BLACK MAGIC AND EPR OXIMETRY:  
From Lab to Initial Clinical Trials


Abstract: EPR oximetry is a technique that can make repeated non-invasive measurements of the pO₂ in tissues. To extend the application of EPR oximetry to humans, India ink is the probe of choice because appropriate India inks have EPR signals whose line widths are sensitive to changes in oxygen concentrations, and, most importantly, India ink already has been used extensively in humans as a marker in the skin, lymphatics, various organs during surgery, tumors, and for decoration as tattoos.

We have developed an India ink that has good sensitivity to oxygen, high stability in tissues, good signal intensity, and minimal toxicity. In this article we describe the various properties of this India ink, results obtained from our animal experiments, and our first preliminary clinical results, which are part of the first systematic clinical use of EPR oximetry. The clinical results indicate that it is possible to do repeated measurements over several months and probably years after the injection of the ink, indicating that long-term follow-up studies are feasible. We are very encouraged with these results and are confident that EPR oximetry using India ink will be a non-invasive, fast, and reliable technique for pO₂ measurements in clinical studies.

1. INTRODUCTION

It would be very useful to have a method to directly and repetitively measure the partial pressure of oxygen (pO₂) in tissues. This would be especially desirable for planning and evaluating therapy for tumors and vascular insufficiencies. A variety of
techniques are available for measuring $pO_2$ in tissues; however, none of these techniques has been shown to have the properties needed for optimal experimental and clinical use (i.e. sensitivity, accuracy, ease, and ability to make measurements repeatedly). EPR oximetry is one of the most promising techniques for accomplishing this goal. It is based on the fact that molecular oxygen can interact with paramagnetic materials such as nitroxides, lithium phthalocyanine, coals such as fusinite, chars, and India ink, affecting their EPR spectra in a reproducible manner that is proportionate to the amount of oxygen. In the studies described here we use India ink, measuring the oxygen-induced change in the line width of the EPR spectrum, which is calibrated against different known $pO_2$ values.$^{1,2}$

In order to extend the application of EPR oximetry to humans, India ink is the probe of choice because appropriate India inks have EPR signals whose line widths are sensitive to changes in oxygen concentrations$^{3, 4}$; most importantly, India ink already has been used extensively in humans (as a marker in the skin, lymphatic, mucosal tissue, and tumors, and for decoration as tattoos)$^{5-8}$.

To facilitate the application of EPR oximetry in human subjects, we have developed an India ink that has good sensitivity to oxygen, high stability in tissues, good signal intensity, and minimal toxicity. In this article we describe the various properties of this India ink, results obtained from our animal experiments, and our first preliminary clinical results, which are part of the first systematic clinical use of EPR oximetry.

2. MATERIALS AND METHODS

2.1. India Ink

We identified an effective India ink, Higgins black magic waterproof ink (No. 4465), through a search of local commercial sources of inks. This ink has an excellent signal to noise ratio. To increase the signal intensity, the ink was concentrated to 20% of its original volume by heating at approximately 90°C-100°C for six to seven hours. We also studied this ink after dialysis to remove soluble components that might be involved in potential toxicity. Prior to injection, the ink was autoclaved for one hour at 121°C. The calibration of the ink was done against 0%, 1%, 2%, and 5% perfused oxygen concentrations at 37°C on a 1.2 GHz spectrometer. The change in line width followed a second order polynomial relation with changes in the oxygen concentration. The coefficients of the fit were determined and used to convert the line widths obtained into $pO_2$ values.

2.2. Animal Preparation and Experimental Protocol

Nine male Sprague-Dawley rats, 200-250 g, (Charles River Laboratories, Wilmington, MA) were used in this study. The experimental techniques and protocol were approved by the Dartmouth College Animal Care and Use Program. The rats were anesthetized using 2.5%-3.0% isoflurane, and approximately 15-20 µl of the India ink was injected intramuscularly or subcutaneously in rats, in the left or right hind limbs, respectively (day 0). The intramuscular injection of the ink was approximately 3 mm deep from the surface of the skin. These injections were done using a 1 ml syringe fitted with a 23 gauge