1. Introduction

The aim of this chapter is to introduce objective pursued by this thesis, that is, the detection and the quantification of the ischemia injury, and to summarize the clinical methods already available and those under research.

Tissue electrical impedance monitoring is one of the methods under research. In this chapter, a short review on previous studies denoting a relationship between ischemia and electrical impedance has been included. Further information on bioimpedance monitoring can be found in Annex A.

This thesis has been developed within the framework of two EU funded projects that are referenced through the report.

1.1. Ischemia

Ischemia is a physiological term denoting insufficient blood flow for normal cellular function. It impedes the delivery of the necessary oxygen (hypoxia) and nutrients to the tissue cells.

In a non-ischemic animal tissue, cells primary use aerobic respiration where glucose is converted with the presence of O_2 into water, CO_2 and energy. The energy is used to build up a molecule called Adenosine Tri-Phosphate (ATP). ATP can provide energy for other processes such as muscle contractions. CO_2 is a byproduct of the reaction that is removed by blood circulation. During ischemia, there is a decrease in the oxygen and glucose available to the tissue as well as a decrease in the removal of carbon dioxide from the tissue due to inadequate blood flow. As a result of the decrease of the available oxygen, the tissue employs an anaerobic metabolic pathway. In this process glucose is also broken down to generate ATP but the efficiency is reduced (less ATP is obtained per glucose molecule) and lactic acid is generated, causing a decrease in the tissue pH. The reduced availability of ATP implies also a decrease in the available metabolic energy.

Animal cells keep their volume constant by means of an active mechanism that implies the consumption of ATP. Almost one-third of the energy requirement of a typical animal cell is consumed in fueling this mechanism. Briefly, the content of proteins and other non-diffusible macro-molecules within cells creates an osmotic pressure that is counter-balanced by extracellular concentration of sodium ions. The extracellular concentration of sodium, however, is not the result of an impermeability of plasma membranes to sodium ions, but the consequence of a continuous active extrusion of sodium from the cell interior to the exterior in order to compensate for the influx diffusion of sodium due to the its low intracellular concentration and the electro-negativity of the non-diffusible molecules and proteins inside the cell. When the supply of metabolic energy becomes insufficient to sustain the usual rate of extrusion of sodium from the cell, as in the case of ischemia, sodium ions permeate into the cell and are not removed by active ion pumps. As a result, a net gain in intracellular sodium and chloride concentration takes place. This increase in solutes within the cell draws water osmotically from the extracellular compartment into the cell, resulting in a marked swelling of cells [1].

The final consequence of a large and severe ischemia period is cell necrosis (cell death[1]). The cell swells so much that the integrity of the membrane is disrupted, it breaks up (lysis) and denatured proteins and DNA fragments are liberated to the extracellular medium. Because of that, the cell-death process is then typically followed by a local inflammatory response.

[1] There is another sort of cell death process which is called apoptosis ('programmed cell death') and is considered to be the smart cell death mechanism, although adjacent necrosis can be an unwanted trigger for apoptosis in cases of severe ischemia.

The sodium extrusion mechanism is based on the Na^+-K^+ pumps or Na^+ pumps. These protein structures are found in the plasma membrane of virtually all animal cells. Each pump operates as an antiporter, actively pumping sodium out of the cell against its steep electrochemical gradient and pumping K^+ in. Because the pump hydrolizes ATP to pump Na+ out and K+ in, it is also known as a Na^+-K^+ ATPase [2].

Non-animal cells employ other techniques to cope with their osmotic problems. Plant cells and many bacteria are prevented from bursting by the semirigid cell wall that surrounds their plasma membrane. In amoebae the excess water that flows in osmotically is collected in contractile vacuoles, which periodically discharge their contents to the exterior.

There are some protective cell responses induced by hypoxia. One of them is angiogenesis: the development of new blood vessels from existing vessels. A shortage of oxygen, in practically any type of cell, causes an increase in the intracellular concentration of a gene regulatory protein called hypoxia-inducible factor 1 (HIF-1). HIF-1 stimulates transcription of a gene whose product is a protein known as vascular endothelial growth factor (VEGF). The VEGF protein is secreted, diffuses trough the tissue, and acts on nearby endothelial cells to stimulate the development of new capillaries. As the new vessels form, bringing blood to the tissue, the oxygen concentration rises, HIF-1 activity declines, VEGF production is shut off, and angiogenesis stops. Of course, this is a long-term regulation process with cycle times of several days [2].

