Oxygen-Sensing Paint-On Bandage: Calibration of a Novel Approach in Tissue Perfusion Assessment

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Abstract

Background
Knowledge of tissue oxygenation status is fundamental in the prevention of postoperative flap failure. Recently, the authors introduced a novel oxygen-sensing paint-on bandage that incorporated an oxygen-sensing porphyrin with a commercially available liquid bandage matrix. In this study, the authors extend validation of their oxygen-sensing bandage by comparing it to the use of near-infrared tissue oximetry in addition to Clark electrode measurements.

Methods
The oxygen-sensing paint-on bandage was applied to the left hind limb in a rodent model. Simultaneously, a near-infrared imaging device and Clark electrode were attached to the right and left hind limbs, respectively. Tissue oxygenation was measured under normal, ischemic (aortic ligation), and reperfused conditions.

Results
On average, the oxygen-sensing paint-on bandage measured a decrease in transdermal oxygenation from 85.2 mmHg to 64.1 mmHg upon aortic ligation. The oxygen-sensing dye restored at 81.2 mmHg after unclamping. Responses in both control groups demonstrated a similar trend. Physiologic changes from normal to ischemic and reperfused conditions were statistically significantly different in all three techniques (p < 0.001).

Conclusions
The authors’ newly developed oxygen-sensing paint-on bandage exhibits a comparable trend in oxygenation recordings in a rat model similar to conventional oxygenation assessment techniques. This technique could potentially prove to be a valuable tool in the routine clinical management of flaps following free tissue transfer. Incorporating oxygen-sensing capabilities into a simple wound dressing material has the added benefit of providing both wound protection and constant wound oxygenation assessment.
Introduction

Knowledge of the oxygenation status of tissue and the early detection of vascular compromise are fundamental in the prevention of postoperative flap failure after microsurgical reconstruction. As the field of autologous tissue transfer has evolved, technology development has been mirrored by a substantial decrease in the number of flap losses. One of the major factors contributing to this finding has been the implementation of monitoring devices in the perioperative period. Although autologous free tissue transfer is considered a reliable procedure, large studies have demonstrated flap loss rates up to 5.6 percent.1–4 Because the likelihood of flap loss is higher within the first 24 to 48 hours after surgery, this time window is of crucial importance in the prevention of complete flap failure.5 Various noninvasive and invasive modalities have emerged as adjuncts to standard clinical monitoring to allow for early detection of flap compromise; however, no current method is ideal for use in all types of autologous tissue transfer.6,7

One technology is the use of near-infrared tissue oximetry (ViOptix T.Ox Tissue Oximeter; ViOptix, Inc., Fremont, Calif.) for monitoring of real-time flap perfusion, which has been used by a number of centers with encouraging outcomes.8–10 It currently serves as the standard of care for free flap perfusion monitoring in the postoperative period in our hospital.

Recently, we introduced a novel, noninvasive, sensitive, easy-to-use paint-on bandage that allows for the assessment of tissue perfusion based on a newly developed oxygen-sensing molecule incorporated into a commercially available liquid bandage matrix.11–13 The bandage is applied to the skin surface, and a camera-based imaging device is used to acquire images that can be processed to provide a two-dimensional oxygenation map of the tissue area covered by the bandage, and monitor changes in transdermal pO2. This technique potentially has its primary application in the monitoring of transplanted flaps and viability assessment of other human tissue. In this study, we extend validation of our oxygen-sensing bandage by comparing it to the use of near-infrared tissue oximetry, in addition to Clark electrode measurements, as one step closer to translation into a clinically applicable device.

