MEASUREMENT OF TISSUE OXYGENATION IN ISOLATED RAT HEARTS USING NEAR INFRARED SPECTROSCOPY

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Abstract: New techniques involving Near Infrared Spectroscopy (NIRS) and imaging are rapidly evolving for a large number of new clinical applications. These techniques, based upon near-infrared light transmission through biological tissue, aim to monitor the hemoglobin and myoglobin concentration changes due to particular physiological state. Clinical applications regard, for instance, the monitoring of muscles and cerebral oxygenation, functional brain activation studies and heart perfusion research. Recently, some works presented tissue oxygenation studies in beating or arrested isolated porcine hearts. In our work we present the design and realization of a dedicated NIRS system for the myocardial perfusion analysis of isolated, saline solution perfused beating rat hearts; in this case the absence of blood allows for unambiguous measurement of tissue myoglobin oxygenation. The presented prototype is portable, low cost, battery operated and permits the measurement of both oxy and deoxy myoglobin concentration changes during imposed regional or global ischemia and reperfusion.

Introduction

Several non-invasive techniques, like positron emission tomography (PET), single photon emission computer tomography (SPECT) and magnetic resonance imaging (MRI), are currently used to image tissue damaged by an ischemia. Unfortunately, these methods are difficult to use under particular condition as open-heart surgery, because of equipment size and characteristics. An amply used technique is X ray angiography, but it uses ionizing radiation and does not provide any information about tissue oxygenation depending on capillary flow.

In order to measure the tissue oxygenation in isolated hearts, the Near InfraRed Spectroscopy (NIRS) technique is considered. This technique has been used widely to measure hemoglobin (Hb) oxygenation in skeletal muscle and brain tissue [1]: it makes use of the near-IR spectra of hemoglobin/myoglobin and its oxygenated counterpart.

The more effective penetration of near infrared light into tissue respect to the visible light, and the very different in the IR absorption spectra of oxy and deoxy hemoglobin and myoglobin (Mb), are the reasons for which this technique is used in tissue oxygenation measurements application. In fact, IR light absorption is proportional to the concentration of these absorbing species so it is possible to have information on the tissue perfusion using IR light emitters and detectors.

Since the NIR absorption of hemoglobin and myoglobin is the same, the NIRS technique can be also used to measure Mb oxygenation in isolated heart perfused with saline solution [1]. The myoglobin oxygenation level in the beating isolated heart responds clearly to variations in the oxygenation perfusion and differences in O_2 consumption are evident comparing the behaviour of the heart.

The manifestations of an ischemia, such as tissue deoxygenation, can be used as diagnostic tools for the assessment, visualization, and localization of ischemia and infarction.

The aim of this work is the design and realization of a prototype using the NIRS technique to provide information regarding myocardial O_2 perfusion of isolated, saline solution perfused beating rat hearts: in this report we describe the prototype and the experimental protocol for its preliminary test.

Materials and Methods

The near infrared region extended from 750 to 2000 nm: light in the 750-1100 nm range has the characteristic of penetrating biological tissue for few centimeters. In this spectral region the major chromophores (pigments which absorb the light) of biological tissue are hemoglobin and myoglobin, which have a near infrared spectrum essentially identical.

Absorption spectra of these metalloproteins are sensitive to oxygenation so near infrared spectroscopy can yield information on tissue oxygenation and perfusion. For this reason NIRS can be used to measure Mb oxygenation in isolated hearts and to obtain a map of perfusion areas during particular physiological state as regional or global ischemia and reperfusion.

Some papers in literature report several cases of NIRS study on isolated arrested blood perfused porcine hearts [2] or saline solution perfused isolated rabbit hearts [3].

We present a study on a saline solution perfused beating isolated rat heart using a home-made prototype.

ISSN: 1727-1983

The designed device is constituted by two light emitting diodes (LED) which emit two infrared wavelength lights (780 nm OD 780W, Opto Diode Corporation and 880 nm SFH 845P, Siemens). The two wavelengths chosen are about the peak absorption wavelength of deoxy Hb/Mb and oxy Hb/Mb respectively (Figure 1).

Light at wavelength lesser than 700 nm do not penetrate enough into the tissue and at wavelength greater than 900 nm the water absorption effect comes predominant.

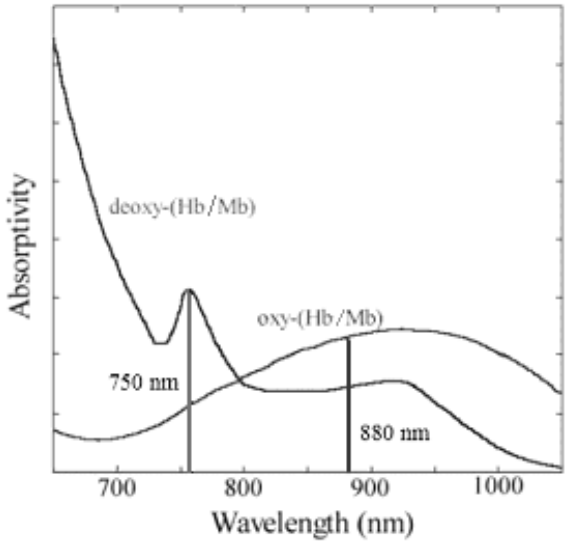

Figure 1: Absorption spectra of oxy and deoxy Hb and Mb

To receive the light that passed through the tissue, we used a PIN photodiode (SFH 203P, Siemens) that converts infrared light photons in an electric current.

