Methodology for Biomaterial Oxygen Imaging Using Trityl Based Pulse Electron Paramagnetic Resonance

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Statement of Purpose: Oxygen is an important indicator of the physiologic state of tissues and bioartificial devices. Although oxygen point measurements using polarographic needle electrodes or luminescence-based optical sensors can reveal local oxygenation, three-dimensional oxygen maps deliver the complete information and thus can better assist the development of artificial cell and tissue replacement devices. This is especially important for samples where metabolically active cells can create steep oxygen gradients on the scale of hundreds of microns.

For optically opaque samples magnetic resonance methods are found to be the most suitable. Pulse electron paramagnetic resonance oxygen imaging (EPROI) is an emerging technique to provide three-dimensional partial oxygen pressure (pO_2) maps of tissues and biological samples (1). EPROI provides oxygen maps with high pO_2 resolution (~1 torr), 0.5-1 mm spatial resolution within 1-10 minutes. Similar to MRI, EPROI uses magnetic field gradients to generate spatial images. EPR uses much smaller magnetic fields (mT range) but requires much faster electronics to deal with million-time shorter electron relaxation times as compared to protons. The signal-to-noise (SNR) ratio of an EPR signal increases with the magnetic field, however with increased losses at higher frequencies, the penetration depth reduces. Table 1 provides the relationship between the magnetic field and penetration depth. The 25 mT is a sweet spot for small animal and in vitro oxygen imaging. Over the past decade, EPROI has advanced at a rapid pace, from continuous wave to pulse acquisition to deliver oxygen images in live mice, rats, and rabbit limbs.

EPROI used in our laboratories measures the relaxation maps of water-soluble oxygen-reporting trityl molecules and utilizes a linear relationship between pO_2 and spin-lattice relaxation rates. Trityls are stable non-toxic radicals with high solubility in water (18). Figure 1 provides the pO_2 calibration of common biomaterials at 25 mT acquired using JIVA-25, O2M's oxygen imager. The pO2 maps can assist with quality control of biomaterials for regenerative medicine applications as shown in Figure 2. Currently, there are two leading methods for EPR oxygen imaging, **(a) inversion recovery electron spin echo (IRESE) (1), and (b) single point imaging (SPI) (2).** IRESE provides pO_2 maps with higher accuracy due to the use of spin-lattice relaxation imaging but at the expense of spatial fidelity and resolution, while SPI provides oxygen maps with less absolute accuracy, but with better spatial resolution and lower RF power. Figure 3 demonstrates the spatial and pO_2 images obtained using these two methodologies. In this presentation, we will provide basic principles of EPROI for the Society for biomaterials community.

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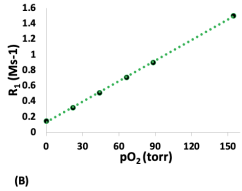
References: 1. Epel et al., J. Mag reson. 280:149-157. **2.** S. Subramanian, N. Devasahayam, R. Murugesan, K. Yamada, J. Cook, A. Taube, J.B. Mitchell, J.A.B. Lohman, M.C. Krishna, Magnet Reson Med, 48 (2002) 370-379.

Frequency	~250 MHz	~750 MHz	1.5 GHz
Magnetic Field	~ 9 mT	~25 mT	~ 50 mT
Penetration depth	> 10 cm	6-8 cm	1-1.5 cm
Object	Mouse, rat, rabbit, monkey, human	Mouse, rat full body, rabbit limbs	Surface parts
SNR	1 a.u.	5.2 a.u.	14.7 a.u.

Table 1: Penetration depth vs the resonance frequency of an EPR imager. The 25 mT is a sweet spot for small animal oxygen imaging.

Values at 37 °C	Intercept (10^6s-1)	Slope (torr/10^6s-1)	Temp Coeff. (in physiologic temp range)
PBS	0.1246	112.93	Intercept: -0.00092, Slope: -2.27
DiH2O	0.1138	108.42	Intercept: -0.0006, Slope: -1.07
DMEM media with 1 M cells	0.1195	114.05	
1% Gelatin	0.1191	114.13	Intercept: -0.00224, Slope: -2.3
2% Agarose	0.1253	123.82	
2% Alginate	0.1223	119.58	

(A)



(B)

Figure 1: (A) pO_2 calibration of common biomaterials with inversion recovery T_1 measurements at 25 mT. (B) An example fit curve between R_1 ($1/T_1$) and equilibrated 0, 3, 6, 9, 12 and 21% oxygen tension in the sample. The sample in this case is 1mL PBS in a 10 mm tube with 1 mM trityl concentration.

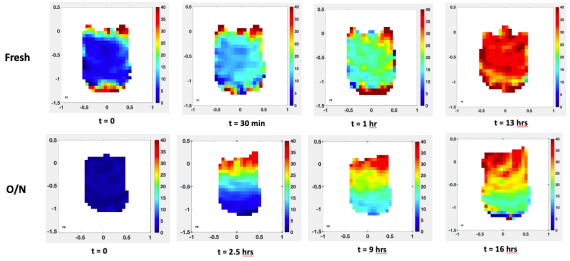


Figure 2: pO_2 maps of fresh (top panel) vs overnight (bottom panel) stored 1% gelatin, 1 mL in a 10 mm tube with 1 mM trityl. The sample was deoxygenated first by bubbling N_2, then 5% O2 gas mixture was circulated on top of the sample.

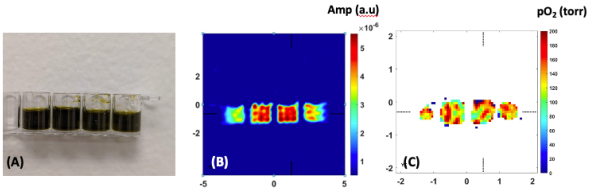


Figure 3: (A) A single strip well plate with 1% gelatin and 1 mM trityl at ambient air and at room temperature, (B) amplitude map of wells acquired using SPI, and (C) pO_2 map acquired using IRESE.