Materials and Methods

Animals
Sprague-Dawley rats weighing 275 to 300 g were approved for use. The animal surgery protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the Beth Israel Deaconess Medical Center, and all procedures were performed at this animal facility.
Oxygen-Sensing Paint-On Bandage

The oxygen-sensing paint-on bandage used in this study is described in our previous work. Briefly, the paint-on bandage was formulated by mixing ethanol solutions of esterified Oxyphor R2 (4.2 mM) (Oxygen Enterprises, Ltd., Philadelphia, Pa.) and Coumarin 500 (10 mM) (Exciton, Dayton, Ohio) with the New-Skin liquid bandage (Prestige Brands, Tarrytown, N.Y.) at a volumetric ratio of 2:1:10. The breathable transparent film dressing Tegaderm (1622W; 3M, Saint Paul, Minn.) was used as the barrier layer covering the sensing bandage to reduce the oxygen exchange between the sensing bandage and room air (Fig. 1).

A custom camera equipped with blue light flash units was used to illuminate and then capture the emission from the sensing bandage. The green-channel image of the reference dye emission was captured with a 510/10-nm filter. The red-channel image was captured using a 700/70-nm filter with the camera delay time set to eliminate the skin autofluorescence background. Ratiometric images were constructed by plotting the intensity ratio between the red- and green-channel images, and a tissue oxygenation map was obtained by applying an intensity calibration to the ratiometric image.

![Figure 1. Oxygen-sensing paint-on bandage. (Above, left) Apply oxygen-sensing paint-on bandage as a liquid. (Above, center) Bandage solidifies into a breathable thin film. (Above, right) Apply barrier Tegaderm film to slow oxygen exchange with room air. (Below, left) Bandage glows green when tissue is well-perfused and (below, center) red when tissue oxygenation is compromised.](image-url)
**Surgical Protocol**

General anesthesia was induced with 3% isoflurane in 100% oxygen at a flow rate of 1 liter/minute and maintained by administration of 2% isoflurane at a 1-liter/minute flow rate. Mechanical ventilation was adjusted to ensure a stable partial pressure of oxygen of 60 to 90 mmHg. The rats were positioned supine and fixed in an X-shape configuration. The abdomen, thigh, and ventral side of the animal’s hind limb were shaved and prepared aseptically for surgery. A linear skin incision of 3 cm was made at the linea alba, the intra-abdominal organs were retracted, and the retroperitoneal cavity was opened to achieve full exposure of the descending aorta and vena cava. The infrarenal aorta and vena cava were circumferentially dissected for placement of the surgical vessel clamp later in the experiment.

Tissue oxygenation was measured on the left and right hind limbs under normal perfusion. In this experiment, we used two methods to serve as controls. A near-infrared imaging device (ViOptix T.Ox Tissue Oximeter) was attached to the ventral side of the right upper hind limb to assess soft-tissue oxygen saturation. Furthermore, a Clark electrode (Unisense, Aarhus, Denmark) was inserted into the gastrocnemius muscle of the left hind limb to monitor the real-time intramuscular oxygen tension (intramuscular oxygen tension). Figure 2 demonstrates an overview of the experimental setup.

After steady continuous baseline measurements were obtained, the oxygen-sensing bandage was applied onto the shaved skin superficial to the region monitored by the Clark electrode on the left hind limb. The bandage was allowed to air-dry for 1 minute and a transparent barrier layer was applied to slow the rate of oxygen exchange between room oxygen and the sensing bandage. Oxygen tension (tissue pO2) images were generated by calculating the intensity ratio between the red- and green-channel images as described previously.\footnote{11}

Equilibrium images were captured on the left hind limb for 20 minutes at a rate of one photograph per minute, and bilateral hind limb ischemia was induced by clamping the infrarenal descending aorta and vena cava bundle. Subsequently, photographs of the experimental oxygen-sensing bandage were repeated at a rate of 1 image per minute for 20 minutes in conjunction with continuous oxygen monitoring to assess tissue oxygen tension under increasing ischemic conditions. After 20 minutes of ischemia, the surgical clamp was removed and the imaging procedures were repeated for another 20 minutes. Three experimental trials were performed for each of the six rats used. After surgery, the animals were terminated. Tissue pO2 tension value at each time point was generated using the intensity calibration curve. After the bandage is applied, the bandage oxygen tension equilibrates with the tissue underneath in approximately 20 minutes. The equilibration curve
was fitted from the first 20-minute baseline measurement. The deviation of transdermal oxygen tension during aortic ligation and reperfusion was plotted by comparing the measured oxygen tension-time trace to the equilibration curve. Continuous recordings were made with both near-infrared tissue oximetry and Clark electrode.

