A new biomedical device for in vivo multiparametric evaluation of tissue vitality in critical care medicine

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ABSTRACT

Real time Monitoring of mitochondrial function in vivo is a significant factor in the understanding of tissue vitality. Nevertheless a single parameter monitoring device is not appropriate and effective in clinical diagnosis of tissue vitality. Therefore we have developed a multiparameteric monitoring system that monitors, in addition to mitochondrial NADH redox state, tissue microcirculatory blood flow, tissue total back-scattered light as an indication of blood volume and blood oxygenation (HbO_2). In the present communication a new device named "CritiView" is described. This device was developed in order to enable real time monitoring of the four parameters from various organs in the body. The main medical application of the CritiView is in critical care medicine of patients hospitalized in the Intensive Care Units (ICUs) and intraoperatively in operating rooms. The physiological basis for our clinical monitoring approach is based on the well known response to the development of body emergency situation, such as shock or trauma. Under such conditions a process of blood flow redistribution will give preference to vital organs (Brain, Heart) neglecting less vital organs (Skin, G-I tract or the urinary system). Under such condition the brain will be hyperperfused and O_2 supply will increase to provide the need of the activated mitochondria. The non-vital organs will be hypoperfused and mitochondrial function will be inhibited leading to energy failure. This differentiation between the two types of organs could be used for the early detection of body deterioration by monitoring of the non-vital organ vitality. A fiber optic sensor was embedded in a Foley catheter, enabling the monitoring of Urethral wall vitality, to serve as an early warning signal of body deterioration.

Keywords: Mitochondrial function, NADH fluorescence, Laser Doppler Flowmetry, Critical Care Medicine, Hemoglobin Oxygenation, Multiparametric monitoring, CritiView

1. INTRODUCTION

Mitochondrial function is a prerequisite condition for the continuous supply of energy in order to perform all cellular functions in the body. Most of the Oxygen consumed in the body is utilized by the mitochondria [1], which produces 95% of the ATP in normal tissues. As shown in Fig. 1, the levels of pO_2 needed for normal mitochondrial function is around 1mmHg. The exact level of pO_2 exists in the mitochondria are not known due to the lack of direct monitoring technique for in vivo measurement. The pO_2 levels at the other body locations, shown in Fig. 1 are well known and documented as shown by the average values. The determination of pO2 at the tissue levels is practical by using an appropriate oxygen electrode, representing the average levels of O_2 in the intravascular, extracellular and intracellular compartments.

The best approach to evaluate intracellular or intramitochondrial O_2 levels is to measure the spectroscopic nature of the various components of the respiratory chain [2]. In this publication Chance concluded that "For a system in equilibrium, NADH is at the extreme low potential end of the chain, and this may be the oxygen indicator of choice in mitochondrial and tissue as well". The same conclusion was published by Lübbers stating "The most important intrinsic luminescence indicator is NADH, an enzyme of which the reaction is connected with tissue respiration and energy metabolism [3]. The pioneering work by Chance & Williams [4] opened up an era of 50 years of intensive research dealing with mitochondrial function In vivo. The evaluation, in vitro as well as in vivo conditions, was done by monitoring NADH redox state using the auto-fluorescence approach [5]. Although the importance of normal mitochondrial function in various tissues is appreciated [6,7], the research effort done in this importance field was not significance. Most of the published material regarding in vivo monitoring of mitochondrial NADH was contributed by not more than 20 groups of scientists around the world. In his review article Scheffler ignored the contribution of in

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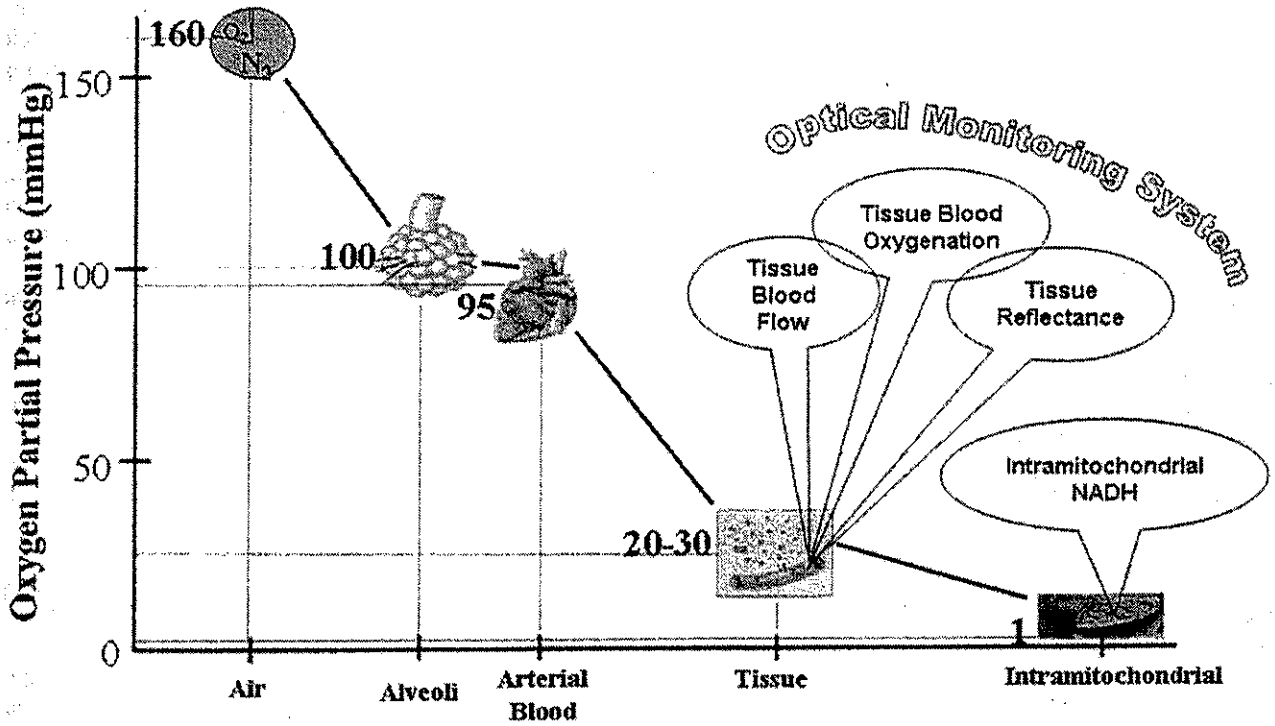