The electronic part acts the light sources driving and the detector signal acquisition and pre processing.

The two LEDs are powered by two square waves with frequencies of 27 KHz (880 nm) and 57 KHz (780 nm), in order to recognize one signal for each light on the output signal from the only one detector. A mixed circuit splits the signal received by the detector in two waves relative to the two different wavelengths of emitted lights.

The whole system is battery operated and is provided with a plastic chassis (Figure 2).

We designed a mechanical support for the probes in order to embrace the heart: the circular support is made of black PVC and has three holes for the two LEDs and the detector, respectively, placed with an angle of 120° one to each other. Figure 3 shows the probes support schematic diagram.

The signals received to the readout electronic circuit are acquired by an acquisition card (DAQ6024E National Instruments) and visualized on a PC monitor using LabView software (National Instruments).

Figure 2: System prototype

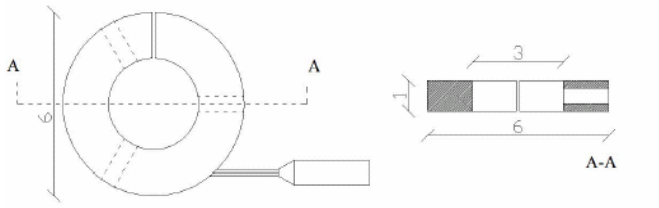

Figure 3: Mechanical support for the NIRS probes (dimensions unit = mm)

We created an interface in LabView for the visualization of the two signals on a graph relative to the two modulation frequencies. The interface calculates the concentration changes of oxy Myoglobin (MbO_2) and deoxy Myoglobin using the modified Lambert-Beer law [4] for each of the two chosen wavelenght:

$$I = I_0 \cdot e^{-\varepsilon \cdot C \cdot l \cdot DPF + G} \qquad (1)$$

where I is the detected light intensity, I_0 the incident light intensity, ε the absorption coefficient of the medium, C the concentration of the medium, l the distance between light emitter and detector, DPF the differential path length factor and G the constant attenuation factor relative to the optical properties and geometry of the tissue.

Preliminary tests were made with several substances with different IR absorption spectra in order to evaluate the prototype range of sensibility: we essentially used water, saline solution and human venous blood.

Finally, we defined the experimental protocol for a rat isolated heart test: the animal was treated in accordance with the "Guiding Principles for Research Involving Animals and Human Beings" published by The American Physiological Society [5].

The heart was isolated from Wistar rat and placed in a Krebs-Henseleit bicarbonate (KHB) solution bath with the following mmol/litre composition: 118 NaCl, 24 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 0.5 EDTA, e 5 glucose.

ISSN: 1727-1983

After foreign tissues removal, the aorta was cannulated and the heart was perfused according to Langerdoff at 37°C with a constant flow of KHB oxygenated with a mixture of 95% O_2 and 5% CO_2; the perfusion pressure was set at 65 mmHg.

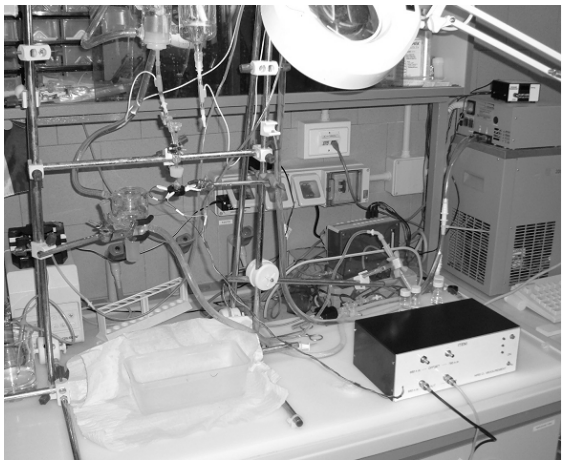

Figure 4: Langerdoff apparatus and the prototype for the experiment on a rat isolated heart

The flow of KHB perfusion solution was measured and visualized on a monitor during all phases of the experiment. Figure 4 shows the Langerdoff apparatus and the designed system: the heart chamber for the thermoregulation had to be removed for the placement of the probes support.

The experimental protocol mimicked an ischemic condition followed by reperfusion by stopping and then restoring the oxygenation in the perfusion solution, respectively: the variation in oxy and deoxy Mb concentration was visualized on a PC monitor.

Figure 5: Signal acquired during the venous human blood dilution test

Results

Figure 5 shows the acquired signal relative to the 880 nm channel for a human venous blood dilution test: 5 ml of venous human blood was diluted with 1 ml of physiologic solution.

