Available online at www.sciencedirect.com
SCIENCE DIRECT
Biosensors and Bioelectronics 21 (2006) 1015–1022
BIOSENSORS & BIOELECTRONICS
www.elsevier.com/locate/bios

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

M. Dinguizli [a], S. Jeumont [a], N. Beghein [a], J. He [a], T. Walczak [b], P.N. Lesniewski [b], H. Hou [b], O.Y. Grinberg [b], Artur Sucheta [b], H.M. Swartz [b], B. Gallez [a,*]

[a] *Biomedical Magnetic Resonance Unit, Laboratory of Medicinal Chemistry and Radiopharmacy, Université Catholique de Louvain, Brussels, Belgium*
[b] *EPR Center for the Study of Viable Systems, Dartmouth Medical School, Hanover, NH, USA*

Received 20 January 2005; received in revised form 18 March 2005; accepted 18 March 2005
Available online 15 April 2005

Abstract

Electron paramagnetic resonance (EPR) oximetry is a powerful technology that allows the monitoring of oxygenation in tissues. The measurement of tissue oxygenation can be achieved using lithium phthalocyanine (LiPc) crystals as oxygen reporters. In order to have biocompatibility for the sensing system and to assure long-term stability in the responsiveness of the system, we developed films of Teflon AF 2400® with embedded LiPc crystals. These systems can be used as retrievable inserts or parts of an implantable resonator or catheter. Atomic force microscopy studies revealed that the surface of the films was regular and planar. The response to oxygen of the sensor (EPR linewidth as a function of pO_2) remained unchanged after implantation in mice, and was not affected by sterilization or irradiation. The use of resonators, holding LiPc embedded in Teflon AF 2400®, implanted in the gastrocnemius muscle of rabbits allowed the monitoring of oxygen during several weeks. Several assays also demonstrated the biocompatibility of the system: (1) no hemolytic effect was noted; (2) no toxicity was found using the systemic injection test of extracts; (3) histological analysis in rabbit muscle in which the films were implanted for 1 week or 3 months was similar to standard polyethylene biocompatible devices. These advanced oxygen sensors are promising tools for future pre-clinical and clinical developments of EPR oximetry. These developments can be applied for other applications of biosensors where there is a need for oxygen permeable membranes.

Keywords: EPR; Oxygen permeation; Oxygenation; Biocompatibility; Polytetrafluoroethylene; Biosensor

1. Introduction

Tissue oxygenation is a key parameter related to many physiological and pathophysiological processes in the 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. The assessment of tissue oxygenation is, therefore, of great physiological and clinical interest. In vivo electron paramagnetic resonance (EPR) oximetry is a powerful technology that permits the continuous monitoring of oxygenation in tissues. The measurement is based on the variation of the EPR linewidth, which is very sensitive to changes in partial pressure of oxygen (pO_2) (Dunn and Swartz, 2003). This can be achieved using paramagnetic carbon particles (chars (Clarkson et al., 1998), charcoals (Vahidi et al., 1994; James et al., 1997; Jordan et al., 1998), carbon blacks (Lan et al., 2004) or lithium phthalocyanine (Liu et al., 1993) as oxygen reporters. EPR oximetry has already been used in animals to study oxygenation in brain

* Corresponding author. Present address: CMFA/REMA, Avenue Mounier 73.40, B-1200 Brussels, Belgium. Tel.: +32 2 7642792; fax: +32 2 7642790.
E-mail address: gallez@cmfa.ucl.ac.be (B. Gallez).

0956-5663/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2005.03.009

(Hou et al., 2003), heart (Kuppusamy and Zweier, 2004), gastrointestinal tract (He et al., 1999), skeletal muscle (Jordan et al., 2004), liver (Gallez et al., 1998; James et al., 2002), kidneys (James et al., 1996), skin (Krzic et al., 2001), and tumors (Goda et al., 1996; Gallez et al., 1999, 2004; Jordan et al., 2000, 2002, 2003, 2004; Sersa et al., 2001). Considering the unique information provided by this technology, it is certainly a valid goal for moving this technology into the clinic (Swartz et al., 2004). For that purpose, it would be desirable to avoid the time-consuming and expensive process involved in the tests necessary for direct implantation of the particulate material in tissue, by enclosing it in biocompatible membranes (Gallez and Mäder, 2000). This would also facilitate assuring that the oxygen sensor will retain its oxygen sensitivity over long periods. We recently succeeded in stabilizing unstable paramagnetic carbon materials, demonstrating that the effectiveness of the coating to preserve responsiveness in tissue (Gallez et al., 1999; He et al., 2001). In the present study, our interest was focused on lithium phthalocyanine (LiPc) crystals. This material has very desirable features, a high spin density, a linear response of the EPR linewidth as a function of pO_2 between 0 and 100% (Liu et al., 1993) oxygen, and a very narrow EPR linewidth, which leads to high sensitivity (as the signal intensity is inversely proportional to the square of the EPR linewidth).

