}}\right] \quad [6]$$

where μ_s' is the reduced scattering coefficient, $\mu_a^{initial}$ is the initial absorption coefficient, and $\mu_s'^{initial}$ is initial scattering coefficient. In this study, the concentration changes of total-hemoglobin {$\Delta[HbT]$} is defined as

$$\Delta[HbT] = \Delta[HbO_2] + \Delta[HbR] \quad [7]$$

C. Experiments for traumatic brain injury rat models

Animals preparation

Adult male Sprague-Dawley rats weighing 375±25 g were prepared in the experiment. All rats were reared on a 12-hour light/12-hour dark cycle, and allowed to access to food and water freely. All experimental procedures were conformed to the guidelines of National Institute of Health, Taiwan, and were approved by Animal Care and Use Committee of Chi-Mei Medical Center to minimize discomfort to animals during surgery and recovery periods. All rats (number of rats =16) were randomly assigned to four impacts (sham, 1.6, 2.0, and 2.4 atm), and monitored by the wireless NIRS system, ICP and MAP. All rats were anesthetized with sodium pentothal (25 mg/kg, i.p.; Sigma Chemical Co., St Louis, MO, USA) and a mixture containing ketamine (44 mg/kg, i.m.; Nan Kuang Pharmaceutical, Tainan, Taiwan), atropine (0.02633 mg/kg, i.m.; Sintong Chemical Industrial Co., Ltd., Taoyuan, Taiwan), and xylazine (6.77 mg/kg, i.m.; Bayer, Leverkusen, Germany). All rats would be sacrificed in the third day post-surgery.

Experiment design for traumatic brain injury

In this study, fluid percussion injury (FPI) model was used to reproduce TBI in the rats (16). Before FPI experiment, the rat was anesthetized first. And then its head was placed in a stereotaxic frame, and its ears was inserted by ear bars to tight its head. In order to keep the rat core temperature at 37 ℃, a rectal temperature probe attached to the thermostatic controller was inserted into the rat's colon. Next, the fur on the rat head was trimmed, and the scalp was incised sagittally. Afterwards, a circular craniotomy on the skull was drilled to set the impact point which was located at the anterior-posterior −3 mm and lateral +4 mm from the bregma. The leur-lock connector connected with a sealed and fluid-filled reservoir was secured into the craniotomy (Figure S1).

After that, the monitoring devices, such as the wireless NIRS system, ICP, and MAP, were installed and began to monitor for 2 hours. Then, a pendulum struck the reservoir to generate a fluid wave to impact the rat brain, and a respiratory treatment procedure was carried out to help rat's respiration immediately after FPI experiment. Finally, after all the experiment finished, the monitoring device, connector and acrylic were removed from the rat, and the incisions were sutured.

The mainly monitoring position of NIRS probe was focus at striatum region of rat's brain, and maximum penetrating depth was about 8 mm which located at anterior-posterior −0.5 mm and lateral +3.5 mm from bregma (Figure S2). In this study, the changes of 30-second HbO_2 and HbR were recorded as the data baseline before the FPI experiment.

In order to measure the mean arterial blood pressure, the right femoral artery was cannulated with polyethylene tubing, and a 3-way stopcock connected with the pressure transducer and the data acquisition system (PowerLab/8sp, ADInstruments) were also used. In addition, a pressure-monitoring catheter of ICP monitor (Codman ICP Express Monitor, Codman & Shurtleff, Inc.) was located at anterior-posterior −0.8 mm and lateral +4 mm from the bregma and was installed on the stereotaxic frame. When the experiment began, the data of MAP and ICP would be recorded as the baseline first. Thereafter MAP and ICP values would be recorded every 10 minutes.

In order to monitor the short-term changes of MAP and ICP, the data during impact and 4.5 minutes after impact were also recorded. Figure S3 shows the location of the NIRS probe, the ICP catheter, and the leur-lock connector.