Fig. 1. Oxygen gradient in the body starting at the highest level in the lung (100mmHg) and the lowest levels in the mitochondria (about 1mmHg). The optical measurements of 4 parameters done by the CritiView represent events occur in the microcirculation level as well as in the intracellular space.

vivo monitoring of mitochondrial function (i.e. NADH fluorescence) to the achievements and perspectives. During the last 30 years our group had published about 150 articles in this important subject. We found that monitoring of mitochondrial NADH fluorescence alone is very practical but it is not enough to understand the metabolic state of the tissue. We therefore developed the multiparametric monitoring approach to evaluate the functional state of various tissues or organs in the body [8;9]. The other parameters that could be monitored provide information originate mainly from the vascular compartment. For this reason the correlation between those 3 parameters maybe significant under certain conditions such as ischemia (decrease in blood supply to the tissue). The decrease in blood to the tissue (i.e. ischemia) will be accompanied by a decrease in HbO_2 as was in blood volume (increase reflectance). The intramitochondrial NADH will show a significant increase as published earlier [10-12]. The contribution of HbO_2 monitoring to the understanding of tissue energy metabolism is not clear since it was not monitored intensively with the other 3 parameters namely, blood flow, tissue reflectance and mitochondrial NADH [13-15]. In our previous communication [16] we presented preliminary results using the Tissue Spectroscope (TiSpec-3) The aims of the present study were to upgrade the monitoring system and technology published previously [8;16;17] into the CritiView that could be applied in critical care medicine.

2. METHODOLOGY

2.1 Animal Preparation and experimental protocols

All experimental protocols were approved by the institutional animal care committee under the instruction of the national institute of health.

Male Wistar rats (200-240 g) were used. The rats were anesthetized by Equithesin (E-th = Chloral hydrate 42.51 mg; Magnesium sulfate 21.25 mg; Alcohol 11.5%; Propylene glycol 44.34%; Pentobarbital 9.72 mg) IP injection 0.3 ml/100 gr body weight. The animals were kept anesthetized during the operation as well as during the entire monitoring period, by IP injections of E-th 0.1 ml every 30 minutes. We have been using this anesthetic for approximately 25 years and it has never shown significant effects on mitochondrial activity. Furthermore, we have not

been able to change the response of the rat brain to spreading depression by using an increased dose of equithesin, suggesting that this is a safe drug [18]. The addition of small volumes of E-th every 30 minutes kept the animal in a stable state. Body heat was measured by a rectal probe (YSI) and was regulated to be at the range of 35-37°C using a heating blanket.

The rat was anesthetized and placed in a head holder. After a midline incision of the skin, a hole (5 mm in diameter) was drilled in the parietal bone of the right hemisphere. The dura mater remained intact and an appropriate light guide holder was placed in the hole. Two stainless steel screws in the right parietal bone were used, with dental acrylic cement, to fixate the probes, which were positioned by a micromanipulator on the cortex. In order to induce cortical spreading depression, a small push-pull cannula was placed epidurally 2mm anterior to the light guide holder. The two common carotid arteries were isolated just before brain surgery, and ligatures of 4-0 silk threads were placed around them.

The experimental animals were exposed to the following perturbations:
Anoxia: The animals were exposed to oxygen deficient atmosphere by spontaneous breathing of N_2, for a short (25 sec) period. Terminal anoxia was induced by 100% N_2 until the animal stopped breathing.
Cortical Spreading Depression (SD): The surface of the dura was washed by 0.5-1.0 M KCl solution.
Ischemia: Reversible occlusion (~1min.) of one or 2 common carotid arteries (by constricting them with threads) led to brain ischemia in the rats.

2.2 The Monitoring System-"CritiView"

The new device named CritiView is based on the principles of monitoring the 4 parameters as described below:

2.2.1 Mitochondrial function

Mitochondrial function is evaluated by monitoring of NADH fluorescence. The measurement is done by excitation of the tissue by UV light (375nm) and measuring the emitted fluorescence signal (420-480nm). The principles of this method was established 50 years ago [2] and the main changes that were introduced since than were the type of light source used as well as the usage of bundle of optical fibers to connect the tissue to the fluorometer. The main light source that was used was Hg arc lamp having a strong emitted line at 366nm absorbed by the reduced form, NADH and not by the oxidized form (NAD^+). The introduction of 375nm LED by Nichia as a new light source in the present instrument (CritiView) affects significantly the size and the price of this component in this medical device.

The use of optical fibers led to a more flexible connection between the monitored tissue and the fluorometer. This enabled us to monitor the unanesthetized brain [10] or at different locations in the same animal [19]. The fluorometer of NADH is a good indicator for the decrease in oxygen availability in the cellular compartment. As shown in Fig. 2 exposing the brain to anoxia (100% N_2) led to a significant increase in the NADH fluorescence spectrum.

2.2.2 Tissue Reflectance

The continuous changes in tissue absorption properties affect the intensity of tissue reflectance. It was found that this parameter could be used as a correction tool for the artifacts measured in the fluorescence signal [2;8;20]. As seen in Fig. 2 the main factor affecting the reflectance signal (R) changes by blood volume [10;19]. When ischemia is induced, the decrease in blood volume will lead to an increase in the R signal. During the recovery phase from ischemia the hyperemic response (increase blood volume) will be recorded as a decrease in the R signal.

2.2.3 Tissue Blood Flow

Monitoring of microcirculatory tissue blood flow using the laser Doppler approach has started more than 25 years ago [21;22] and is widely used in various animal model as well as in clinical applications [23]. The main advantage of the LDF approach is the real time data provided by the optical nature of the signal. As of today, the large number of publications (more than 5,000) in experimental animals and patients indicate its applicativity (for more details see Ref.8).