Our choice for the coating polymer was guided by the need for both high oxygen permeation and biocompatibility for use in human subjects. The oxygen permeability should not be dependent on experimental conditions or environmental characteristics. The use of polytetrafluoroethylene is widely reported to produce gas permeable membranes. In this class, Teflon® AF has been used as lens protectors (Werner et al., 1999), and in the field of gas sensors and gas separation systems (Pinnau and Toy, 1996; Dasgupta et al., 1998; Alentiev et al., 1997). Moreover, due to their inert chemical properties and high hydrophobicity, these membranes possess anti-adhesive properties and limited protein adsorption. These features confer good biocompatibility as demonstrated by biocompatibility assays for applications in the lens (Werner et al., 1999).

The purpose of the present work was to develop and test films in Teflon® AF 2400 holding paramagnetic LiPc crystals. These films could be used as retrievable inserts or components of implantable resonators. We characterized the effect of coating on the oxygen sensitivity of LiPc. We investigated the stability of the responsiveness to oxygen after sterilization, irradiation (to mimic an application to monitor tumor pO_2 during the radiotherapy), and after long residence in vivo. As the purpose of implantation in vivo differs from their use as lens protectors because of the differences in the sites of use, we carried out several biocompatibility assays according to standardized methods (ISO 10993-6, 1994; ASTM F756-00, 2000; USP, 2000): hemolytic assay, systemic injection test, and histology after implantation in muscle.

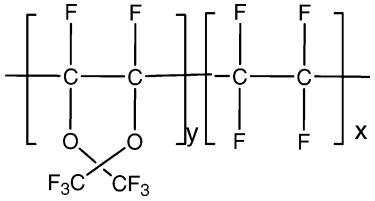

Fig. 1. Chemical structure of Teflon® AF 2400.

2. Materials and methods

2.1. Preparation of the coated sensors

2.1.1. Films preparation

Films were made with Teflon® AF 2400 (Fig. 1), a random copolymer of tetrafluoroethylene and 2,2-bis(trifluoromethyl)-4,5-difluoro-1,3-dioxole, obtained from Aldrich (Steinheim, Germany). The Teflon® AF 2400 powder was dissolved in FC 75 solvent (3M Company, Zwijndrecht, Belgium) and stirred overnight. Films were prepared by solvent evaporation at 70 °C on pre-cleaned microscope slides. Crystals of LiPc were incorporated during the solvent evaporation. The dimension of the group of crystals inserted in a film was less than 1 mm^3. The films holding LiPc obtained were coated several times (10 cycles of coating) with the same polymer solution. Films were kept in an oven at 70 °C overnight to evaporate all the solvent.

2.1.2. Inclusion of LiPc in resonators

The construction of implantable resonators is described elsewhere (Walczak et al., submitted for publication). Briefly, it uses a coaxial microcable with an external diameter of 0.8 mm. The film holding LiPc is put at one end of the resonator within a loop with a diameter of less than 1 mm. The opposite end of the resonator terminates in a larger, 8–10 mm diameter loop that allows inductive coupling with the resonator. The microloop is placed in the tissue of interest, while the coupling loop is placed subcutaneously at a depth that is compatible with delivering microwaves into the resonator and receiving the reflection signal with an external pick up system. The process of coating was similar to that described above, and then, the crystals (two or three crystals) were incorporated during the next solvent evaporation. Finally, the loops were coated (10 cycles of coating) with the same polymer solution. Film's dimensions inside the small loop do not exceed 0.8 mm^2 of surface and 0.1 mm of thickness.

2.2. Characterization of the films

2.2.1. Microscopic verification

Optical microscopy was used to assure the absence of cracks, bubbles, or other defaults in the films and in the loops of the resonators. All films and resonators were checked at different locations, especially near the crystals and the wire of resonators.

2.2.2. Atomic force microscopy (AFM)

AFM images were obtained in air in the contact mode, using a commercial microscope (Nanoscope III, Digital Instruments, Santa Barbara, CA, USA) equipped with Si_3N_4 triangular levers (ThermoMicroscopes, Sunnyvale, CA, USA; nominal radius curvature = 20 nm).

2.3. Performance of the coated sensors and resonators

2.3.1. EPR measurements

Calibrations (EPR linewidths as a function of the pO_2) were made at 9.3 GHz with a Bruker EMX EPR (Rheinstetten, Germany) spectrometer equipped with a variable temperature controller BVT-3000. Films with crystals were placed in 4 mm quartz tubes, open at both ends and placed in the center of the EPR cavity. Gas with known concentrations of nitrogen and air (obtained using a gas mixer Aalborg®, Orangenburg, NJ, USA), equilibrated at 37 °C, was flushed over the samples, and the spectra were recorded every minute until equilibration was achieved. The oxygen content in the gas was analyzed using a Servomex oxygen analyzer OA540. Typical spectrometer parameters were modulation amplitude less than one-third of the peak-to-peak linewidth, incident microwave power 50 μW, and at a 10 kHz modulation frequency.

