6. Conclusions

The principal conclusions of the studies presented in this thesis are:

1. There are some clinically available methods to monitor ischemia but it is difficult to find out methods to monitor its effect on living tissues, that is, the ischemic injury. Measurement of electrical impedance is one of these few methods that could allow true ischemic injury real-time monitoring and probably the only one suitable for long measurement periods.

2. The developed silicon probe is useful for tetrapolar electrical bioimpedance measurements of soft living tissues in the frequency range from 100 Hz to 100 kHz. At lower frequencies, the high electrode-tissue interface impedances are the main error cause whereas at higher frequencies the measurement is distorted because of the parasitic capacitances.

3. The analysis of the inter-electrode separation distances demonstrates that non-constant separation distances can be beneficial in terms of signal-to-noise ratio and spatial resolution.

4. It has been justified that the measurement current of 5 μA is a proper level for the organs under study and for the frequency band of interest. In the myocardium case, higher currents are not only not advisable because of safety reasons but also in order to avoid non-linear phenomena.

5. The developed five-electrode method is able to minimize errors at low frequencies caused by the high electrode-electrolyte interface impedances. Unfortunately, some limitations have been found that made almost impossible its practical implementation for living tissue measurements.

6. Different instrumentation setups for four-electrode impedance measurements were developed. From this experience it can be concluded that digital designs are preferable to analogue approaches. Nevertheless, it must be noted that some analogue circuitry will always be necessary, such as the input differential amplifier, and that the features of those elements will determine to a great extent the overall system performance.

7. The mechanical machining of the silicon substrate to obtain the needle shaped probes by using the wafer saw is a reliable and inexpensive method in comparison to RIE machining.

8. The electrochemical deposition of platinum black on the sub-millimeter electrodes has been optimized for mechanical stability. Electrode surface preparation (cleaning and activation) and ultrasonic agitation are key factors for success

9. The development of probes based on flexible materials seems possible and is very promising in terms of tissue damage minimization. The fact that the

configuration will be not rigid and that the cell constant would not be constant makes more interesting the measurement of parameters such as the phase angle rather than the impedance magnitude.

10. The silicon probe can be inserted into soft tissues by direct puncture and bleeding rarely occurs.

11. The silicon probe even allows measurements in the case that the tissue is completely immersed in a conductive medium such as the preservation solutions.

12. It has been shown the severe heterogeneity of the electrode-tissue interface impedance in the case of small electrodes. Two to five-fold ranges at 1 kHz have been observed.

13. The experimental studies confirm that the electrical impedance parameters in the β dispersion region are related to the ischemia process. Moreover, some tissue alterations not detected by pH nor potassium sensors are manifested by the electrical impedance.

14. Impedance spectroscopy during rat kidney preservation has demonstrated the importance of a parameter often ignored in precedent studies: the α parameter in the Cole model. Time evolution of α during preservation is markedly different between kidneys obtained from beating donors and kidneys which have been subjected to a 45 minutes warm ischemia episode.

15. The developed bi-dimensional bioimpedance simulator is mainly intended for didactical purposes but it can be also applied to validate some hypothesis concerning the interpretation of experimental bioimpedance measurements in the β dispersion region. The most relevant result obtained by using it is that the α parameter in the Cole model can be related to the morphology of the extracellular spaces.

16. By combining the results from experimental studies and from computer simulations it was concluded that the α parameter could be related with the 'tortuosity' of the extracellular spaces. At least, a relation between cytoskeleton condition and this parameter was found. This result is of great relevance because up to know all tissue bioimpedance alterations were attributed to edema events (intracellular or extracellular), ionic imbalances, cell membrane permeability alterations or gap junctions closure or opening.