2.2.4 Microcirculatory blood Oxygenation

For the monitoring of blood oxygenation we utilized a 2-wavelength reflectance technique to analyze the absorption of light in the blood, exploiting the difference in absorption spectra of oxyhemoglobin (hemoglobin saturated with oxygen) and deoxyhemoglobin. The principles of the technique were demonstrated years ago using a time-sharing system [13]. Generally, absorption coefficient of oxyhemoglobin differs from that of deoxyhemoglobin, along most of the spectrum [24]. However in some certain isosbestic wavelength, the absorption curves cross each other and represent

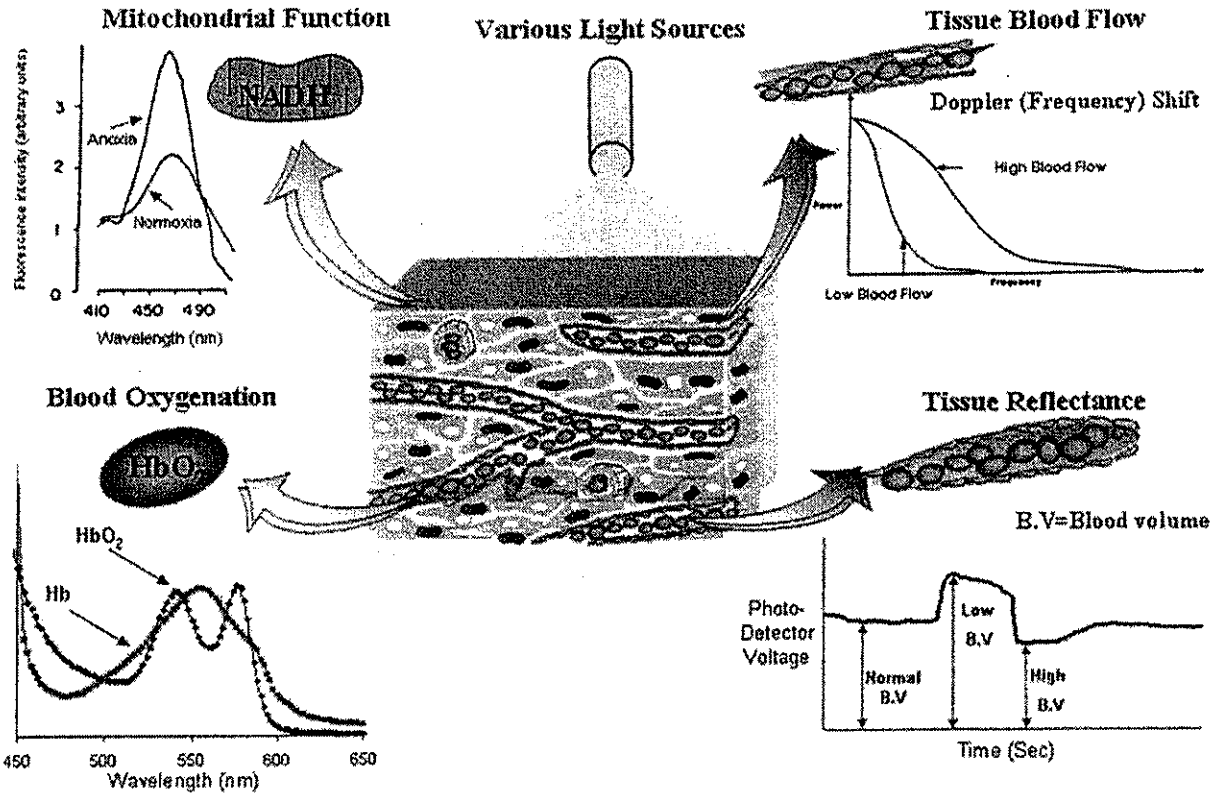

Fig. 2. The principles of tissue vitality monitoring performed by the CritiView. Three of the parameters were monitored from the intravascular compartment while the NADH parameter represents the function of the intramitochondrial space.

the same value. The change in reflectance in an isosbestic wavelength is affected only by blood volume, while a change in reflectance in a non isosbestic wavelength is affected by the oxy-deoxy ratio as well. By subtracting the back-reflectance in these two wavelengths, one can consider the difference between the measurements as qualitative representation of blood oxygenation at the microcirculatory level. As seen on Fig. 2, the former time-sharing system[13;25] used two Hg-lamp emission bands to determine the oxy-deoxy ratio in-vivo by measuring the reflectance in 585nm (R_{585}) and 577nm (R_{577}). Calculating - (R_{577}-R_{585}) yields the relative change in oxyhemoglobin state. In the present study we used the same principle with contemporary 530nm (nearly isosbestic) and 470nm (non-isosbestic) super bright LEDs. The relative change in oxyhemoglobin state is therefore derived by - (R_{470}-R_{530}).
It is important that the two wavelengths are closed and roughly within the same range of absorption coefficient. Thus, the absorption of the tissue may be considered constant and get neutralized.

2.3 Device description

In our last communication [16], we described the TiSpec03 which employed a light source unit (LSU) based on a NIR laser diode for Doppler measurement and a UV LED for reflectance measurement and NADH excitation. Additionally, this device harbored a module for the determination of the oxygenation level of hemoglobin (HbO_2) by two wavelength reflectometry, based on Blue and Green LEDs.

The NADH fluorescence emission spectrum peaks at about 450nm while the blue LED reflection peaks at near by 470nm. Therefore in order to eliminate the signal interference from the strong reflectance signal to the weak fluorescence signal the excitation fibers measuring the NADH and oxygenation parameters where separated by few millimeters. Separate detectors where used for detection of each parameter. In this new CritiView device new detection concept is utilized. This new concept enables detection of NADH fluorescence and the both blue and green reflection by a single detector. Additionally it eliminates the need in the separation of the excitation fibers of NADH and oxygenation.

