Premiere Publications from The Triological Society

Read all three of our prestigious publications, each offering high-quality content to keep you informed with the latest developments in the field.

**The Laryngoscope**

**Editor-in-Chief:** Samuel H. Selesnick, MD, FACS

The leading source for information in head and neck disorders.

Laryngoscope.com

**Laryngoscope Investigative Otolaryngology**

**Editor-in-Chief:** D. Bradley Welling, MD, PhD, FACS

Rapid dissemination of the science and practice of otolaryngology-head and neck surgery.

InvestigativeOto.com

**ENTtoday**

**Editor-in-Chief:** Alexander Chiu, MD

Must-have timely information that Otolaryngologist-head and neck surgeons can use in daily practice.

Enttoday.org
Measuring Flap Oxygen Using Electron Paramagnetic Resonance Oximetry

Marc A. Polacco, MD; Huagang Hou, MD; Periannan Kuppusamy, PhD; Eunice Y. Chen, MD, PhD

<table>
<thead>
<tr>
<th>Objectives/Hypothesis</th>
<th>To determine if electron paramagnetic resonance (EPR) oximetry is a viable technology to aid in flap monitoring.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods</td>
<td>This was a cohort study assessing accuracy and speed of EPR oximetry in detecting ischemia of a saphenous artery–based flap in a rat model, using transcutaneous oximetry as a control. Measurements were obtained under both resting and ischemic conditions for nine Sprague Dawley rats (18 flaps), for 3 postoperative days following flap elevation.</td>
</tr>
<tr>
<td>Results</td>
<td>The mean partial pressure of oxygen prior to tourniquet application was 66.9 ± 8.9 mm Hg with EPR oximetry and 64.7 ± 5.2 mm Hg with transcutaneous oximetry (P = .45). Mean partial pressures of oxygen during tourniquet application were 89 ± 3.2 mm Hg and 85.9 ± 2.9 mm Hg for EPR oximetry and transcutaneous oximetry, respectively (P = .48), and 67.2 ± 6.9 mm Hg and 65.3 ± 6.1 mm Hg after tourniquet release for EPR oximetry and transcutaneous oximetry, respectively (P = .44). The mean ischemia detection time of EPR oximetry was 49 ± 21 seconds.</td>
</tr>
<tr>
<td>Conclusions</td>
<td>Offering timely, accurate, and noninvasive tissue oxygen measurements, EPR oximetry is a promising adjunct in flap monitoring.</td>
</tr>
<tr>
<td>Key Words</td>
<td>Flap, tissue, oxygen, ischemia, electron paramagnetic resonance.</td>
</tr>
<tr>
<td>Level of Evidence</td>
<td>NA</td>
</tr>
</tbody>
</table>

INTRODUCTION

Flap necrosis is an important complication in head and neck and plastic reconstructive surgery. Methods to mitigate risk include refinement of surgical technique, thoughtful patient selection, use of vasodilators and anticoagulants, cooling tissue, and close postoperative monitoring.¹ It is common practice to monitor flap viability postoperatively, especially that of free flaps, as ischemia and flap failure risk is highest in the first 24 to 48 hours following surgery. Early detection of flap failure is essential in increasing odds of flap survival by timely re-exploration.²⁻⁴ As a testament to the importance of early detection, more than 20 methods of monitoring surgical flaps have been described in the literature.⁵ Physical examination, bedside Doppler ultrasonography, implantable Doppler ultrasonography, laser Doppler, implantable temperature probes, oxygen saturation probes, pH monitoring, and confocal microscopy, and glucose/lactate monitors are just a few among the plethora of techniques previously described.⁵ Most recently, oxygen-sensing paint-on bandages have been described that glow green when tissue is well perfused and red when there are low levels of oxygen.⁶⁷ Due to ease of use and availability, the most commonly implemented methods of flap monitoring are often physical examination, which may include assessment of color, capillary refill, temperature, and pinprick testing; bedside Doppler; and implantable Doppler. However, each of these methods carries inherent weaknesses secondary to subjectivity and reliance on provider experience.⁵