2.3.2. Stability of the coated sensors

Dry heating at 120 °C for 8 h was used to sterilize the films. Calibration curves (EPR linewidth as a function of the pO_2) were made before and after the sterilization. The influence of implantation in vivo was also tested. Films were implanted in the gastrocnemius muscle of mice for 3 weeks. Calibration curves were built before and after placement in vivo. Resistance to irradiation in vivo was also checked. A single dose of 80 Gy was delivered in vivo to a gastrocnemius muscle, in which a film holding LiPc was implanted. The animals were kept under gas anesthesia (isoflurane) (1.8% (v/v)) during irradiation by X-rays (1.2 Gy/min, Philips Medical RT 250). Calibrations curves were made before and after in vivo irradiation.

2.3.3. Performances of resonators holding LiPc implanted in rabbits

Three New Zealand white male rabbits (about 4.5 kg) were used in this experiment. The animals were-anesthetized by nose cone using 2–2.5% of isoflurane in 26% oxygen. Both hind legs were shaved and the skin was prepared with a betadine and 70% alcohol scrub. Implantable resonators were placed in both hind limbs using an aseptic technique. Two-centimeter cross incisions were made on the skin at the external side of the leg, about 3 cm from each other. The tip of the resonator with LiPc crystals was placed in the muscle through one of the incisions at a depth of 10–15 mm. The transmission line part of the resonator was fixed in the surrounding tissue. The large coupling loop was placed subcutaneously in the other incision. Both incisions were closed with sterile sutures. The rabbits were periodically re-anesthetized with 2% isoflurane and 26% oxygen. pO_2 measurements in normal (base line) and in compressed (for 10 min) muscles were performed for several weeks after implantation, using a 1.2 GHz EPR spectrometer (Swartz et al., 2004). The acquisition parameters were chosen to avoid overmodulation and power saturation of the EPR signal. Decrease of blood flow and pO_2 in the muscle was performed with a rubber band.

2.4. Biocompatibility assays

2.4.1. Hemolysis test

The test was adapted from the standard practice for assessment of hemolytic properties of materials (ASTM, 2005American Society for Testing and Materials (ASTM) F756-00). Briefly, solutions of whole human blood in PBS were incubated for 3 h in the presence of a copper foil (positive control), a polyethylene plate (USP88, negative control), and samples of polymeric films (Teflon® AF 2400, with and without LiPc crystals), and coated resonators. The solutions were then centrifuged at 740 × *g* for 15 min, and the optical density of the supernatant was measured at 540 nm.

2.4.2. Systemic injection test

This test is designed to evaluate systemic responses to the extracts of materials following injection into mice (USP, 2000). The extracts in 0.9% NaCl or sesame oil were prepared as recommended by USP, 2000. The extract in NaCl was injected intravenously (50 ml/kg), and the extract in oil was injected IP (50 ml/kg). Five mice (male NMRI mice) were injected per extract and per group. The animals were observed immediately after the injection, and then at 24, 48 and 72 h after injection.

2.4.3. Implantation test

The implantation test is designed for the evaluation of polymeric materials in direct contact with living tissues. The test was carried out according to the standardized assays described in ISO 10993-6. The implants (pieces of cable of 1-cm length, coated with Teflon® AF 2400, and polyethylene implants (negative controls, USP88) were aseptically inserted in the paravertebral muscle. The animals were sacrificed 7 days and 90 days after the implantation. Muscles were excised and the implant was removed. The tissue were fixed with 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin and Trichrom Masson for further histological studies.

3. Results and discussion

3.1. Preparation and characterization of the films

The solvent evaporation procedure described in the present paper results in solid transparent films holding particles of oxygen sensors. During the optimization of the procedure,