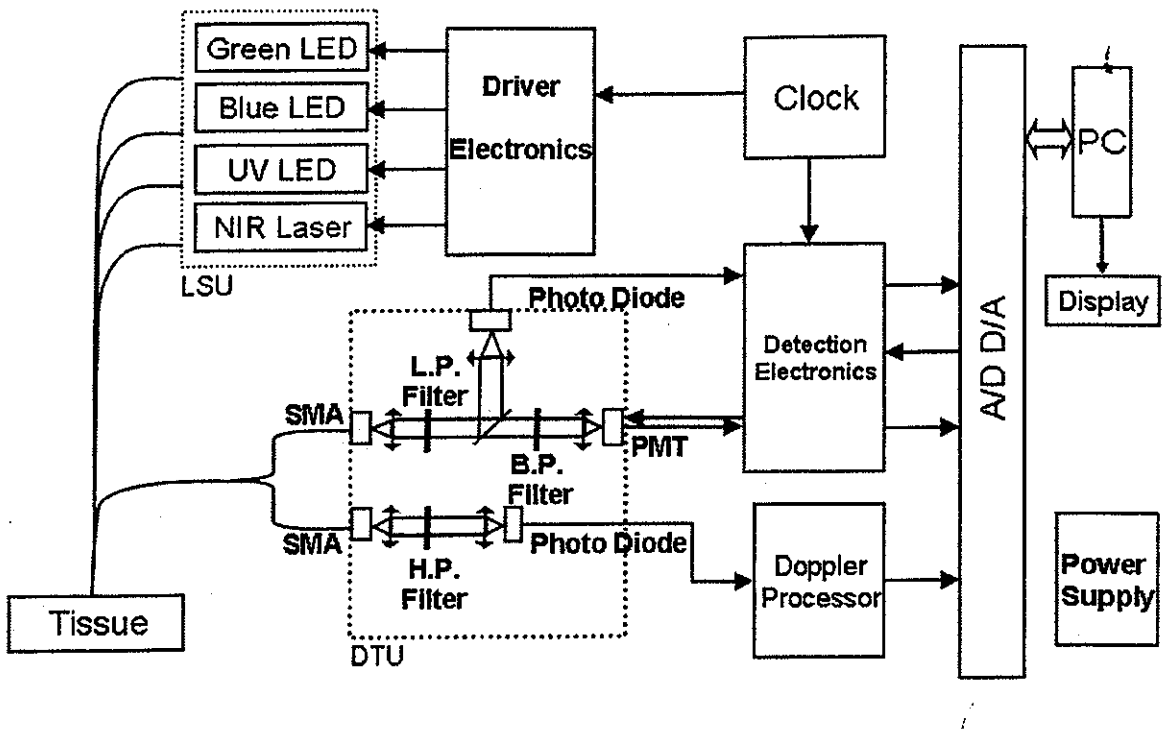

Fig. 3. Blocks diagram of the current CritiView used in the present study (for details see text).

2.3.1 Light Source Unit (LSU)

The light source unit of the CritiView comprises a 785nm CW laser diode which serves for laser Doppler measurement, a 375nm LED for NADH fluorescence excitation and for total back scatter (or reflection) measurement, a 470nm LED and 530nm LED for oxygenation measurement. Each one of the discrete light sources is fed by the appropriate electronics drivers.

2.3.2 Detection Unit (DTU)

The detection system is based on two channel detection. On one channel, the 785nm light is collimated after emitting the fibers, then filtered by high-pass filter and focused into the active area of a fast photodiode with a joined pre-amplifying circuit. The detected signal processed by analog Doppler processor and the result values are digitized into the PC by A/D converter. On the other channel, the light is collimated and filtered by low pass filter in order to eliminate the 785nm reflection. Then the light is splited according to wavelengths by the dichroic mirror. The Back-reflected 375nm light is reflected by the dichroic mirror towards focusing lenses and on the active area of a second pre-amplified photodiode. The NADH fluorescence around 450nm and the reflections at 470nm and 530nm are passing through the dichroic filter. These wavelengths pass through interference filter specified for NADH emission and detected by photomultiplier (PMT). Since the reflection intensities of 470nm and 530nm signals are much stronger then the NADH fluorescence the actual irradiation intensity of these light sources is reduced significantly in order to adjust the reflection intensity levels to be similar to the fluorescence level. This enables to utilize single PMT detector to detect all three wavelengths. This arrangement also enables to eliminate the need in two separate excitation fibers. The detected signals processed by detection electronics that consists of sample and hold circuits that synchronized with the respective excitation pulses. The output of the detection electronics is acquired into PC by A/D converter.

In order to connect the experimental animal or the patients to the monitoring system we used various types of probes. In our previous communication[8;17] we described in many details the basic structure of the probes used for brain studies in experimental animals as well as in clinical environment. In addition a probe used in the neonate intensive care unit was shown. Fig. 4 shows the most advanced probe used in monitoring patients in the adult ICU.

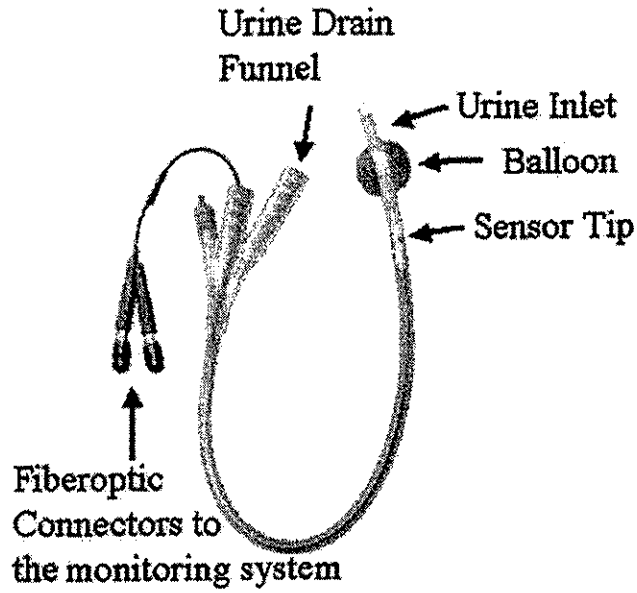

Fig. 4. A picture of a three-way Foley catheter used in patients hospitalized in the Intensive Care Unit (ICU): The balloon is filled with physiological saline after the insertion of the catheter into the bladder.

3. RESULTS

The new device – CritiView was tested in a group of rats exposed to changes in O_2 supply, (anoxia, ischemia) as well as during increase in O_2 demand (cortical spreading depression). In the 5 figures presented in this communication 4 signals are presented. The upper trace CBF is measured by the laser Doppler flowmetry (LDF) and the trace represent the flux of red blood cells in the microcirculation. The 2nd and the 3rd traces are measured by the mitochondrial fluorometry/reflectometry. The NADH signal is calculated by subtraction the reflectance signal from the fluorescence as used by various investigators (for details see10;19).

The Y-scale of all parameters is expressed as percent change of the signal calibrated to 100% in the initial stage of the monitoring.

In Fig. 5, the responses to an anoxic episode are shown. As expected the decrease in HbO_2 was accompanied by an increase in the NADH as well as a decrease in CBF. During the recovery phase a large hyperemic response was noted in the HbO_2 and CBF. The decrease in the reflectance signal also indicates on increase in blood volume. The changes in the NADH were quite symmetrical in term of the percent change during the anoxia and the recovery to the same baseline.

In Fig. 6 a typical responses to 2 wave of Spreading Depression are shown. The oxidation cycles (decrease in NADH) are typical to this event correlated to the large increase in CBF. In this rat, the HbO_2 signal shows also an increase in brain oxygenation. As seen the mitochondrial response was much shorter as compared to the response recorded in the CBF.