Another mechanism for the regulation of local blood flow acts faster: when either the rate of tissue metabolism increases or the availability of oxygen is reduced, the vessels that perfuse this tissue (metarterioles and arterioles) become dilated and local blood flow increases. The basis of this mechanism is still not fully understood. There are two basic theories: 1) cells release some compounds (vasodilator substances) in response to oxygen deficiency that cause the vessels to dilate and 2) the lack of oxygen relaxes the vascular muscle contraction and more blood is made available to flow [3].

Ischemia can be caused naturally by vascular accidents. The myocardial infarction and the brain stroke are the most known fatal consequences of severe ischemic injuries [3]. However, ischemia can also be induced artificially in some therapeutic maneuvers. That does not mean that ischemia is beneficial for the living organism[3], but it is a consequence of the applied medical methods. Two examples of artificially induced ischemia are the heart arrest during cardiac surgery and the cold preservation of grafts during transplantation. Obviously, in these cases it would be very interesting to have

[2] Its importance in controlling cell volume is indicated by the observation that many animal cells swell, and often burst, if they are treated with *ouabain*. This Na^+-K^+ pump inhibitor is a natural substance used by some African peoples as a dart poison [2].

[3] There are some exceptions. For instance, ischemic preconditioning is a case in which ischemia can be considered beneficial for the living tissue. This surgical technique, still under study, consists in inducing a short-lasting ischemia before a later large ischemia period (e.g. for organ transplatation) [4;5]

some method available for monitoring the amount of tissue damage caused by ischemia.

Table 1.1. Changes due to ischemia.

parameter	change due to ischemia
extracellular pH	decrease
extracellular K^+	increase
extracellular Na^+	decrease
ATP	decrease
lactate	increase
pO_2	decrease
pCO_2	increase
cell volume	increase
extracellular volume	decrease
VEGF	increase
adenosine (vasodilator)	increase

As it will be explained later, there exist some methods to monitor the ischemia process (i.e. blood perfusion level) and a few more are available to monitor the effect of ischemia on the living tissues. The scope of this thesis is focused on the monitoring of ischemia induced damage by measuring the electrical impedance of living tissues (also referred as electrical bioimpedance). An indirect relationship between ischemia and bioimpedance is generally accepted: the cell swelling resulting from inhibition of energy metabolism narrows the extracellular space and, consequently, reduces the width of the electrical path for low frequency currents (plasma membranes are considered as dielectric), thus decreasing conductance and increasing resistance. However, the electrical bioimpedance characteristics still lack a complete explanation in terms of biological structures and physiological events. Other cause-effect connections have also been proposed between physiology and bioimpedance.

For a general introduction to the electrical bioimpedance field and its relation to ischemia, annex A has been included. This document is specifically written to introduce life-sciences practitioners to the electrical bioimpedance field. Other relevant books and reviews on this topic are suggested for physicists and engineers: [6-11]

1.2. Thesis framework and scope

This thesis has been developed within the framework of two EU funded projects. Both of them are related with the monitoring of ischemic processes using silicon probes containing multiple sensors.

Roughly, the objectives pursued by this thesis have been:

- to develop and analyze the methods and the instrumentation to measure electrical impedance of living tissues in a clinically applicable manner, specially under events of induced ischemia.
- to obtain experimental results denoting a relationship between tissue electrical impedance and ischemia damage.
- to provide, when possible, physiological interpretations of the observed experimental results.

1.2.1. MicroCard project

(LTR-23485, TIC98-1634-CE)
'Si-Based Multifunctional Microsystem Needle For Myocardial Ischemia Monitoring'

This project was created with the ultimate goal of exploring the capabilities of silicon technologies for health care monitoring.

The MicroCard project proposed the implementation of a demonstrator consisting of a multi-sensor silicon needle probe able to monitor different myocardium parameters known to be related with ischemia: the electrical bioimpedance, the extracellular K^+ [12] concentration and the extracellular pH [13]. The device would contain the electronics needed to acquire the information from the sensors and the wireless transmission capabilities to send such information to a monitor located in the operating theatre.