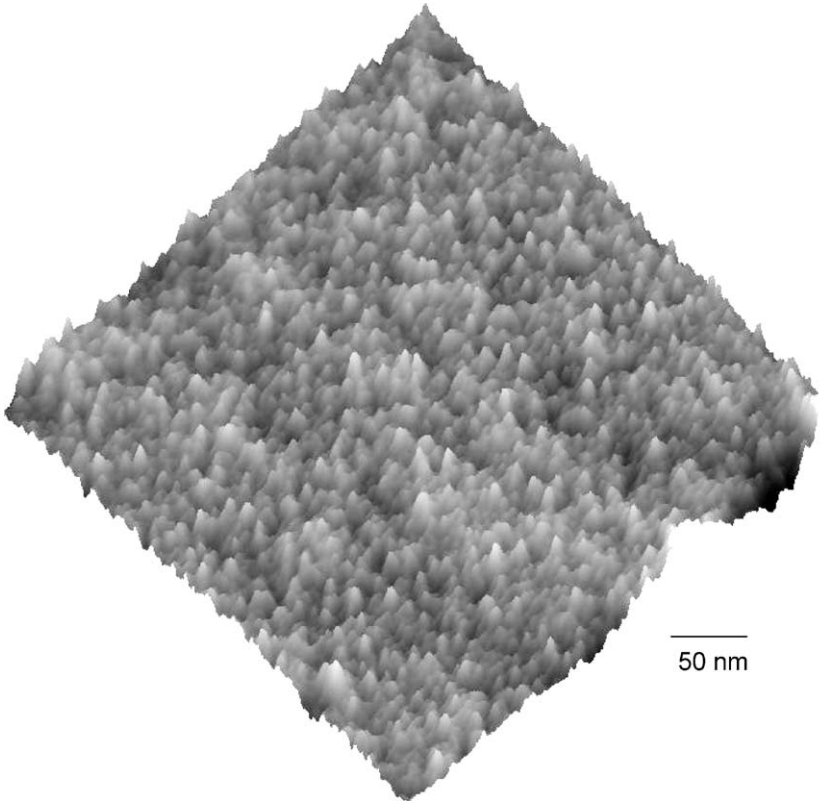

Fig. 2. AFM image of film. Note the planar and regular structure of the film.

we found that the viscosity of the solution (depending on the initial concentration of Teflon® AF 2400) and the kinetics of evaporation were important to control in order to avoid formation of micro-bubbles in the films. These bubbles should be avoided because they can act as small gas reservoirs and so disturb the measurement or the kinetics of equilibrium. We found out that an optimal preparation was to use an initial concentration of 3% (w/v) Teflon® AF 2400 with evaporation carried out in an oven at 70 °C. Optical microscopy checks revealed planar surfaces without defaults and bubbles. The regularity of the films was confirmed by the AFM analysis. Several fields of view were recorded in several films and examined by an operator trained in the field. All of them were planar without defaults (valleys of less than 5-nm height). A typical image is shown in Fig. 2. We found that it was necessary to use at least five cycles of coating to confer high mechanical resistance to the film, and to avoid cracks or damage after insertion and removal from the tissues.

3.2. Performances of the coated oxygen sensors

As the sensitivity of LiPc to oxygen is strongly dependent on the crystal structure (Bensebaa et al., 1992), we first checked the influence of the preparation procedure on the oxygen sensitivity of the paramagnetic materials. No change in the calibration curve was observed between crystals before and after inclusion in films (data not shown). The sterilization procedure did not affect the sensitivity to oxygen. The kinetics of equilibrium with gas phase was very rapid when changing the oxygen atmosphere. We did not observe any significant change in the kinetics in the gas phase when using up to 10 cycles of coating. The kinetics of equilibrium of the oxygen sensor with its environment is shown in Fig. 3 (for LiPc in films coated with 10 cycles of coating). As it can be observed, the equilibrium is reached within 2 min. Therefore, all the in vivo experiments were carried out using the resonators prepared with 10 cycles of coating, which represents the best compromise between mechanical resistance and rapidity of the equilibrium. We checked the performances

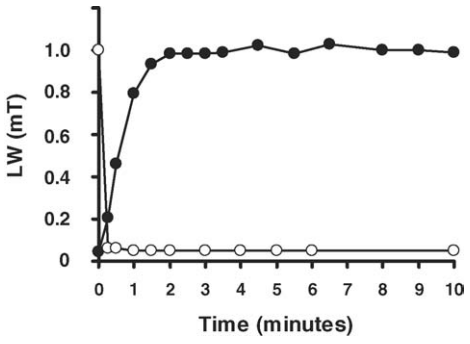

Fig. 3. Kinetics of equilibrium of LiPc cystals inserted in Teflon® AF 2400 films (10 cycles of coating) when changing the oxygen environment from air to nitrogen and from nitrogen to air.

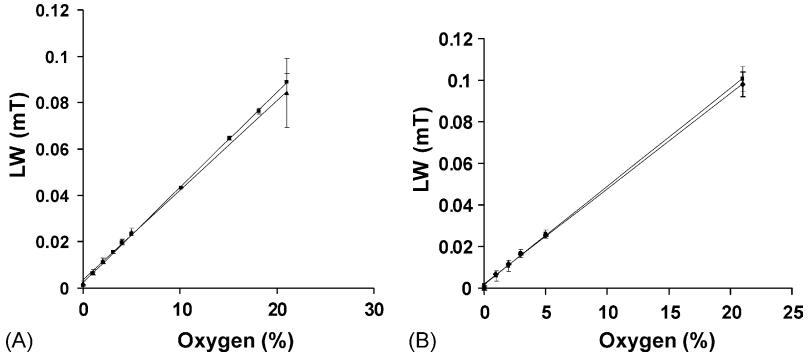

Fig. 4. Responsiveness of LiPc crystals in films: (A) (■) before and (▲) after implantation in vivo (3 weeks of residence); (B) (■) before and (♦) after irradiation at 80 Gy in vivo.

of the embedded sensors in vivo. In Fig. 4a, it is shown that the residence in muscles for 3 weeks did not change the calibration curves. When this type of film was used as component of a resonator implanted in the rabbit muscle, the pO_2 measurements recorded in normal muscles and in compressed muscles were reproducible over a period of 7 weeks (Fig. 5).