It is well established that oxygen is necessary for tissue viability and wound healing. In normal healing, hypoxia in surgical wounds is caused by microvascular injury and signals influx of inflammatory cells, which in turn activate proangiogenic, profibrotic, and pro-inflammatory cytokines, including tumor necrosis factor α, basic fibroblast growth factor, platelet-derived growth factor, transforming growth factor β, and vascular endothelial growth factor.⁸⁹ Although certain degrees of hypoxia are tolerated and indeed required for wound healing, an imbalance of hypoxia and the subsequent inflammatory response leads to poor wound healing or irreversible tissue damage and necrosis. Unlike Doppler ultrasound and other indirect monitoring techniques mentioned above, measuring tissue oxygen would provide a direct metric of tissue viability. Although there are techniques (OxyLite and Eppendorf) available that provide direct measurement of tissue oxygen concentration (partial pressure of oxygen, or PO₂), none are suitable for repeated use in a clinical setting due to invasiveness and artifact created through repeated tissue trauma.¹₀⁻¹⁵
Electron paramagnetic resonance oximetry (EPR oximetry) allows for direct, repeated, noninvasive measurements of oxygen through injection of a paramagnetic source into tissue. Although it has been used primarily to measure tumor oxygenation in response to radiation and chemotherapy, interest in utilizing it to measure tissue oxygen in wound healing and surgical flaps is growing. Using transcutaneous oximetry (TcO$_2$) as a control, we demonstrate the accuracy and ease with which EPR oximetry may be used to monitor flap oxygenation and viability.

**MATERIALS AND METHODS**

**EPR Oximetry Using OxyChip**

EPR oximetry uses an implantable oxygen-sensing probe, called OxyChip, in the tissue. The probe, in the shape of a cylinder of approximately 0.3 mm diameter and 1 mm length, prepared and sterilized as previously described, is implanted into the tissue using a 23-G needle. Once placed, the chip is stable and allows repeated pO$_2$ measurements in a small volume of tissue (~0.1 mm$^3$) for weeks to months.

**Animal Preparation**

All animal use was approved by the Institutional Animal Care and Use Committee at the Geisel School of Medicine at Dartmouth College (Hanover, NH). A total of 10 male Sprague-Dawley rats, each weighing 200 g to 250 g, were obtained from Charles River Laboratories (Wilmington, MA). One rat was utilized for piloting chip injection and surgical technique, and the remaining nine were utilized for data collection.

**Chip Injection and Surgical Procedure**

Seventy-two hours prior to surgery, an OxyChip was injected into the center of the inner thighs on the hind legs of each rat. This procedure was performed under anesthesia after shaving each leg and sterilizing the skin with alcohol. The surgical procedure used to raise saphenous artery–based flaps was adapted from previously described methodology. Once anesthesia had been obtained, the rat was placed on a heating pad located on a sterile blue towel in a supine position. The left hind leg was then shaved to remove hair, and the skin was cleaned with Betadine antiseptic solution. The hind leg was taped in a stretched position to increase tension on the skin. A marking pen was used to outline a 4-by-1-cm area along the inner thigh from ankle to upper thigh. A scalpel with a 15C blade attached was used to create an incision along the length of the marked region. To expose the femoral artery, the skin flap was elevated using Stevens tenotomy scissors (Fig. 1). Dissection was continued along the saphenous artery and medial tarsal branches until the ankle was reached. Assessment of the septocutaneous perforators branching off the saphenous artery was performed. Once the flap had been elevated and hemostasis confirmed, 5-0 fast gut suture was used in a running fashion to reapproximate the skin edges. Each rat was allowed 2 weeks to recover before surgery was performed on the contralateral leg.

**Oxygen Measurements**

**Postoperative day 0.** Transcutaneous oximeter (TCM400) probes were calibrated according to the manufacturer’s instructions (Radiometer; Copenhagen, Denmark). A standard fixation ring was placed on the flap, with care taken to ensure that all edges were airtight. Four drops of contact liquid were placed in the well, and the electrode was fastened securely into place. The oximeter was allowed to run for 20 minutes to achieve fluid warming (to 44°C) and TcO$_2$ stabilization. TcO$_2$ measurements were then recorded for 5 minutes after stabilization was complete. Once TcO$_2$ measurements were complete, the TcO$_2$ probe and fixation ring were removed, and the rat was placed between the magnets of a custom-built L-band (1.2 GHz) spectrometer with a surface-loop resonator (Fig. 2). The location of the loop wire was adjusted until a characteristic EPR signal from the OxyChip was obtained, and measurements were taken every 5 seconds over a course of 5 minutes. The TcO$_2$ and EPR oximetry measurements were taken in this fashion prior to flap elevation and immediately following.