The device was intended for an early detection of myocardial ischemia injury during cardiac surgery, while the heart is being artificially arrested (i.e. extracorporeal circulation). During this period, the electrocardiogram (ECG) is not available as a monitoring technique and there are not established clinical methods to assess the damage caused to the myocardium. It is known that the post-operative mortality in patients undergoing cardiac surgery is markedly related to the effectiveness of myocardial protective techniques applied during the operative phase of extracorporeal circulation. Thus, the introduction of a device capable of monitoring ischemia damage during extracorporeal circulation would have a remarkable importance in the cardiac surgery field. This implies also a substantial economic impact, since the number of cardiac operations per year world-wide lies now in is the range of one million.

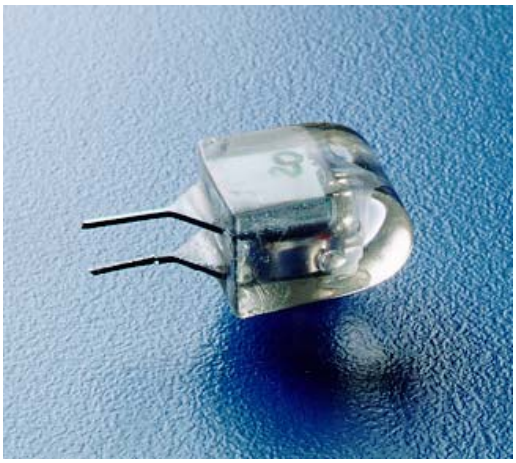

Figure 1. 1. MicroCard non-functional prototype.

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The decision to include pH and K^+ sensors was determined by the large experience of the CNM in the devolvement of Ion-Selective Field Effect Transistors (ISFET) [14-18]. The ISFET devices are sensitive and selective to pH but their target parameter can be substituted by placing specific membranes on its active area [19;20]. In this way, it is possible to create sensors for a wide range of ions and even for some organic molecules such as urea or glucose.

However, the development and use of the pH and K^+ sensors based on ISFETs was more complex than expected, since the application field imposes some constraints that cannot be overcome by this technology.

On the other hand, the developed electrical bioimpedance probes were successfully used *in vivo* and their results were equivalent to those obtained with previous impedance probes.

Some of the outcomes from the MicroCard project are presented in [21-29]

Project partners:

1. Coordinator: Centro Nacional de Microelectrónica. Instituto de Microelectrónica de Barcelona (CNM-IMB) - Bellaterra, Spain.
2. Centro Nacional de Microelectrónica. Instituto de Microelectrónica de Sevilla (CNM-IMSE) - Sevilla, Spain.
3. University of Ulster, Northern Ireland BioEngineering Centre (NIBEC) - Jordanstown, United Kingdom.
4. Scuola Superiore di Studi Universitari e di Perfezionamento S. Anna (SSSA) - Pisa, Italy.
5. Hospital Universitari Vall d'Hebron (HVH) - Barcelona, Spain.
6. Academisch Medisch Centrum University of Amsterdam (AMCA) - Amsterdam, The Netherlands.
7. D+T Microelectronica, A.I.E. - Bellaterra (D+T) , Spain.

1.2.2. MicroTrans project

(IST 99 13047)
'Micro-Probe Multi-Sensor for Graft Viability Monitoring during Organ Preservation and Transplantation' (http://www.cnm.es/~mtrans)

The objective of the MicroTrans project was to provide the medical community with an innovative method to monitor organs during transplantation. The ultimate goal was to enhance the viability assessment[4] of organs for transplantation, expanding thus the number of available grafts and their possible destinies. Originally, the monitoring of organ behavior during the initial postoperative period for early detection of non-functionality was also included as a project objective.

Direct evaluation of organ function after transplantation is, to date, the only reliable procedure for the assessment of correct preservation of the organ during transportation. Although some viability indicators have been proposed and tested (e.g. nucleotides, energetic charge or histology), they are extremely invasive (requiring various tissue biopsies) and time-consuming (imposing a considerable time lag that can stretch out to a couple of days). Therefore, these indicators are seldom used in the clinical practice and have been mostly relegated to experimental studies.

When a graft reaches the receptor, the main criteria to proceed with implantation are the observance of standard transport time ranges and a visual inspection by an expert surgeon. This crude evaluation protocol does not take into account any incidents that may have arisen during transportation nor, most importantly, does it take into consideration the inherent variability in the response of organs to sustained ischemia during transportation. This problem becomes aggravated by a growing tendency to make use of sub-optimal organs. As transplantation demand peaks in developed countries, donor numbers remain essentially constant. This has motivated healthcare institutions to approve transplantation of organs coming from non-heart-beating donors (NHBD)[5]. In these particular cases, the organs suffer an ischemic insult of unknown severity and duration which can compromise the viability of the grafts.