To simulate a situation where the sensor will be used in vivo to monitor the changes in pO_2 after irradiation to guide radiation therapy treatment, the response of the sensor was checked before and after irradiation in vivo. We observed that irradiation of 80 Gy (this is more than the usual total dose used in radiotherapy regimens, which in practice would be delivered in a series of smaller doses) did not change the sensitivity of the sensor (Fig. 4b).

The long-term stability observed in vivo is particularly valuable for chronic studies using in vivo EPR oximetry. A very useful advantage in using coated materials is to provide a long-term response to oxygen in tissues. The oxygen sensitivity of uncoated LiPc crystals was indeed found unstable in specific tissues such as muscle (Liu et al., 1993).

3.3. Biocompatibility

To our knowledge, the use of Teflon® AF 2400 has not been reported in applications other than the eye lens. Therefore, it was important to check the biocompatibility of these films after implantation, and when used in the configuration of films holding LiPc or covering the loop of implantable resonators. As one possible application of the implantable resonator could be to monitor the pO_2 in blood, we checked the absence of hemolytic properties. In Fig. 6, the results of the hemolysis assay are presented. The Teflon® AF 2400 film alone or containing LiPc did not induce a significantly higher hemolysis than the USP88 standard. In these conditions, the foil of copper, used as a positive control, led to significant hemolysis. We also verified that extracts from films (alone or holding LiPc) did not induce any systemic toxicity. No abnormal sign was observed using this test. No loss of weight was observed in animals injected with these extracts. Finally, histological analysis was performed after implantation. Histological sections of rabbit muscles implanted with the coated wire are shown in Fig. 7. For both time of residence in tissues (7 days and 3 months), there is a presence of a small inflammatory reaction, which is similar to the reaction observed after the implantation of the USP88 polyethylene implant

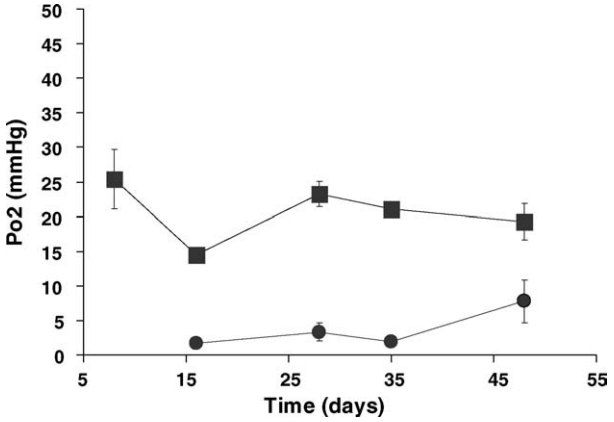

Fig. 5. Measurements of pO_2 in anesthetized rabbit muscle using implantable resonators holding LiPc crystals and embedded in Teflon® AF 2400. The measurements were recorded before (■) and after (●) compression of the muscle. Data are mean ± S.E.M.

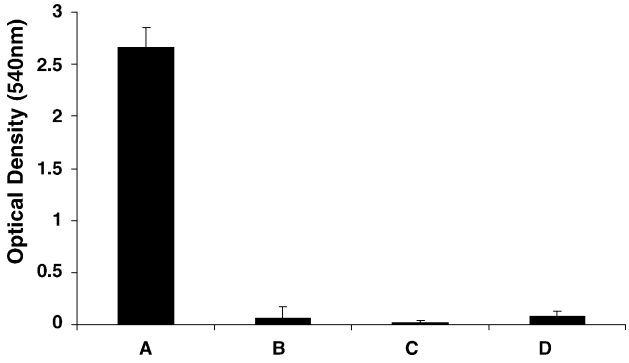

Fig. 6. Hemolysis assay: (A) optical density recorded at 540 nm of diluted plasma after incubation of the blood in the presence of a foil of copper (positive control); (B) polyethylene (negative control); (C) film of Teflon® AF 2400; (D) film of Teflon® AF 2400 holding LiPc crystals.