![Figure 2](image.png)

**Figure 2.** Experimental setup and surgical procedures.

**Statistical Analysis**
All analyses were performed using IBM SPSS Version 22.0 (IBM Corp., Armonk, N.Y.) including GraphPad Prism Version 6.04 (GraphPad Software, Inc., San Diego, Calif.). Data are presented as mean, median, and standard deviation. The clinical response in tissue oxygenation to ischemia-induced conditions and subsequent reperfusion are compared between groups and presented in graphs. Continuous data were compared using unpaired t tests. A value of p < 0.05 was deemed significant.
Results

As demonstrated in Figure 3, the results could be divided into three regions. Region A represents the baseline tissue oxygenation in rats under normal physiologic conditions. Clamping the aorta resulted in a rapid drop in soft-tissue oxygen saturation, transdermal oxygen tension, and intra-muscular oxygen tension (region B), which stabilized at a plateau level. Unclamping resulted in a prompt increase in reperfusion and reoxygenation, which demonstrated an initial peak before stabilizing at the baseline level again (region C).

![Image](image-url)

*Figure 3.* (Above) Example of transdermal oxygen tension (pO2) maps calculated from oxygen-sensing bandage measurements (n = 1 rat). (Below) Three regions: (left) average baseline measurement, (center) after ligation of descending aorta, and (right) after removing clamp on descending aorta (n = 6 rats)

The mean referenced intramuscular oxygen tension baseline after 20 minutes of stabilization, recorded by the Clark electrode, was 78.2 ± 10.5 mmHg (median, 75.9 mmHg). Ligation of the aorta resulted in a significant decrease in intramuscular oxygen tension to a new mean baseline of 14.1 ± 16.5 mmHg (median, 3.2 mmHg; p < 0.001) (Fig. 4). Near-infrared tissue oximetry recordings of the soft-tissue oxygen saturation followed a comparable trend; baseline readings were 69.1 ± 14.1 percent (median, 67.5 percent), which significantly dropped to 44.9 ± 19.5 percent (median, 53.8 percent) after surgical ligation. On average, the oxygen-sensing paint-on bandage measured a decrease in transdermal pO2 from 85.2 ± 15.9 mmHg (median, 83.3 mmHg) to 64.1 ± 13.8 mmHg (median, 63.7 mmHg) upon clamping the
aorta, corresponding with a value of $p < 0.001$. In summary, surgical ligation resulted in an extremely significant decrease in registered oxygenation for all three modalities.

Unclamping the aorta showed a conversely analogue response. After an initial peak in oxygenation registration, Clark electrode measurements reached equilibrium at $73.7 \pm 15.2$ mmHg (median, 68.7 mmHg). Similarly, near-infrared tissue oximetry recordings reached a plateau phase at $64.9 \pm 14.7$ percent (median, 70.0 percent) after an initial peak. Finally, the oxygen-sensing molecule restored at $81.2 \pm 17.8$ mmHg (median, 82.7 mmHg). Physiologic changes from clamped to unclamped conditions were statistically significantly different in all three techniques ($p < 0.001$).

**Figure 4.** (Left) Mean data obtained from Clark electrode measurements ($n = 6$ rats). (Right) Mean data obtained from near-infrared (NIR) tissue oximetry ($n = 6$ rats)

**Discussion**

Survival of free flaps after microsurgical transplantation relies on the maintenance of adequate tissue perfusion and oxygenation. Notably, the time to detection of flap compromise has been demonstrated to be a significant predictor of flap salvage outcome. The assessment of tissue oxygenation is possible based on several principles, all with the intent of pursuing a noninvasive, accurate, and reliable means that is readily applicable to all types of flaps and easy to use. Near-infrared tissue oximetry is a noninvasive technology that allows continuous monitoring of real-time flap perfusion and measures scattering and absorption of calibrated wavelengths of near-infrared light, which is related to the oxygen content of hemoglobin within the monitored tissue. Advantages include its ease of use, reliability,
and sensitivity. The main drawback for implementation of tissue oximetry is still mainly related to costs.