[4] Graft viability may be defined as the ability of the implanted graft to exhibit the same function exhibited by the organ before transplantation or an equivalent fresh organ.

[5] Traditionally, death was defined as the cessation of heartbeat and breathing. However, the ability to keep people alive with ventilators and other life-sustaining technology means that they are excellent sources for transplant organs. Hence death has been redefined in a way that makes it legally possible to remove organs from a person that still breathes and has heartbeat by means of life-supporting systems. Such redefinition led to the concept of brain death. Nowadays a person death is evidenced by a lack of brain electrical activity.

Figure 1. 2. MicroTrans *CoolBox* prototype

During the development of the MicroTrans project several experimental studies were carried out to analyze the significance of electrical impedance, extracellular pH and potassium concentration during the transplantation procedures of heart, liver and kidney. These studies were performed in pigs and simulated the standard extraction and preservation procedures [30] under different conditions (e.g. different preservation solutions and temperatures).

Although the initial postoperative monitoring objective was abandoned during the first stages of the project due to a lack of available resources and time, it must be mentioned that there exist some previous studies in which electrical bioimpedance monitoring has been proposed to detect the inflammatory process related to early rejection [31-33].

Some of the outcomes of the MicroTrans project are presented in [4;34-41]

Project partners:

1. Coordinator: Centro Nacional de Microelectrónica. Instituto de Microelectrónica de Barcelona (CNM-IMB) - Bellaterra, Spain.
2. Corporació Sanitaria Cínic. Fundació Clínic de de Recerca Biomèdica (CSC)- Barcelona, Spain.
3. University of Ulster, Northern Ireland BioEngineering Centre (NIBEC) - Jordanstown, United Kingdom.
4. Scuola Superiore di Studi Universitari e di Perfezionamento S. Anna (SSSA) - Pisa, Italy.
5. Carburos Metálicos, S.A. (CM) - Barcelona, Spain.
6. Kayser Italia, srl. (KI) - Livorno, Italy.
7. Università degli Studi di Pisa. Divisione di Chirugia Generale –Dipartamento di Oncologia (UP-DCGDO) -Pisa, Italy.
8. Academisch Medisch Centrum University of Amsterdam (AMCA) - Amsterdam, The Netherlands.
9. Universitat Autònoma de Barcelona (UAB) - Bellaterra , Spain.

1.3. Precedent studies on tissue bioimpedance monitoring

Since the early studies on electrical bioimpedance, it is known that impedance undergoes substantial changes after the organism death[6] [8]. However, it seems that it was not until the 1970's that impedance measurement was proposed as a possible clinical method to monitor the ischemia injury [42-44]. Curiously enough, during the 1980's the bioimpedance monitoring of tissue seems to have been less pursued by researchers, probably because at the time there was an increasing interest in biochemical sensors and also because some of the bioimpedance research groups oriented their efforts towards impedance imaging. Nevertheless, during the 1990's the interest in bioimpedance monitoring increased substantially, and several papers were published pointing to a marked relationship between ischemia and electrical impedance. Table 1.2 lists some recently published works on electrical bioimpedance that are related to warm ischemia monitoring (e.g. after clamping and artery or after excising a tissue sample) or related to preservation monitoring (e.g. by using cold solutions for graft preservation or by using cardioplegic solutions [7]).

The favourite frequencies or frequency bands for electrical bioimpedance measurements related to ischemia are located within the so called β dispersion region [8]. This broad frequency band goes from some tens or hundreds of Hz to some tens of MHz and two main reasons are argued in its favour: 1) it is a 'comfortable' frequency range, not too high and not too low, to develop the necessary electronic instrumentation and probes and 2) the impedance measurements performed in this frequency regions have demonstrated a high sensitivity to ischemia, whilst higher frequencies do not seem to show much sensitivity [45;46]. However, it must be mentioned that the α dispersion region (< 10 Hz) could be specially valuable in some tissues, such as liver [47].