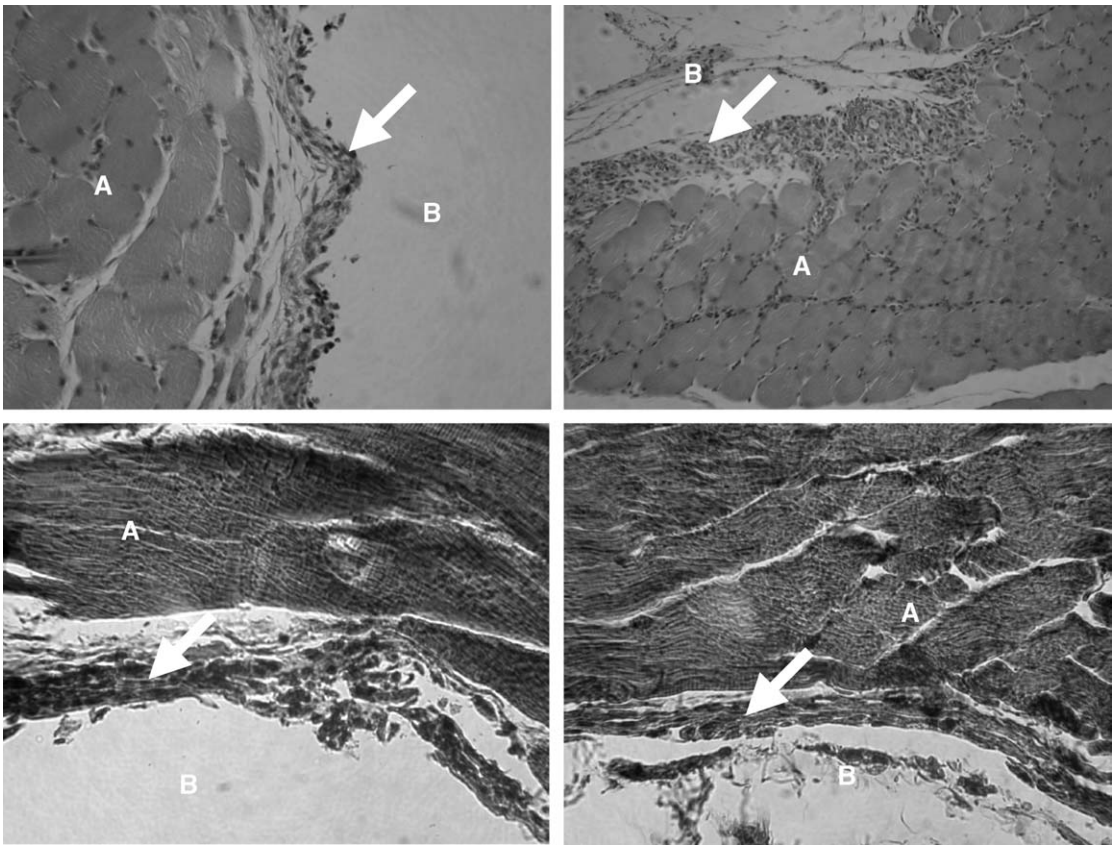

Fig. 7. Histological aspect of rabbit muscles after residence of implants. Left: tissue implanted with polyethylene USP88 (negative control). Right: tissue implanted with the wire coated with the Teflon® AF 2400. Top: 7 days of implantation. Bottom: 90 days of implantation. (A) Muscle and (B) empty place after removal of the implant. The arrows indicate the presence of a thin capsule formed by few layers of inflammatory cells. Note that the implantation of coated wire did not induce a larger inflammatory response than the USP88 implant.

for short-term. We observed a thin capsule formed by one to three layers of fibroblasts surrounding the implants. The morphology of muscle around the implants did not present any abnormal sign. All together, these biocompatibility assays using standardized methods demonstrated that there was no larger reaction using the LiPc sensor inserted in Teflon® AF 2400 films than observed using other standard or commercial materials.

4. Conclusion

We have described procedures that incorporate LiPc crystals in Teflon AF 2400® films. We demonstrated that the performances of the sensors were preserved in the conditions used for the preparation and sterilization. The stability of the response to oxygen was maintained over a long period. The biocompatibility was demonstrated using standardized methods, as there was no larger reaction than observed using other standard or commercial materials. With the start of measurements with EPR in humans (Swartz et al., 2004), this oxygen sensing system appears to be a very suitable system for the safe and stable implantation of oxygen responsive paramagnetic materials for appropriate applications, such as measurements in peripheral vascular disease, wound healing, and cancer therapy. These developments can be applied for other applications of biosensors where there is a need of oxygen permeable membranes.

Acknowledgments

This work was supported by research grants of the Belgian National Fund for Scientific Research 7.4503.02, by the Fonds Joseph Maisin, by the “Communauté Française de Belgique – Actions de Recherches Concertées – ARC 04/09-317”, and by NIH grants P01 EB002180 and P41 EB002032. The authors acknowledge Sylvie Derclaye, from the Laboratoire de Chimie des Surfaces (Université Catholique de Louvain, Louvain-la-Neuve, Belgium), for the technical help in AFM.

References

Alentiev, A.Y., Yampolskii, Y.P., Shantarovich, V.P., Nemser, S.M., Platé, N.A., 1997. High transport parameters and free volume of perfluorodioxole copolymers. J. Membr. Sci. 126, 123–132.

American Society for Testing and Materials. Standard practice for assessment of hemolytic properties of materials. ASTM F756-00, 2000.

Bensebaa, F., Petit, P., Andre, J.J., 1992. The effect of oxygen on phthalocyanine radicals. 1. ESR study of lithium phthalocyanine spin species at different oxygen concentrations. J. Phys. Chem. 96, 5739–5745.