In correspondence of the dilution, the electric signal was quickly increased like we expected: the dilution, in fact, caused a decrement of Hb concentration and hence a decrement in O_2 concentration, so the transmitted infrared light intensity increased.

In the isolated rat heart experiment the output signals from the designed prototype are the Mb oxy and deoxy concentration variations regarding the whole heart; the two emitters and the detector are placed with an angle of 120 degrees between each other. Since the heart's size is quite small, the infrared light penetrates into the myocardium and the results regard an integration of several contribution from all heart volume. In the first phase of experiment, after 1 minute of baseline condition (beating heart with normal perfusion), the oxygenation of the solution was stopped and a global ischemic condition was achieved. Under this hypoxic condition, the oxy-Mb level decreased quickly, with an half-time ($t_{1/2}$) of about 6 sec.

Figure 6 shows the relative level of MbO_2 respect to the baseline condition (beating heart with normal perfusion): the upper graph shows the raw signal while the lower graph is the signal after a low pass filter operation (high cut frequency= 1Hz) acts by the implemented algorithm.

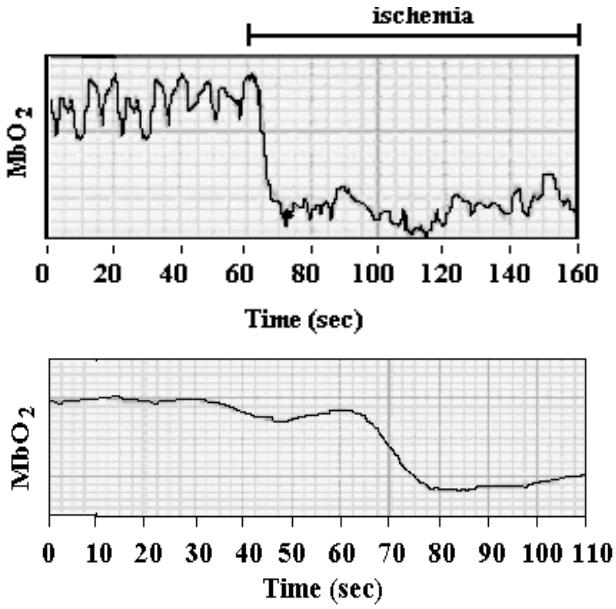

Figure 6: MbO_2 relative level during ischemic condition: no processed signal (up); low-pass filtered signal (bottom)

After 100 sec of ischemic condition, the perfusion solution was reoxygenated. Unfortunately, the electronic signal did not recover as theoretically expected probably because the oxygen reperfusion itself has the potential to initiate both reversible and irreversible tissue injury following ischemia [6]. There are many mechanisms suspected of causing reperfusion injury, including the release of nitric oxide, reactive oxygen and reactive nitric oxide species. Some of them, namely nitric oxide and superoxide anion, react each other at a very fast rate (approaching the diffusion rate) [7] to form peroxinitrite, which causes irreversible

ISSN: 1727-1983

inhibition of the mitochondrial respiratory chain and triggers apoptosis of cardiac myocytes [8].

In our test, the ishemia/riperfusion cycle was repeated two times and, finally, the perfusion solution was completely stopped.

Discussion

The results obtained with our prototype regarding the MbO_2 level, are coherent with the data reported on literature in a similar experimental case [9].

In this work are reported only the results relative to the MbO_2 variation changes in the rat isolated heart: the results regarding the Mb variations changes, in fact, were not good. Probably, this fact was due to the readout electronic circuit of the 780 nm channel, that is more affected from visible light and power line frequency electric noise. Moreover, changes in deoxy Mb concentration are much less strict. However, these problems can be eliminated using optical filters for the visible light or specific detectors, and electrical filter for the noise frequencies.

The very small size of the used heart did not permit a careful localization of the hypoxic perfusion in the myocardial area: the three NIRS probes were placed in contact with the heart and they covered a measurement area that corresponded to the whole heart zone.

Examining a larger heart, like a pig heart, the position of the probes can be chosen more accurately in order to obtain optimal information on the ischemic zone.

Conclusions

In this work-in-progress we describe a prototype for the measurement of oxy and deoxy Mb level in an isolated rat heart using NIRS technique, and the experimental protocol for its preliminary test.

Final system will be able to allow a real time monitoring of the changes in myocardial tissue oxygenation within the beating heart.

An isolated rat heart perfused according to Langerdoff method was examined during an hypoxic/re-oxygenation procedure: device output signal reflected the expected MbO_2 level time course during the ischemia condition.

Future developments will regard the optimization of the 780 nm channel for the detection of deoxy Mb variation changes and a generic optimization of the electronic readout circuit to also measure the concentration changes during reperfusion condition.

Further optimization of the protocol could involve a measurement synchronism with the cardiac cycle in order to eliminate motion artifacts.

We are preparing in our laboratories a prototype for the 2D reconstruction of the myocardial perfusion map: the NIRS probes support rotates of 180° around the examined organ (human finger or isolated rat heart) and the system acquires a fixed number of measurements. A mathematical algorithm will have to act the reconstruction of the 2D organ image.

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ISSN: 1727-1983