In Vivo pO_2 Assessment of Implantation sites Using Solid Probe Oxygen Imaging

Navin Viswakarma[1], Eliyas Siddiqui[1], Sonny Patel[1], Safa Hameed[1], Boris Epel[1,2], and Mrignayani Kotecha[1*]
[1]Oxyegn Measurement Core, O2M Technologies, 2201 W Campbell Park Dr, STE 310, Chicago, IL 60612
[2]Department of Radiation and Cellular Oncology, University of Chicago, 5841 S Ellis Ave, Chicago IL 60637
*Contact: mkotecha@o2map.com

Statement of Purpose

The purpose of the present study was to assess the native pO_2 of subcutaneous (SC) and intraperitoneal (IP) sites in mice using solid LiPc-PDMS probe pulse electron paramagnetic resonance oxygen imaging (EPROI). Both SC and IP sites are regularly used for device implantation in cell encapsulation therapy (1,2). LiPc is a water-insoluble crystalline probe whose relaxation rates are highly sensitive to the local oxygen concentration. To facilitate direct *in vivo* oxygen imaging, we encapsulated LiPc crystals in polydimethylsiloxane (PDMS), an oxygen-permeable and bioinert polymer, and developed an implantable cylindrical shaped solid probes (3-5). While LiPc-PDMS or similar probes have been used in repeated spectroscopic or average oxygen measurements since the late 1990s leading up to clinical studies, it has not been used for oxygen imaging of implanted devices. One LiPc-PDMS probe of 2 mm diameter and 10 mm length was implanted in subcutaneous or intraperitoneal sites (left or right side) in each animal. The pO_2 imaging of implanted LiPc-PDMS probes were performed weekly by O2M's preclinical oxygen imager, JIVA-25™ (Figure 1A and B) for six weeks. At week 6, the probes were recovered and histological exam was performed. We report here, first-ever solid probe oxygen imaging of implanted devices and pO_2 values of subcutaneous and intraperitoneal sites.

Materials and Methods

Fabrication of LiPc-PDMS probe: The LiPc crystals were purchased from Clin-EPR, LLC. The PDMS elastomer (A-103), crosslinker (A-103-C) and platinum accelerator (A-317) were purchased from Factor II, Inc., Lakeside, Arizona. The LiPc-PDMS probes with 10 mm length and 2 mm diameter were fabricated following the published protocol (3).

Implantation of LiPc:PDMS probes in mice: All animal experiments were performed under the approved protocol from UIC IACUC. Briefly, LiPc-PDMS probes were implanted subcutaneously (SC) or intraperitoneally (IP) on the left or right ventral side of the mouse (8-week-old male C57BL/6). The sutured skin was allowed to heal for at least 24 h prior to making EPR measurements.

LiPc-PDMS probe calibration and pO_2 imaging: The calibration of the LiPc-PDMS probes was performed using inversion recovery T_1 sequence for 0, 1, 2, 4, 6, and 8% oxygen concentration. Relaxation rates ($R_1=1/T_1$) were calculated using single exponential fit. The slope and intercept were obtained by linear fitting of R_1 and pO_2 (Figure 1C). pO_2 imaging of LiPc-PDMS probes were performed using electron spin echo inversion recovery (IRESE) sequence with following parameters: pulse lengths 60 ns, 8 phase cycles scheme with FID suppression, spin-echo delay 400 ns, equal solid angle spaced 654 projections, 67 baselines, 1.5 G/cm gradient, 8-time delays from 410 ns–15 µs, 55µs repetition time, overall 10 min image duration. Images were reconstructed using filtered back-projection in isotropic 64 × 64 × 64 cube with 0.66 mm voxel linear size.

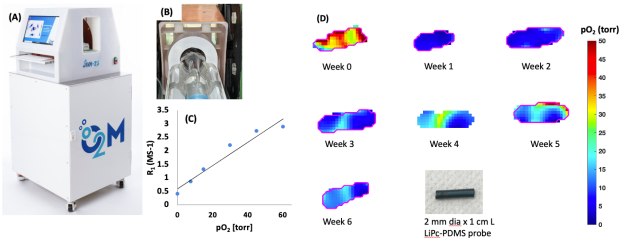

Figure 1: (A) JIVA-25™ is a 25 mT instrument and provides pO_2 maps in live tissues *in vitro* and *in vivo*, (B) An animal inside the 32x35 mm resonator that was used for the measurements, (C) calibration curve of LiPc-PDMS probe at 37 °C, oxygen was set at 0, 1, 2, 4, 6, and 8% and R_1 ($1/T_1$) were measured when the equilibrium was reached. (D) Weekly pO_2 maps of LiPc-PDMS probe implanted in mouse #1 subcutaneously. The last inset shows the picture of the probe.

	pO_2 (torr) mean ± std (# of voxels)			
	Mouse #1 Right – subQ (n=1)	Mouse #2 Left subQ (n=1)	Mouse #3 Right IP (n=1)	Mouse #4 Left IP (n=1)
Week 0	26 ± 10.11 (n631)	6.89 ± 2.27 (n519)	21.44 ± 8.92 (n408)	3.82 ± 1.81 (n383)
Week 1	4.56 ± 1.80 (n560)	6.26 ± 2.27 (n589)	9.54 ± 3.77 (n479)	1.63 ± 1.48 (n513)
Week 2	5.31 ± 3.01 (n 613)	1.41 ± 1.17 (n 334)	2.98 ± 1.16 (n 466)	1.65 ± 1.11 (n557)
Week 3	10.77 ± 5.62 (n588)	5.17 ± 2.76 (n531)	3.33 ± 1.19 (n512)	2.07 ± 0.81 (n570)
Week 4	15.69 ± 9.14 (n519)	7.42 ± 3.70 (n584)	4.71 ± 1.66 (n559)	6.10 ± 2.99 (n596)
Week 5	15.32 ± 7.54 (n642)	10.59 ± 5.23 (n531)	2.59 ± 1.45 (n531)	3.43 ±1.86 (n525)
Week 6	11.14 ± 3.73 (n602)	11.87 ± 5.03 (n635)	9.03 ± 2.44 (n511)	12.79 ± 2.30 (n612)

Table 1: Weekly pO_2 statistics of implanted probes at left or right side in subcutaneous or intraperitoneal pockets. At week six, all devices showed low pO_2. Tissue examination of explanted at week six devices showed that they were surrounded by fat or muscle tissues.

Results and Discussion

The pO_2 map shown in Figure 2 demonstrated the ability of the implanted LiPC-PDMS probes to provide repeated measurements of *in vivo* tissue oxygenation at SC and IP sites. Figure 1D provides an example pO_2 maps of mouse #1 with SC implantation as a function of time. Table 1 provides 6-week data on pO_2 for all 4 animals in the study. Mouse #1 (right SC) and #3 (right IP) showed normal pO_2 at week 0, but subsequently, all animals showed hypoxic pO_2. The recovered probes after six weeks of measurement showed that the devices were embedded in fat or muscular tissues with little vascularization that is consistent with pO_2 data of all four animals as shown in Table 1.

Conclusion: Our experimental results showed that LiPC-PDMS probe is capable of repeated pO_2 imaging using pulse EPR oximetry using JIVA-25. The pO_2 data show that the average oxygen remained low throughout the study period, and there was no significant difference between SC and IP sites.

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