**Postoperative days 1 to 3.** On the first postoperative day (POD), baseline TcO$_2$ and EPR oximetry measurements were obtained using identical steps for POD 0. After baseline measurements were obtained, a rubber-band tourniquet was applied to the proximal thigh and EPR oximetry measurements were repeated at 5-second intervals for a total of 5 minutes. The rat was then removed from between the magnets and a transcutaneous oximeter was applied, with recordings performed identical to POD 0. Once measurements under ischemic conditions were complete, the tourniquet was removed, and EPR oximetry and TcO$_2$ measurements were repeated for 5 minutes each.
After tourniquet application on PODs 1 to 3, EPR oximetry measurements were obtained every 5 seconds for a total of 5 minutes. The time needed to obtain three consecutive measurements within a 5-mm Hg range (plateau) was measured.

Statistical Analysis
The mean and standard deviation of EPR and TcO₂ oximetry measurements were calculated. To determine statistical differences between EPR and TcO₂ measurements on each POD, two-way paired t tests were utilized. To determine statistical differences in measurements longitudinally for each condition across PODs 0 to 3, a repeated-measures ANOVA was performed. Statistical analyses were performed using SPSS Statistics (IBM Corp., Armonk, NY).

RESULTS
Oxygen Measurements
Mean EPR oximetry and TcO₂ measurements are presented in Table I. On POD 0, there was no tourniquet applied; thus, data in the “Tourniquet Released” column are after flap inset. There were no significant differences in pO₂ values between EPR oximetry and TcO₂ for any POD (P > .05). There was no statistically significant difference in EPR or TcO₂ measurements longitudinally from POD 0 to POD 3 (F[3,15] = 1.28; P > .100). Graphical representations of POD 1 data are included in Figure 3.

Ischemia Detection Speed
The mean time to plateau was 49 ± 21 seconds. EPR oximetry plateau time was measured after tourniquet removal with a mean of 45 ± 19 seconds. Detection speed was not compared with TcO₂, as standardization time alone required a minimum of 15 to 20 minutes.

Complications
There were no complications related to equipment or OxyChip extrusion. On POD 1, the flap on the left leg of one rat was absent, reducing the total flap number to 17. Given the acuteness of which the flap was removed, it was most likely self-removed, although we did not encounter this complication for the other leg on the same rat or for any other rats used in the study. The open wound was treated by local advancement and primary closure.

DISCUSSION
EPR oximetry is a unique technology for directly measuring tissue oxygen continuously and noninvasively.

<table>
<thead>
<tr>
<th>Pre–Tourniquet Application</th>
<th>Tourniquet Applied</th>
<th>Tourniquet Released*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPR Oximetry, mm Hg</td>
<td>TcO₂, mm Hg</td>
<td>P Value</td>
</tr>
<tr>
<td>POD 0</td>
<td>63.9 ± 4.6</td>
<td>62.6 ± 5.9</td>
</tr>
<tr>
<td>POD 1</td>
<td>68.9 ± 11.5</td>
<td>64.7 ± 4.9</td>
</tr>
<tr>
<td>POD 2</td>
<td>68.4 ± 11.6</td>
<td>66.8 ± 5.0</td>
</tr>
<tr>
<td>POD 3</td>
<td>66.5 ± 8.1</td>
<td>64.8 ± 4.9</td>
</tr>
<tr>
<td>Mean</td>
<td>66.9 ± 8.9</td>
<td>64.7 ± 5.2</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
* A tourniquet was not applied on POD 0, and thus data in row 1 reflect measurements obtained after flap elevation.
EPR = electron paramagnetic resonance; NA = not applicable; POD = postoperative day; SD = standard deviation; TcO₂ = transcutaneous oximetry.
In this study, we showed that there was no significant difference in flap oxygen measurement accuracy between EPR oximetry and TcO₂ under both ischemic and nonischemic conditions. Despite measuring different parameters, values between EPR and TcO₂ were similar, which could be secondary to the thinness of the flap model utilized or proximity of the OxyChip or TcO₂ electrode to perforators. However, EPR oximetry offers several advantages. First, TcO₂ measures pO₂ at the skin surface through a warming process that causes hyperemia and increases oxygen diffusion, providing an estimate of arterial pO₂. A disadvantage is that the measurements rely on arterial pO₂ instead of tissue pO₂. Moreover, TcO₂ requires 20 minutes of calibration and tissue surface warming prior to obtaining measurements, whereas EPR oximetry consistently produces accurate readings in <50 seconds.