Fig. 7 shows repetitive cycles of SD recorded from another rat that was presented in Fig. 6. The 5 oxidation cycles seen in the NADH signal are very symmetrical and similar to each other. The changes in CBF and HbO_2 were less stable and were correlated to each other. The baseline was shifted as passing from the 1st to the 5th cycle. When the response to the 6th SD cycle was started (vertical line) the right carotid artery was occluded (Rocc1). As seen, the response to the SD was completed under the small change in blood supply and an oxidation cycle was recorded but with a slower rate of recovery. During the occlusion the HbO_2 shows the decrease was followed by an increase in the signal correlated to the increase in CBF. After recovery from the SD response, the effect of anoxia was typical to the brain. After the recovery to normoxia the last response to the SD stimulus was recorded. Figures 8 and 9 present responses to SD in normoxia brain (Fig. 8) and in partial ischemic brain (Fig. 9).

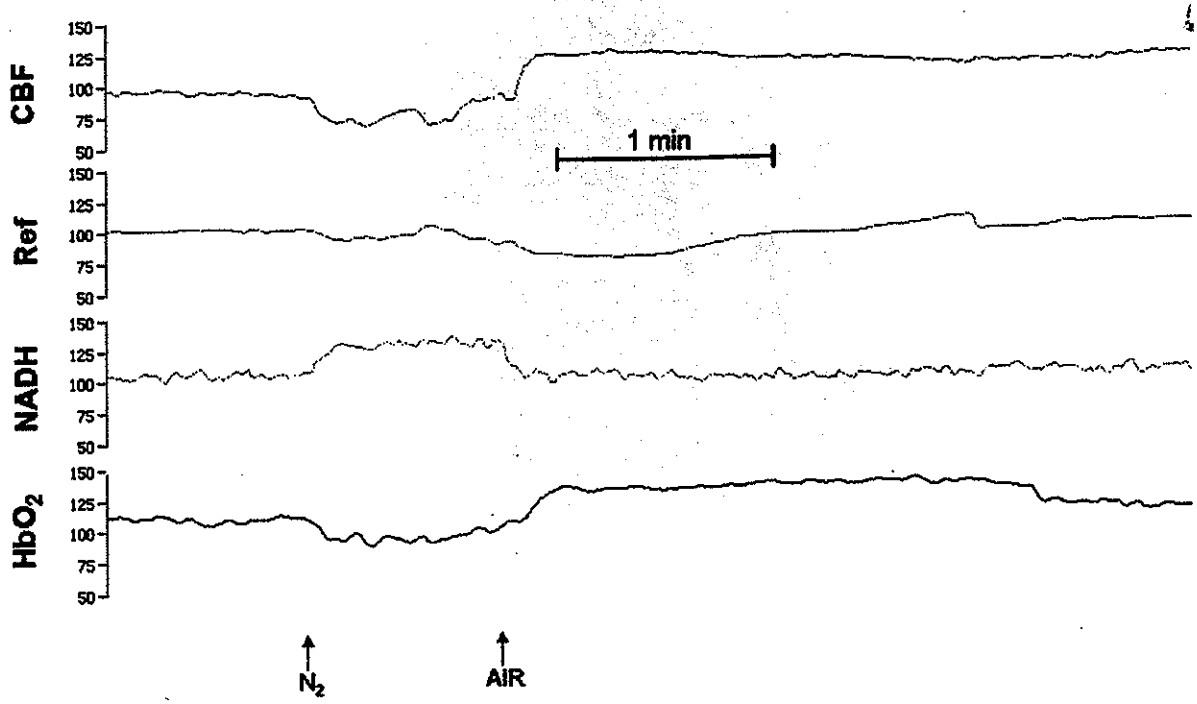

Fig. 5. Responses to complete deprivation of O_2 by breathing the rat with 100% O_2. CBF-cerebral blood flow, Ref-reflectance or total back-scattered light at the 375 excitation wavelength, NADH-net change in the NADH, HbO_2-microcirculatory blood oxygenation. The Y-scale of all parameters is expressed as percent change of the signal calibrated to 100% in the initial stage of the monitoring.

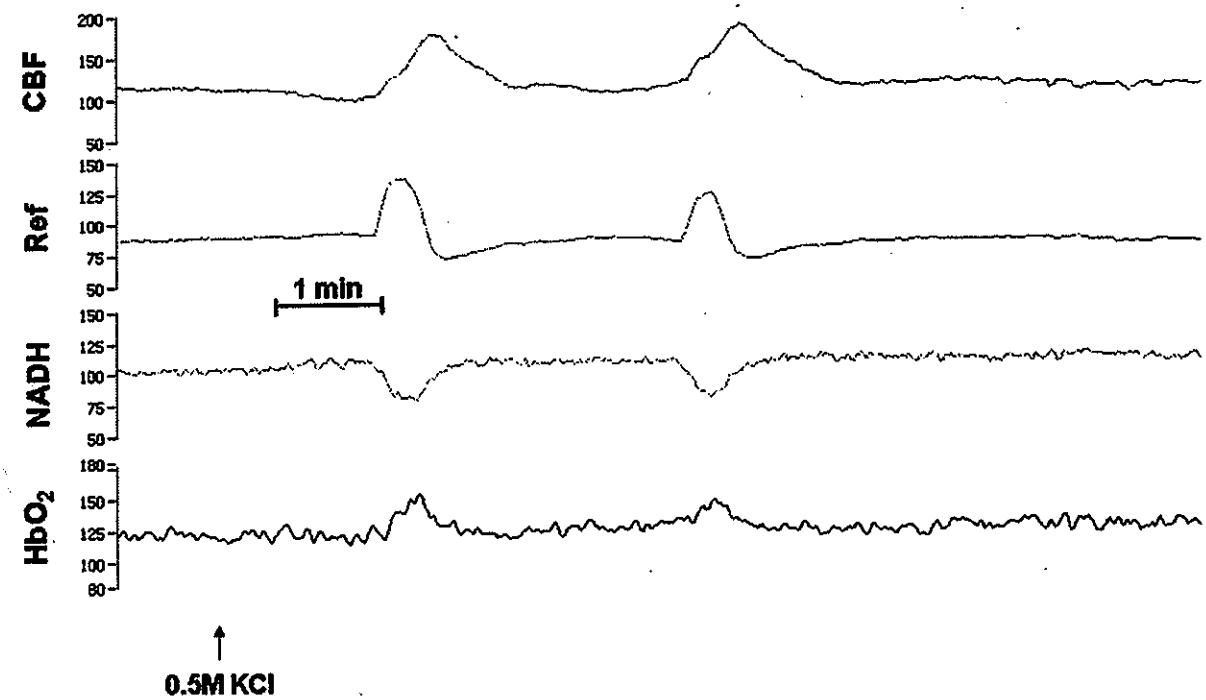

Fig. 6. Two responses to cortical spreading depression (SD) are shown. Abbreviations are as in Fig. 5.