Much research has been done to improve noninvasive tissue monitoring techniques. Detection of light reflected by tissues has been previously studied by Jeffcoate et al., who explored the use of hyperspectral imaging, based on light scattering, to predict healing in diabetic foot ulcers and demonstrated a positive association between oxygenation of hemoglobin assessed by hyperspectral imaging and time to healing, suggesting that it may prove to be a valuable tool in the prediction of ulcer healing.

Historically, handheld Doppler ultrasound, temperature probes, implantable Doppler probes, color duplex sonography, microdialysis, and near-infrared tissue oximetry have been implemented in the field of plastic surgery to monitor flaps. In this study, we describe a recently developed oxygen-sensing paint-on bandage that relies on phosphorescence-quenching–based detection of skin oxygenation. Among the currently known oxygen-responsive materials, metalloporphyrins have presented an interesting focus because of favorable structural versatility and photophysical properties that translate into valuable oxygen-sensing characteristics. In this study, we show that the oxygen-sensing probe within the bandage follows a comparable pattern in the detection of tissue oxygenation to the use of near-infra-red tissue oximetry, a current technique in flap perfusion assessment at our institution. Surgical ligation of the aorta led to a rapid decrease in measured skin oxygen in a fashion similar to that of near-infrared imaging. Within minutes, the oxygen-sensing bandage was able to detect inadequate tissue perfusion, indicating a clinically relevant response to detect flap compromise. In fact, the oxygen-sensing liquid bandage decreased even more rapidly after clamping compared with the near-infrared imaging device. This explorative study demonstrates the potential of paint-on films and bandages based on real-time sensing to identify perfusion compromise at an early stage. Restoration of blood flow to the extremities resulted in a steep increase in oxygen registration, again replicating the pattern of the near-infrared imaging recordings.

In addition to near-infrared imaging, we conducted conventional oxygen tension measurements using a Clark electrode. A Clark electrode oxygen probe sensor for electrochemical measurements is based on polarography: a typical amperometric transducer in which the rate of a chemical reaction is detected by the current drained through an electrode. It measures net tissue oxygen tension and has found many clinical applications in monitoring oxygen tension (e.g., in newborns). If tissue perfusion decreases, for example, while partial pressure of oxygen remains constant, cutaneous oxygen tension will decrease, therefore linking peripheral perfusion and tissue oxygenation. In this study, the intramuscular oxygen tension measured by the Clark electrode under normoxic and ischemic
conditions were consistent with the previously reported values measured by polarographic electrodes, suggesting accurate and reliable monitoring of tissue oxygenation by the oxygen-sensing bandage.29,30

Detection of decreased oxygenation by the oxygen-sensing bandage and near-infrared imaging was delayed by a few minutes compared with intramuscular surveillance of oxygen tension using the Clark electrode. This is likely because of the slower oxygen consumption by the skin compared with the muscular tissue where the Clark electrode is inserted. Therefore, these traces are not expected to overlap at every time point. However, the consistent patterns of change identified with each of the studied modalities are of particular importance. Although one might suggest that these methods assess different aspects of microvascular function and cannot be directly compared, this is overcome by the presentation of steady iterations in microvascular flow in terms of change from the baseline values. Although differences are less apparent on analysis of absolute values, the consistency of the changes we observed between these distinct measures of tissue perfusion strongly suggest that these findings are robust.