Due to ease of use and availability, the most commonly implemented methods of flap monitoring are often physical examination, bedside Doppler, and implantable Doppler, although none are without weaknesses. Physical examination is subjective, relies on provider experience, and with shift changes lends greater risk to slower flap ischemia identification. Bedside Doppler methods rely on ability of hospital staff to locate the pedicle of the flap reliably every 1 to 2 hours during the first 24 hours postoperatively, followed by every 4 hours for the next 24 to 48 hours, depending on surgeon protocol. Inexperienced staff may not be able to locate a pulse reliably or may inadvertently identify a pulse from an adjacent vessel. Moreover, as in physical examination, this method is burdensome to providers, who may be performing serial monitoring while caring for other patients. In the last 10 years, implantable Doppler has gained popularity, as this method allows for continuous monitoring and improvement in salvage rates and has been particularly useful for monitoring buried flaps. However, implantable Doppler devices may dislodge, malfunction, or injure an anastomosis upon removal. Furthermore, an increase in false-positive rates of vascular occlusion detection have been reported.

Lastly, other emerging noninvasive modalities for measuring oxygen such as photoacoustic imaging and paint-on bandages, though useful in the right situation, harbor inherent weaknesses. Both carry the disadvantage of relying on surrogate markers to measure tissue oxygen concentration, and although photoacoustic imaging is capable of performing measurements at a depth of several centimeters, the bandages lack the ability to produce measurements from deep tissue.

EPR oximetry circumvents these weaknesses by providing reliable and repeated oxygen measurements of tissue. Moreover, EPR oximetry has the capability of measuring deep tissue that cannot be directly observed, making it a potentially powerful tool for assessing viability of buried flaps. If effectively translated into a clinical setting, EPR oximetry has the potential to be utilized for measuring flap pO₂ analogous to pulse oximetry measuring arterial sO₂%. However, there are disadvantages of EPR oximetry that warrant discussion. First, EPR oximetry requires injection of an oxygen-sensing chip. Although the size of the chips used in this study was small, injection via needle insertion is required, and patients may be averse to needles and injection of a foreign body. Additionally, there is potential for chip migration, although the authors have not experienced this complication in either animal models or humans. After injection, the chip may remain implanted for long periods without adverse effects; however, should a patient request chip removal, excision would be required. Another disadvantage of EPR oximetry is that currently measurements require chip placement between magnets of a nonmobile machine that operates similarly to magnetic resonance imaging. Although a portable model is in development for bedside EPR oximetry, until this is a reality, flap monitoring using EPR in a clinical setting is not realistic.

In addition to being one step closer to clinical applicability, this study opens the door for EPR oximetry use in studying flap viability and interventions moving forward. EPR has already been shown to be effective in...
measuring wound response to treatments aimed at improving oxygenation.33,34 In addition, this technology could elucidate exactly how much ischemic insult various types of tissue may tolerate, as well as lay groundwork for studies to establish guidelines concerning when flap salvage is indicated. These advances may translate into flap-survival improvement and a reduction in patient morbidity and mortality.

CONCLUSION

There are currently no technologies on the market for directly measuring both superficial and deep tissue oxygen continuously, accurately, and noninvasively, although EPR oximetry is able to circumvent these challenges. Using a rat model, we have demonstrated that EPR oximetry has the potential to provide a viable and reliable modality for flap monitoring.

Acknowledgments

The authors thank the New England Otolaryngology Society and Dr. Jay Bucky.

BIBLIOGRAPHY

17. Post JM, Kuppusamy P. Hyperoxia oximetry as a therapeutic supplement for treatment of triple negative breast cancer. Front Oncol 2018;8:527.