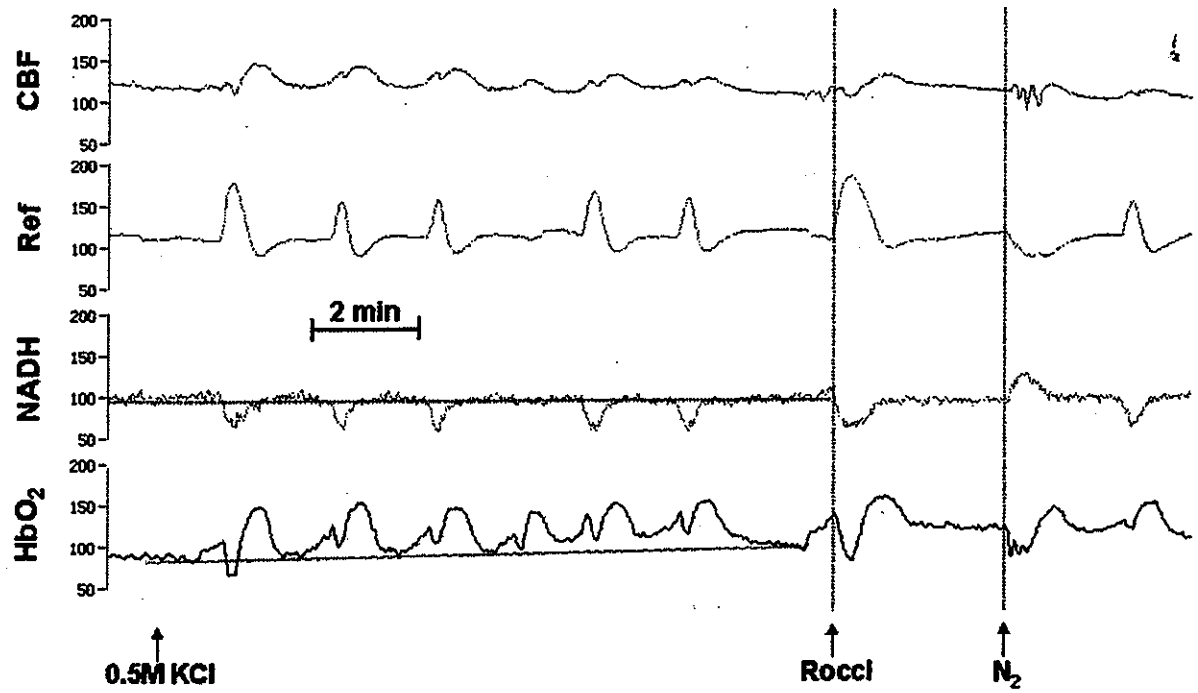

Fig. 7. Repetitive cycles of cortical spreading depression (SD) measured in the normoxic brain and the effect of partial ischemia on the response to SD. Abbreviations are as in Fig. 5.

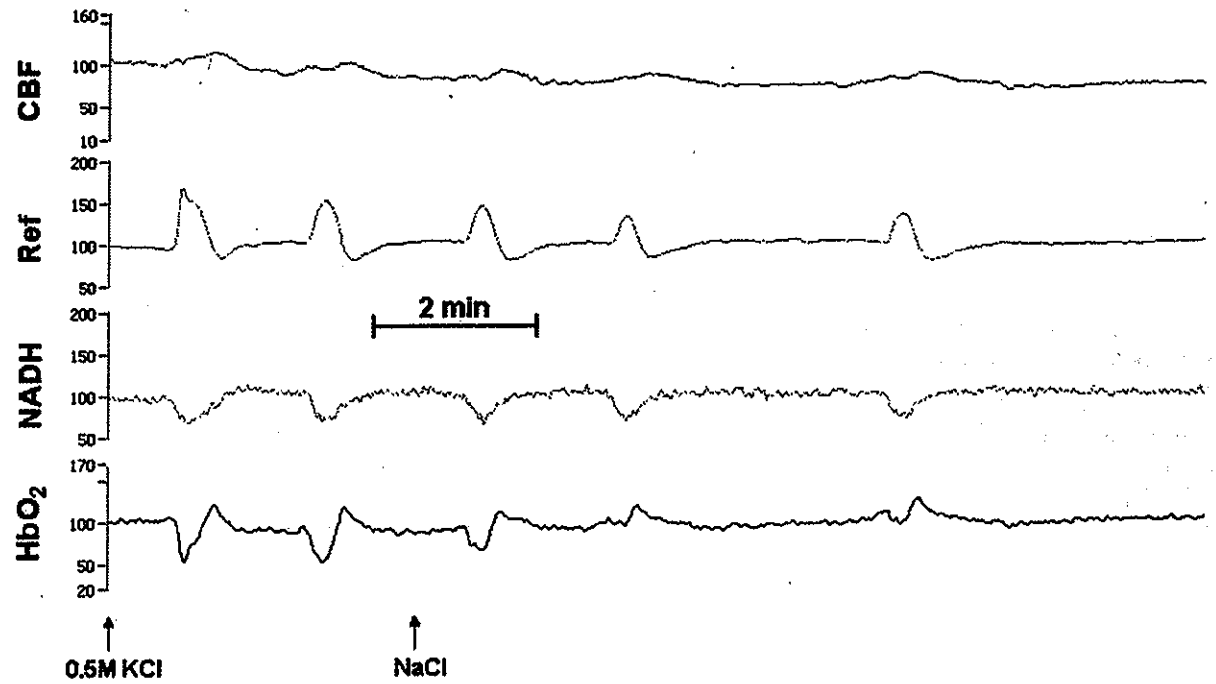

Fig. 8. Repetitive responses to cortical spreading depression in the normoxic brain. Abbreviations are as in Fig. 5.