This study is subject to several limitations. In general, temperature and skin quality (e.g., in irradiated skin and after prior nicotine exposure) are important factors in skin oxygenation. Increased skin temperature is known to lead to a shift of the oxygen dissociation curve, which results in decreased oxygen solubility. The oxygen-sensing bandage was applied to skin and therefore subject to vasomotor response of skin blood vessels that can potentially interfere with oxygen measurements. Near-infrared imaging might be more sensitive to temperature because of the change in oxygen dissociation curves. Vasomotor response in the skin can somewhat decouple the skin’s oxygen tension to that of deeper tissue layers; thus, any transcutaneous measurement such as the oxygen-sensing bandage will be potentially affected by change in temperature.

Despite these limitations, the oxygen-sensing liquid bandage is composed of a U.S. Food and Drug Administration–approved wound dressing known to be biocompatible, thereby providing an excellent clinical basis for future clinical trials and use. The system can not only predict localized tissue necrosis but actually detect necrosis by monitoring the oxygen consumption rate of the tissue in contact. Previously, we have demonstrated the bandage’s ability to visualize tissue necrosis in burn wounds.11 Future clinical applications could include burn depth determination, amputation level determination, chronic ischemic wounds monitoring, skin-based monitoring after composite allotransplantation, assessment after vascular injury, and others.
The barrier film is required in the current application, as it helps to slow oxygen exchange with room air so that the sensing bandage is responsive to the oxygenation from the tissue. The bandage is applied as a liquid, so its application is not restricted to a flat surface, and can be used on complex tissue topology. No wiring is needed between the bandage and the readout device, as it is “hands-off.” Another advantage over near-infrared oximetry is that instead of point measurements, the bandage is able to provide two-dimensional, real-time maps of large tissue regions. With optimized illumination intensity and time interval, long term recording of tissue oxygenation maps can be achieved without causing significant saturation or photobleaching of the sensor dye within the bandage.

The camera used in this study was a monochromic camera, equipped with bandpass filters (green or red) that selectively pass emitted light with the color of interest. Therefore, the brightness of the black-and-white images can be directly used for analysis. It should be noted that a camera with color chips can be used as well; any image-processing software that separates red, green, and blue image components can be used for quantitative analysis. In our previous work, we have demonstrated that the color change of the sensor molecules can be captured using a consumer digital single-lens reflex camera, or even smartphone cameras, rendering this a feasible technique for clinicians in their future practice. Because of its potential to be engineered for use on smartphone cameras, it has the benefit of telemedical tissue perfusion screening, obviating the need for in-hospital assessment.

The authors do not anticipate cost to be a barrier in translating this technology, as the material cost per bandage should be less expensive than existing tools. In addition, the readout device can be manufactured at the cost of a standard “point-and-shoot” consumer camera on product engineering. The commercially available bandage can be worn for up to 1 week, which is longer than the time required for the proposed use case. One potential barrier of the current iteration, however, is that the sensing bandage requires approximately 20 minutes’ equilibration time on application, which may not be suitable for certain acute clinical applications. Further engineering can reduce this equilibration time if found problematic in clinical settings.

Newly developed oxygen-sensing phosphors also have promise to markedly improve the performance and ease of use of these bandages. This recent study led to the creation of four new oxygen-sensing porphyrins, two of which have strong visible emission that can be seen with the naked eye in sunlit rooms. The incorporation of these new oxygen-sensing molecules into the bandage will greatly improve signal-to-noise ratios and sensitivity, and will allow for further simplification of the camera equipment, or ultimately the assessment of trans-dermal pO2 with naked eye by comparing the bandage emission to a calibrated color scale.
Conclusions

Analysis of tissue oxygenation using the oxygen-sensing paint-on bandage is simple and non-invasive and could therefore become a valuable tool in the routine clinical management of flaps following free tissue transfer. Incorporating oxygen-sensing capabilities into a simple U.S. Food and Drug Administration–approved wound dressing material has the added benefit of providing both wound protection and constant wound oxygenation assessment. The oxygen-sensing bandage demonstrates a comparable trend in oxygenation recordings similar to conventional oxygenation assessment methods. This finding potentially provides an excellent base for future research toward optimizing detection of impending free flap compromise and improving clinical outcome following reconstructive procedures.
References