The most noticeable impedance change during ischemia is an increase of the impedance magnitude at low frequencies[8]. As it has been mentioned, this fact is generally explained as being the result of cell swelling caused by the decrease of energy metabolism. Quite recently [48-51] it has also been proposed that the closure of inter-cellular junctions (gap junctions) during ischemia plays an important role in this sense. With the exception of a few terminally differentiated cells such as skeletal muscle cells and blood cells, most cells in animal tissues are in communication with their neighbors via gap junctions. Each gap junction is made up of proteins (connexins) on the plasma membrane that form channels between adjacent cells. These channels (pores of about ∅ 1.5 nm) allow inorganic ions and other small water-soluble

[6] The organism death or the excision of a tissue sample are examples of severe ischemia.

[7] Cardioplegic solutions are used in heart surgery for the arrest of heart activity. Due to their high potassium content they cause an arrest of the myocardium electrical activity.

[8] Such an impedance magnitude increase is always accompanied by a phase angle decrease (impedance phase in living tissues is always negative), particularly noticeable at slightly higher frequencies (10 kHz to 100 kHz). This is an interesting fact since impedance phase is, up to a point, not dependent on electrode geometry and, therefore, the results could be more universal and reliable. Unfortunately, phase angle is often ignored by researchers.

molecules to pass directly form the cytoplasm of one cell to the cytoplasm of the other, thereby coupling the cells both electrically and metabolically. This cell coupling has important functional implications, many of which are only beginning to be understood. Individual gap junctions flip between open and closed states, thus regulating the overall permeability of the cell. This regulation depends on many factors such as pH, Ca2+ and certain neurotransmitters [2].

Some ischemia events are usually accompanied by temperature changes. The temperature plays an important role in the ionic conductance: the viscosity of the solvent decreases as the temperature rises, increasing ion mobility and, consequently, decreasing resistivity. There exist a linear relation between temperature and ionic conductance that lies roughly around 2%/ºC. Thus, it is possible to mathematically compensate the temperature dependence or, at least, to minimize it by using a temperature sensor (the conductivity-temperature coefficient is not fixed and should be determined for each kind of tissue [52]).

Ischemia not only means a lack of nutrients and oxygen but also an accumulation of metabolic byproducts in the tissue. This explains the decrease of impedance magnitude at high frequencies (10 MHz) [47] since those metabolic byproducts are in part acids (lactic acid and carbonic acid) that increase the overall ionic conductivity of intra and extracellular fluids. In fact, the increase of ionic conductivity caused by the metabolic byproducts is currently used to detect bacterial metabolism and growth [53;54].

The electrical bioimpedance measurements in the β dispersion region are usually characterized according to the Cole equation (here expressed as in [6], see chapter 4 and annex A for further details):

$$\mathbf{Z} = R_\infty + \frac{\Delta R}{1 + (j\omega\tau)^\alpha} \quad , \qquad \Delta R = R_0 - R_\infty \tag{1.1}$$

where **Z** is the impedance value at frequency ω, j is the complex number $(-1)^{1/2}$, R_∞ is the impedance at infinite frequency, R_0 is the impedance at zero frequency, τ is the characteristic time constant and α is a dimensionless parameter with value between 0 and 1. During ischemia, a significant increase of R_0 can be observed, and is typically attributed to the causes explained above. The time constant τ also increases and some authors [55;56] link it to an equivalent cell membrane capacitance that increases due to the surface enlargement resulting from cell edema. However, the α parameter still lacks a successful physical interpretation and the observed evolution of this parameter during ischemia, frequently observed as a broadening of the dispersion width, remains unexplained. During the research involved in the MicroTrans project, we found out that the α parameter could be particularly useful for cold preservation monitoring and, for that reason, we tried to get some insight into the workings of this parameter and its relationship with ischemia (see chapters 4 and 5).

Table 1.2. Recent published works on ischemia monitoring by using electrical impedance.