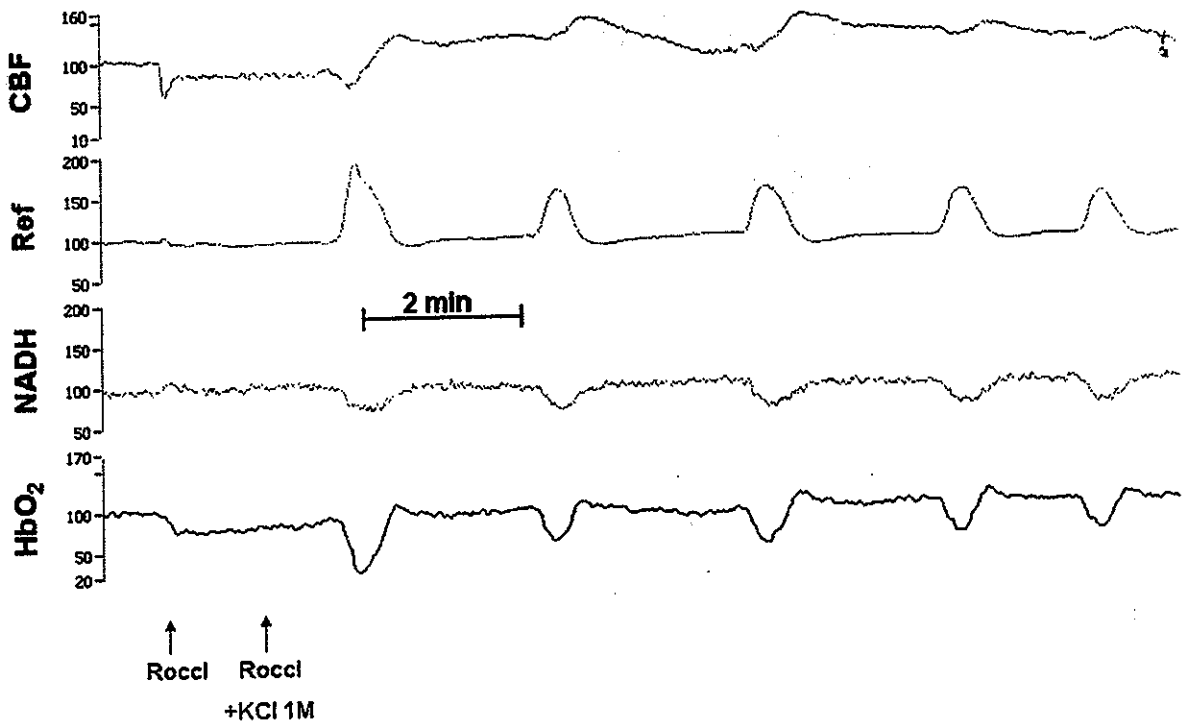

Fig. 9. The effect of partial ischemia (Roccl) on the responses to repetitive cycles of spreading depression. Abbreviations are as in Fig. 5.

4. DISCUSSION

The involvement of mitochondrial function in various pathophysiological conditions, developed in experimental and clinical situation, is widely documented. The relationship between mitochondrial and neuronal survival was reviewed recently [7] The aim of the present study was to describe the new medical device – CritiView which enables the monitoring of microcirculatory hemoglobin oxygenation in addition to the three parameters measured before [16], namely tissue microcirculatory blood flow, reflectance and mitochondrial NADH redox state. Tissue vitality is correlated to oxygen or energy balance defined as the ratio between O_2 supply and demand. Energy or oxygen supply mechanisms are identical in all tissues and therefore could be monitored by the same technique – CritiView, in various body organs. In order to understand the functional state of a tissue, exposed to various pathophysiological conditions, the more parameters to be monitored the better the diagnosis of the tissue will be.

Until recently, most of the monitoring devices used in experimental animals or in clinical environment were based on a single parameter. Out of the suggested 4 parameters monitored, laser Doppler flowmetry is the most distributed technology [26-30] in experimental and clinical studies. Most of the 5,000 studies were done by monitoring of tissue blood flow in a single organ (skin, brain, heart etc.). Few medical devices were developed in order to monitor microcirculatory blood oxygenation (HbO_2). This kind of measurement is different from the widely used pulse oximeter that can measure oxygenation of blood that flows in large arteries. The changes in local O_2 consumption will affect local oxygenation but the pulse oximeter will not show any change. Monitoring of intramitochondrial NADH redox state in vivo was started close to 50 years ago [31]. The multiparametric monitoring approach was started close to 20 years when we were able to combine the measurement of NADH and PO_2 [32], NADH and CBF [11] or NADH, CBF and HbO_2 [14-16]. Only recently we were able to upgrade the laboratory type of monitoring system into a medical device that was also approved by the FDA [8]. In the present version of our device we add to the three monitored parameters (TiSpec) the microcirculatory HbO_2, which will improve the diagnosis value of the CritiView. The possibility to correlate the systemic blood oxygenation (pulse oximetry) to the microcirculatory HbO_2 will add a new dimension to

medical diagnosis made by the CritiView. This new device was also upgraded in the level of laser safety. The previous model (TiSpec) was classified as class 3B according to IEC standards (60825-1), while by improving the detection sensitivity (CritiView) the device is classified as class 1. Also the smaller dimensions of the CritiView will enable clinical team to have a monitoring unit near each bad in the operating rooms or in the intensive care units.

As seen in the results presented in this study, the most stable and representative parameter of tissue energy metabolism is the mitochondrial NADH redox state. The main factors affecting mitochondrial NADH are substrate and O_2 availability, levels or turnover of ADP which are determined by metabolic activity of the tissue. The CBF and HbO_2, which are interconnected, are more sensitive to instability of regulation of blood flow, which is affected by many factors (NO, CO_2, pH) and not only by oxygen consumption.
We can conclude that monitoring mitochondrial NADH is the most appropriate parameter in evaluation of tissue energy metabolism. The addition of the other three parameters is also significant to the diagnosis of tissue vitality.