Clarkson, R.B., Odintsov, B.M., Ceroke, P.J., Ardenkjaer-Larsen, J.H., Fruiamu, M., Belford, R.L., 1998. Electron paramagnetic resonance and dynamic nuclear polarization of char suspensions: surface science and oximetry. Phys. Med. Biol. 43, 1907–1920.

Dasgupta, P.K., Genfa, Z., Poruthoor, S.K., Caldwell, S., Dong, S., 1998. High-sensitivity gas sensors based on gas-permeable liquid core waveguides and long-path absorbance detection. Anal. Chem. 70, 4661–4669.

Dunn, J.F., Swartz, H.M., 2003. In vivo electron paramagnetic resonance with particulate materials. Methods 30, 159–166.

Gallez, B., Debuyst, R., Dejehet, F., Liu, K.J., Walczak, T., Goda, F., Demeure, R., Taper, H., Swartz, H.M., 1998. Small particles of fusinite and carbohydrate chars coated with aqueous soluble polymers: preparation and applications for EPR oximetry. Magn. Reson. Med. 40, 152–159.

Gallez, B., Jordan, B., Baudelet, C., 1999a. Microencapsulation of paramagnetic particles by pyrroxylin in order to preserve their responsiveness to oxygen when used as sensors for in vivo EPR oximetry. Magn. Reson. Med. 42, 193–196.

Gallez, B., Jordan, B.F., Baudelet, C., Misson, P.D., 1999b. Pharmacological modifications of the partial pressure of oxygen in murine tumors: evaluation using in vivo EPR oximetry. Magn. Reson. Med. 42, 627–630.

Gallez, B., Mäder, K., 2000. Accurate and sensitive measurements of pO_2 in vivo using low frequency EPR spectroscopy: how to confer biocompatibility to the oxygen sensors. Free Radic. Biol. Med. 29, 1078–1084.

Gallez, B., Baudelet, C., Jordan, B.F., 2004. Assessment of tumor oxygenation by electron paramagnetic resonance: principles and applications. NMR Biomed. 17, 240–262.

Goda, F., Bacic, G., O'Hara, J.A., Gallez, B., Swartz, H.M., Dunn, J.F., 1996. The relationship between partial pressure of oxygen and perfusion in two murine tumors after X-ray irradiation: a combined gadopentetate dimeglumine dynamic magnetic resonance imaging and in vivo electron paramagnetic resonance oximetry study. Cancer Res. 56, 3344–3349.

He, G., Shankar, R.A., Chzhan, M., Samouilov, A., Kuppusamy, P., Zweier, J.L., 1999. Noninvasive measurement of anatomic structure and intraluminal oxygenation in the gastrointestinal tract of living mice with spatial and spectral EPR imaging. Proc. Natl. Acad. Sci. U.S.A. 96, 4586–4591.

He, J., Beghein, N., Ceroke, P., Clarkson, R.B., Swartz, H.M., Gallez, B., 2001a. Development of biocompatible oxygen-permeable films holding paramagnetic carbon particles: evaluation of their performance and stability in EPR oximetry. Magn. Reson. Med. 46, 610– 614.

He, J., Beghein, N., Clarkson, R.B., Swartz, H.M., Gallez, B., 2001b. Microencapsulation of carbon particles used as oxygen sensors in EPR oximetry to stabilize their responsiveness to oxygen in vitro and in vivo. Phys. Med. Biol. 46, 3323–3329.

Hou, H., Grinberg, O.Y., Taie, S., Leichtweis, S., Miyake, M., Grinberg, S., Xie, H., Csete, M., Swartz, H.M., 2003. Electron paramagnetic resonance assessment of brain tissue oxygen tension in anesthetized rats. Anesth. Analg. 96, 1467–1472.

International Standardization Organization, 1994. Biological Evaluation of Medical Devices. Part 6. Tests for Local Effects After Implantation. ISO 10993-6.

James, P.E., Bacic, G., Grinberg, O.Y., Goda, F., Dunn, J., Jackson, S.K., Swartz, H.M., 1996. Endotoxin induced changes in intrarenal pO_2 measured by in vivo electron paramagnetic resonance oximetry and magnetic resonance imaging. Free Rad. Biol. Med. 21, 25–34.

James, P.E., Grinberg, O.Y., Goda, F., Panz, T., O'Hara, J.A., Swartz, H.M., 1997. Gloxy: an oxygen-sensitive coal for accurate measurement of low oxygen tensions in biological systems. Magn. Reson. Med. 38, 48–58.

James, P.E., Madhani, M., Roebuck, W., Jackson, S.K., Swartz, H.M., 2002. Endotoxin-induced liver hypoxia: defective oxygen delivery versus oxygen consumption. Nitric Oxide 6, 18–28.

Jordan, B.F., Baudelet, C., Gallez, B., 1998. Carbon-centered radicals as oxygen sensors for in vivo electron paramagnetic resonance: screening for an optimal probe among commercially available charcoals. MAGMA 7, 121–129.

