Development and evaluation of biocompatible films of polytetrafluoroethylene polymers holding lithium phthalocyanine crystals for their use in EPR oximetry

M. Dinguizli[1], S. Jeumont[1], N. Beghein[1], J. He[1], T. Walczak[2], P. N. Lesniewski[2], H. Hou[2], O. Y. Grinberg[2], A. Sucheta[2], H. M. Swartz[2], B. Gallez[1]

[1]Biomedical Magnetic Resonance Unit, Catholic University of Louvain, Brussels, B, Belgium, [2]EPR Research center for the study of viable systems, Dartmouth Medical School, Hanover, NH, United States

Introduction

Tissue oxygenation is a key parameter related to many physiological and pathophysiological processes in biological systems. Oxygen also plays a major role in therapeutics: for example, the efficiency of cancer treatments such as radiotherapy and chemotherapy dramatically depends on the oxygen tension in tumors. *In vivo* Electron Paramagnetic Resonance (EPR) oximetry is an emerging technology that permits the continuous monitoring of oxygenation in tissues (1). Lithium phthalocyanine (LiPc) is one oxygen reporter specially interesting for *in vivo* measurements (2). One of the majors preoccupations of this work is to produce LiPc in a biocompatible formulation and to preserve its oxygen sensitivity over long periods of time. We focused our approach on the production of films in Teflon® AF 2400 holding LiPc crystals. These films could be used as retrievable inserts or components of implantable resonators. Our choice for the coating polymer was guided by the need for both high oxygen permeation and biocompatibility for use in human subjects.

Material and methods

LiPc crystals were inserted in Teflon AF2400 films by solvent evaporation method. The thickness of the coating was modulated by several cycles of coating using the same polymer. The procedure was also adpated to produce implantable resonators holding LiPc in the loop. Films were characterized with static contact angle, AFM, and optical microscopy. EPR at 9 GHz was used for *in vitro* calibration. 1.2 GHz EPR spectrometer was used for *in vivo* testing. Performance of the coated sensors were tested after sterilization, after residence in tissues, and after irradiation**.**Biocompatibility was verified using standardized methods: hemolysis assay, systemic injection of extracts and histological analysis after *in vivo* implantation.

Results

Solid transparent films, (sizing arround 2-3 mm^2 and weighing 0.1mg or less) holding particles of oxygen sensors were obtained with 3% (W/V) teflon solution. Films obtained appeared plan with out micro-bubbles or defaults. The regularity of the films was confirmed by the AFM analysis. The measurement of the static contact angle confirmed the high hydrophobic character of the film. The response to oxygen of the sensor remained unchanged after sterilization, implantation in muscle of mice or rabbits, and was not affected by the irradiation as shown in Figure. Concerning the biocompatibility assays, no hemolytic effect was noted, no toxicity was found using the systemic injection of extracts. The histological analysis in mouse and rabbit muscle in which the films were implanted for three months was similar to standard biocompatible polyethylene devices.

Conclusions

We demonstrated that these oxygen sensors based on LiPc crystals embedded in Teflon films are promising tools for future pre-clinical and clinical developments for EPR oximetry. Sterilization, irradiation and *in vivo* implantation do not affect the stability and the integrity of the films and of the sensor. Calibration curves were also not changed after the different treatments. The biocompatibility of the systems was also demonstrated. Finally, a long term monitoring of tissue oxygenation *in vivo* is achievable using these systems.

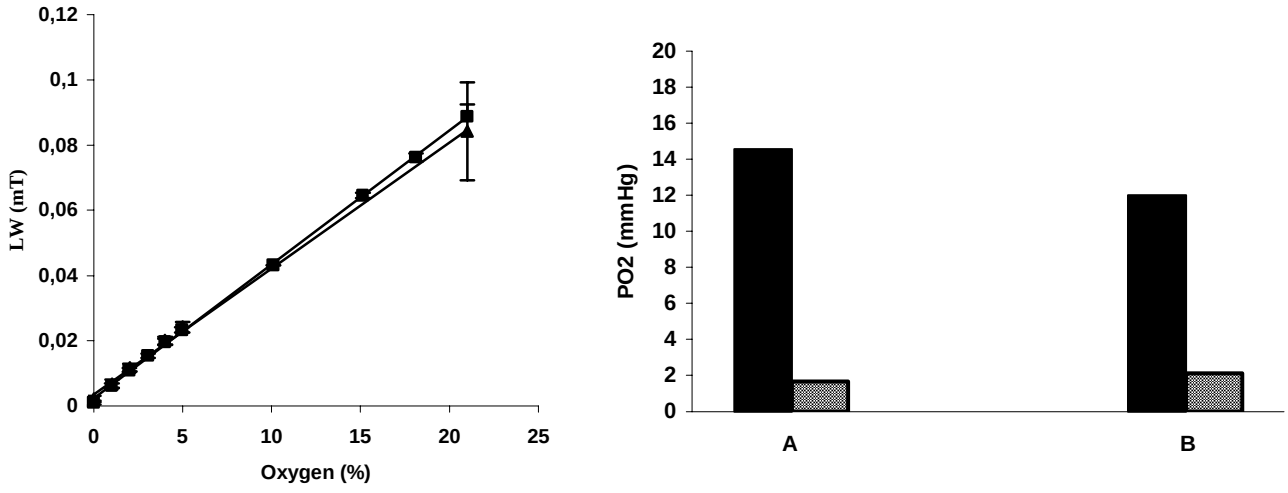

Figure: Stability of response to oxygen of LiPc crystals embedded in Teflon AF2400 films. On the left, calibration before and after residence in tissues. On the right, measurements of pO2 in anesthetized rabbit muscle using implantable resonators. Typical measurements before and after compression of the muscle recorded 2 weeks (A) and 10 weeks (B) after implantation.

References

1. Dunn JF, Swartz HM. In vivo electron paramagnetic resonance with particulate materials. Methods 2003; 30:159-166.
2. Liu KJ, Gast P, Moussavi M, Norby SW, Vahidi N, Walczak T, Wu M, Swartz HM. Lithium phthalocyanine: a probe for electron paramagnetic resonance oximetry in viable biological systems. Proc Natl Acad Sci USA 1993; 90: 5438-5442.