References

1. C. L. Waltemath, "Oxygen, uptake, transport, and tissue utilization", *Anesth. Analg.*, **49**, pp. 184-203, 1970.
2. B. Chance, N. Oshino, T. Sugano, and A. Mayevsky, "Basic principles of tissue oxygen determination from mitochondrial signals", *Oxygen Transport to Tissue, Adv. Exp. Med. Biol.*, H. I. Bicher and D. F. Bruley, Eds. pp. 277-292, Plenum Publishing Corporation, New York, 1973.
3. D. W. Lubbers, "Optical sensors for clinical monitoring", *Acta Anaesth. Scand. Suppl.*, **39**, pp. 37-54, 1995.
4. B. Chance and G. R. Williams, "Respiratory enzymes in oxidative phosphorylation (I- Kinetics of oxygen utilization)", *J. Biol. Chem.*, **217**, pp. 383-393, 1955.
5. B. Chance, P. Cohen, F. Jobsis, and B. Schoener, "Intracellular oxidation-reduction states *in vivo*", *Science*, **137**, pp. 499-508, 1962.
6. I. E. Scheffler, "A century of mitochondrial research: achievements and perspectives", *Mitochondrion*, **1**, pp. 3-31, 2000.
7. D. G. Nichols and S. L. Budd, "Mitochondrial and neuronal survival", *Physiological Rev.*, **80**, p. 360, 2000.
8. A. Mayevsky, T. Manor, E. Pevzner, A. Deutsch, R. Etziony, N. Dekel, and A. Jaronkin, "Tissue spectroscope: a novel in vivo approach to real time monitoring of tissue vitality", *J. Biomed. Opt.*, **9**, pp. 1028-1045, 2004.
9. A. Mayevsky, A. Meilin, G. G. Rogatsky, N. Zarchin, and J. Sonn, "Multiparametric monitoring of the awake brain exposed to carbon monoxide", *J. Appl. Physiol.*, **78**, pp. 1188-1196, 1995.
10. A. Mayevsky, "Brain NADH redox state monitored *in vivo* by fiber optic surface fluorometry", *Brain Res. Rev.*, **7**, pp. 49-68, 1984.
11. A. Mayevsky, E. S. Flamm, W. Pennie, and B. Chance, "A fiber optic based multiprobes system for intraoperative monitoring of brain functions", *SPIE*, **1431**, pp. 303-313, 1991.
12. A. Mayevsky and H. R. Weiss, "Cerebral blood flow and oxygen consumption in cortical spreading depression", *J. CBF Metab.*, **11**, pp. 829-836, 1991.
13. I. J. Rampil, L. Litt, and A. Mayevsky, "Correlated, simultaneous, multiple-wavelength optical monitoring in vivo of localized cerebrocortical NADH and brain microvessel hemoglobin oxygen saturation", *J. Clin. Monit.*, **8**, pp. 216-225, 1992.
14. A. Mayevsky, K. Frank, M. Muck, S. Nioka, M. Kessler, and B. Chance, "Multiparametric evaluation of brain functions in the Mongolian gerbil *in vivo*", *J. Basic Clin. Physiol. Pharmacol.*, **3**, pp. 323-342, 1992.
15. A. Mayevsky, K. H. Frank, S. Nioka, M. Kessler, and B. Chance, "Oxygen supply and brain function *in vivo*: a multiparametric monitoring approach in the Mongolian gerbil", *Oxygen Transport to Tissue XII*, J. Piiper, T. K. Goldstick, and M. Meyer, Eds. pp. 303-313, Plenum Press, 1990.
16. A. Deutsch, E. Pevzner, A. Jaronkin, and A. Mayevsky, "Real time evaluation of tissue vitality by monitoring of microcircultory blood flow, HbO2 and mitochondrial NADH redox state", *SPIE Proc.*, **5317**, pp. 116-127, 2004.
17. E. Pevzner, A. Deutsch, T. Manor, N. Dekel, R. Etziony, I. Derzy, N. Razon, and A. Mayevsky, "Real-time multiparametric spectroscopy as a practical tool for evaluation of tissue vitality in vivo", *Proc. SPIE*, **4958**, pp. 171-182, 2003.
18. A. Mayevsky, N. Zarchin, and C. M. Friedli, "Factors affecting the oxygen balance in the awake cerebral cortex exposed to spreading depression", *Brain Res.*, **236**, pp. 93-105, 1982.
19. A. Mayevsky and B. Chance, "Intracellular Oxidation-Reduction State Measured in Situ by a Multicannel Fiber-Optic Surface Fluorometer", *Science*, **217**, pp. 537-540, 1982.

20. F. F. Jobsis, M. O'Connor, A. Vitale, and H. Vreman, "Intracellular redox changes in functioning cerebral cortex. I. Metabolic effects of epileptiform activity", *Neurophysiology*, **3465**, pp. 735-749, 1971.
21. M. D. Stern, "In vivo evaluation of microcirculation by coherent light scattering", *Nature*, **254**, pp. 56-58, 1975.
22. R. Bonner and R. Nossal, "Model for laser Doppler measurements of blood flow in tissue", *Applied Optics*, **20**, pp. 2097-2107, 1981.
23. A. P. Shepherd, "History of laser-Doppler blood flowmeter", *Laser-Doppler blood flowmeter,* A. P. Shepherd and P. A. Oberg, Eds. pp. 1-16, Kluwer academic, Boston, Dordrecht, London, 1990.
24. S. Prahl, "Optical Absorption of Hemoglobin", http://omlc.bme.ogi.edu/spectra/hemoglobin/index.html, 1999.
25. A. Mayevsky, E. Barbiro-Micahely, H. Kutai-Asis, A. Deutsch, and A. Jaronkin, "Brain physiological state evaluated by real time multiparametric tissue spectroscopy in vivo", *Proc. SPIE*, **5326**, pp. 98-105, 2004.
26. J. E. Batista, J. R. Wagner, K. M. Azadzoi, R. J. Krane, and M. B. Siroky, "Direct measurement of blood flow in the human bladder", *J. Urol.*, **155**, pp. 630-633, 1996.
27. V. Krejci, L. Hiltebrand, A. Banic, D. Erni, A. M. Wheatley, and G. H. Sigurdsson, "Continuous measurements of microcirculatory blood flow in gastrointestinal organs during acute haemorrhage", *Br. J. Anaesth.*, **84**, pp. 468-475, 2000.
28. D. T. Ubbink, I. I. Tulevski, and M. J. Jacobs, "Can the green laser doppler measure skin-nutritive perfusion in patients with peripheral vascular disease?", *J. Vasc. Res.*, **37**, pp. 195-201, 2000.
29. P. Okunieff, X. Wang, P. Rubin, J. N. Finkelstein, L. S. Constine, and I. Ding, "Radiation-induced changes in bone perfusion and angiogenesis", *Int. J. Radiat. Oncol. Biol. Phys.*, **42**, pp. 885-889, 1998.
30. M. Sailer, E. S. Debus, K. H. Fuchs, J. Beyerlein, and A. Thiede, "Comparison of anastomotic microcirculation in coloanal J-pouches versus straight and side-to-end coloanal reconstruction: an experimental study in the pig", *Int. J. Colorectal Dis.*, **15**, pp. 114-117, 2000.
31. B. Chance, "Cellular oxygen requirements", *Fed. Proc.*, **16**, pp. 671-680, 1957.
32. A. Mayevsky, S. Lebourdais, and B. Chance, "The interrelation between brain PO_2 and NADH oxidation-reduction state in the gerbil", *J. Neurosci. Res.*, **5**, pp. 173-182, 1980.