		kind of tissue							
ref.	**year**	**heart**	**liver**	**kidney**	**other**	**ischemia**	**preservation**	**freq. (Hz)**	**research group**
[57]	1987	*				*	*	$10 - 10^7$	Göettingen
[58]	1987	*				*		10^3	Duke
[59]	1993	*				*		$10^3 - 10^4$	Philadelphia
[60]	1994		*			*		$3.10^3 - 3.10^5$	Boston
[49]	1995		*			*	*	$0.1 - 10^7$	Göettingen
[61]	1995	*	*		muscle	*	*	$10 - 10^7$	Göettingen
[56]	1995		*			*	*	$300 - 2.10^3$	Tokushima
[62]	1996		*			*		$10 - 10^7$	Göettingen
[63]	1996	*				*		$10^3 - 10^6$	UPC – HGUVH
[64]	1996	*					*	$20 - 10^6$	Gifu
[65]	1997	*				*		$10 - 10^5$	Worcester
[5]	1997	*				*		10^3	HGUVH
[47]	1998	*	*			*	*	$0.1 - 10^7$	Göettingen
[66]	1998	*	*	*	multiple	*		$10^2 - 10^6$	UPC – HGUVH
[67]	1998				muscle	*		$20 - 10^6$	Gifu
[68]	1998				muscle	*	*	$10^2 - 10^7$	Heidelberg
[69]	1999	*		*		*		$10^2 - 10^6$	UPC – HGUVH
[70]	2000		*				*	$10^4 - 10^8$	Kochi
[71]	2001				muscle	*		$10 - 10^6$	Worcester
[50]	2001	*				*		10^3	AMCA
[72]	2002				muscle	*		$6.10^2 - 5.10^5$	Halifax
[73]	2002	*				*		$10 - 4.10^8$	Heidelberg
[45]	2002		*				*	$20 - 3.10^6$	Gifu
[74]	2002	*				*		$1 - 10^6$	Wisconsin
[55]	2002		*			*		$10 - 10^6$	Wisconsin
[75]	2003				muscle	*		$100 - 10^6$	Worcester
[46]	2003	*				*		$5.10^6 - 3.10^9$	Heidelberg
[76]	2003				intestine	*		$50 - 3.10^5$	UEFA
[77]	2003				brain	*		$4.10^3 - 10^6$	Qeensland
[78]	2003	*				*		7.10^3	HGUVH
[51]	2004	*				*		13.10^3	Heidelberg

List of research groups:

- AMCA: Laboratory of Experimental Cardiology, Academic Medical Center, Amsterdam, The Netherlands.
- Boston: Dep.. of Surgery, Brigham and Women's Hospital and Harvard Medical School, Boston, Ma, USA.

- Duke: Dept. of Surgery, Duke University Medical Center, Durham, NC, USA.
- Gifu: Dept. of Surgery, Gifu University School of Medicine, Gifu, Japan.
- Göettingen: Institute of Physiology, University of Göettingen, Göettingen, Germany.
- HGUVH: Laboratorio de Cardiología Experimental, Hospital General Universitari Vall d'Hebrón, Barcelona, Spain.
- Kochi: Kochi Medical School, Kochi, Japan.
- Halifax: Dept. of Electrical & Computer Eng., Dalhouise University, Halifax, NS, Canada.
- Heidelberg: Dept. of Experimental Surgery, University of Heidelberg, Heidelberg, Germany.
- Philadelphia: Depts. of Medicine, Surgery and Bioengineering, University of Pennsylvania, Philadelphia, USA.
- Queensland: University of Queensland, Queensland, Australia
- Tokushima: School of Medicine, University of Tokushima, Tokushima, Japan.
- UEFA: Universidad del Ejército y Fuerxa Aérea, México, Mexico.
- UPC: Departament d'Enginyeria Electrònica, Universitat Politècnica de Catalunya, Barcelona, Spain.
- Wisconsin: Dept. of Electrical and Computer Eng., University of Wisconsin, Madison, USA.
- Worcester: Worcester Polytechnic Institute, Biomedical Engineering Dept, MA, USA.

It must be said that, up to now and to our best knowledge, no commercial system is available for the clinical monitoring of electrical bioimpedance in the cases of induced ischemia during surgery nor during transplantation. In this sense, the device under development at CNM and in cooperation with Carburos Metálicos (Air Products group) [40] should be a welcome novelty.

1.4. Alternative methods for ischemia monitoring

Ischemia plays a major role in tissue viability and in other clinical disorders. However, there are limited means by which its effect on the tissue (ischemic injury) can be clinically quantified. Quite frequently, physicians are forced to accept the perfusion level or the blood flow as the sole parameter for monitoring.

There are some clinical techniques for monitoring the perfusion in an organ or tissue. Some of them are briefly described below:

Ultrasonic Doppler monitoring
In ultrasonic Doppler monitoring, a miniature probe consisting of ultrasonic transducers is surgically implanted around the artery supplying blood to the affected tissue or organ and the blood flow is monitored thanks to the Doppler effect (the frequency of the reflected sound is shifted according to the velocity of red cells)[79].
This technique is used extensively in surgery to make a rapid assessment of whether blood is flowing, or not, through a certain vessel.