Jordan, B.F., Beghein, N., Aubry, M., Gregoire, V., Gallez, B., 2003a. Potentiation of radiation-induced delay by isosorbide dinitrate in FSAII murine tumors. Int. J. Cancer 103, 138–141.

Jordan, B.F., Gregoire, V., Demeure, R.J., Sonveaux, P., Feron, O., O'Hara, J.A., Vanhulle, V.P., Delzenne, N., Gallez, B., 2002. Insulin increases the sensitivity of tumors to irradiation: involvement of an increase in tumor oxygenation mediated by a nitric oxide-dependent decrease of the tumor cells oxygen consumption. Cancer Res. 62, 3555–3561.

Jordan, B.F., Kimpalou, J.Z., Beghein, N., Dessy, C., Feron, O., Gallez, B., 2004a. Contribution of oxygenation to BOLD contrast in exercising muscle. Magn. Reson. Med. 52, 391–396.

Jordan, B.F., Misson, P., Demeure, R., Baudelet, C., Beghein, N., Gallez, B., 2000. Changes in tumor oxygenation/perfusion induced by the no donor, isosorbide dinitrate, in comparison with carbogen: monitoring by EPR and MRI. Int. J. Radiat. Oncol. Biol. Phys. 48, 565–570.

Jordan, B.F., Sonveaux, P., Feron, O., Gregoire, V., Beghein, N., Gallez, B., 2003b. Nitric oxide mediated increase in tumor blood flow and oxygenation of tumors implanted in muscles stimulated by electric pulses. Int. J. Radiat. Oncol. Biol. Phys. 55, 1066–1073.

Jordan, B.F., Sonveaux, P., Feron, O., Grégoire, V., Beghein, N., Dessy, C., Gallez, B., 2004b. Nitric oxide as radiosensitizer: evidence for an intrinsic role additive to its effect on oxygen delivery and consumption. Int. J. Cancer 109, 768–773.

Krzic, M., Sentjurc, M., Kristl, J., 2001. Improved skin oxygenation after benzoyl nicotinate application in different carriers as measured by EPR oximetry in vivo. J. Control Release 70, 203–211.

Kuppusamy, P., Zweier, J.L., 2004. Cardiac applications of EPR imaging 17, 226–239.

Lan, M., Beghein, N., Charlier, N., Gallez, B., 2004. Carbon blacks as EPR sensors for localized measurements of tissue oxygenation. Magn. Reson. Med. 51, 1272–1278.

Liu, K.J., Gast, P., Moussavi, M., Norby, S.W., Vahidi, N., Walczak, T., Wu, M., Swartz, H.M., 1993. Lithium phthalocyanine: a probe for electron paramagnetic resonance oximetry in viable biological systems. Proc. Natl. Acad. Sci. U.S.A. 90, 5438–5442.

Pinnau, I., Toy, L.G., 1996. Gas and vapor transport properties of amorphous perfluorinated copolymer membranes based on 2,2-bistrifluoromethyl-4,5-difluoro-1,3-dioxole/tetrafluoroethylene. J. Membr. Sci. 109, 125–133.

Sersa, G., Krzic, M., Sentjurc, M., Ivanusa, T., Beravs, K., Cemazar, M., Auersperg, M., Swartz, H.M., 2001. Reduced tumor oxygenation by treatment with vinblastine. Cancer Res. 61, 4266–4271.

Swartz, H.M., Khan, N., Buckey, J., Comi, R., Gould, L., Grinberg, O., Hartford, A., Hopf, H., Hug, H., Iwasaki, A., Lesniewski, P., Salikhov, I., Walczak, T., 2004. Clinical applications of EPR: overview and perspectives. NMR Biomed. 17, 335–351.

United States Pharmacopoeia, 2000. Biological reactivity tests, in vivo. USP 2000; 24:1831–1836.

Vahidi, N., Clarkson, R.B., Liu, K.J., Norby, S.W., Swartz, H.M., 1994. In vivo and in vitro EPR oximetry with fusinite: a new coal-derived, particulate EPR probe. Magn. Reson. Med. 31, 139–146.

Walczak, T., Lesniewski, P.N., Hou, H., Grinberg, O.Y., Gallez, B., Dinguizli, M., Sucheta, A., Swartz, H.M, submitted for publication. Im-

plantable resonators—a new direction for in vivo application of EPR spectroscopy.

Werner, L., Legeais, J.M., Nagel, M.D., Renard, G., 1999a. Evaluation of Teflon-coated intraocular lenses in an organ culture method. J. Biomed. Mater. Res. 46, 347–354.

Werner, L., Legeais, J.M., Nagel, M.D., Renard, G., 1999b. Neutral red assay of the cytotoxicity of fluorocarbon-coated polymethylmethacrylate intraocular lenses in vitro. J. Biomed. Mater. Res. 48, 814–819.