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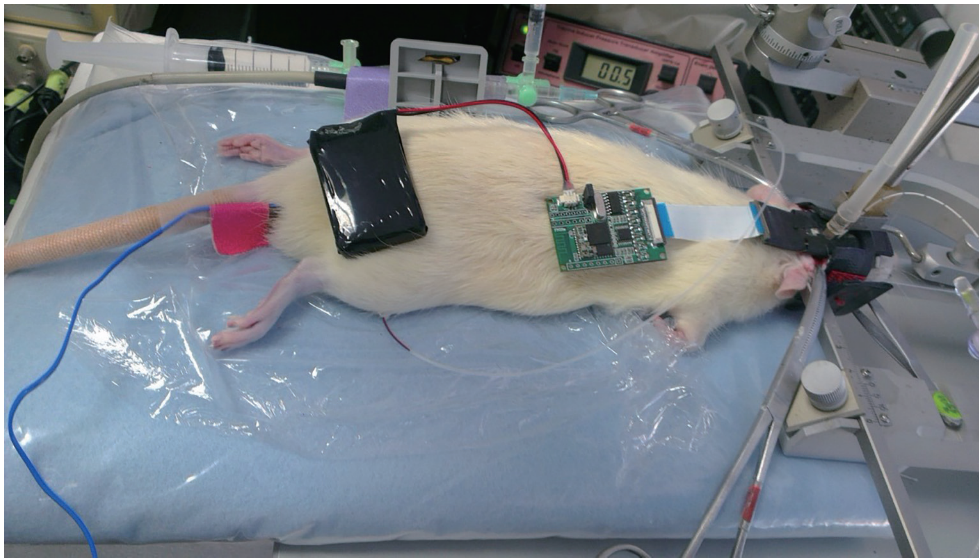

Figure S1 Mice setting.

Figure S2 Experiment equipment grossly during recording.

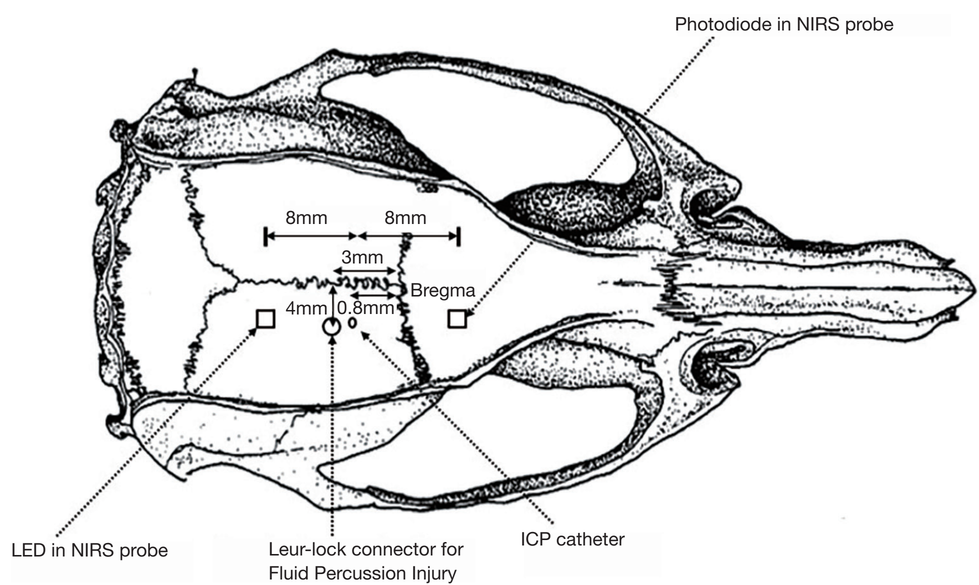

Figure S3 Anatomic position on the skull. NIRS, near-infrared spectroscopy; LED, light emitting diode; ICP, intracranial pressure.

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