Duplex scanning
Duplex scanning is a non-invasive method that combines a B-mode ultrasound imager (i.e. echography) with an ultrasonic Doppler system, in such a way that it is possible to measure the blood flow of a selected single vessel [80].
This technique is quite expensive and it is only valid for large vessels.

Laser Doppler blood flow monitoring
The laser Doppler technique measures blood flow in the very small blood vessels of the microvasculature. This non-invasive technique depends also on the Doppler principle. In essence, low power light from a monochromatic stable laser is applied on the tissue. The incident light is scattered by moving red blood cells and, as a consequence, its spectrum is broadened and the average velocity of the red cells can be calculated [79;81].
The drawback to this method is that flow can only be measured to a depth of 1 to 2 mm.

Fluorescent staining
In fluorescent staining, a bolus of fluorescent dye is injected to the patient or the tissue under study. Within a few moments (seconds) the dye reaches all the perfused portions and the area of interest is exposed to ultraviolet light. In this way, perfusion quality can be assessed from the intensity of fluorescence.
Even though it can yield precise measurements, this method is not continuous, neither quantitative, limiting its scope of application.

Electromagnetic flow-meters
The performance of electromagnetic flow-meters is similar to that of ultrasonic Doopler monitoring devices and their method of action is based mainly on Faraday's law. That is, a voltage is generated when a conductive medium, such as blood, moves through a magnetic field [79].

Arteriography
In a similar way to fluorescent staining, an X-ray contrast material is injected into the blood stream for arteriography. Thus, the vascular system and its related disorders (e.g. thrombosis) can be visualized by using an X-ray imaging device.
This method requires cumbersome equipment and it is not continuous neither quantitative.

Thermal diffusion methods
In almost all mammalian tissues, blood flow accounts for the major part of convective heat transfer. Therefore, it is possible to estimate perfusion by depositing an small amount of thermal energy and observing how temperature evolves with time and diffuses through the tissue [82].

As it has been said, the above techniques are able to monitor the perfusion of living tissues and, therefore, to detect or to foresee ischemia. However, none of these methods provides information on the ischemic injury. The range of available techniques that allow an effective monitoring of the damaging effects of ischemia on tissues is more reduced. Due to it precision and the range of available analysis, biopsy is often the preferred choice. However, when biopsies cannot be taken or the imposed delay by the subsequent analysis of histological properties and biochemical parameters is not acceptable, only the following methods remain available:

Optical inspection
The metabolic and structural changes induced by ischemia can involve some observable alterations of colour and texture. This method is not precise nor qualitative and requires a high degree of expertise. However, due to the lack of alternative methods, it is routinely used by some physicians to determine whether or not an organ is acceptable for transplantation.

MR Diffusion Imaging
Magnetic Resonance (MR) Diffusion-Weighted Imaging (DWI) detects changes in the mobility of free molecular protons [83].
The high-resolution images obtained by this method are suitable for detecting early ischemic changes. Apparently, image brightness is related to the narrowing of the extracellular spaces due to the cell edema caused by the influx of water into the cells because of the breakdown in the cellular energy metabolism.
Unfortunately, the instrumentation associated with this technique is very expensive and cumbersome and, to day, this limits its applicability to certain critical applications such as brain strokes.

On-line monitoring of tissue pH, ionic content and metabolites
Although it is widely known that the pH, the extracellular concentration of some ions and the presence of some metabolites, such as lactate, are significantly altered by ischemia, the related on-line measurement methods are too cumbersome to be applied in the clinical practice and, up to date, they are mostly constrained to experimental studies. However, some recent results by different research groups should be noted in this regard. These encompass the development of planar ion-selective electrodes for *in vivo* measurement of pH and some ions [13], the development of photo-chemical sensors (optodes) for pH and blood gases [84], the use of filtration catheters to transport analytes from sample to sensor [85], and the implementation of miniaturized analytical systems for Point-Of-Care applications (POC) [86]. The POC approach is related with the miniaturization of the fluidic elements of analytical systems, also known as microfluidics. It must be mentioned that the CNM is also conducting some research in this same trend [87;88].

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