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Early detection of necrotizing enterocolitis using broadband optical spectroscopy

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ABSTRACT

Purpose: The definitive diagnosis of necrotizing enterocolitis (NEC) is typically at an advanced stage, indicating the need for sensitive and noninvasive diagnostic modalities. Near infrared spectroscopy (NIRS) has been utilized to noninvasively measure intraabdominal tissue oxygenation and to diagnose NEC, but specificity is lacking, in part because sensors are limited to a narrow band of the electromagnetic spectrum. Here, we introduce the concept of broadband optical spectroscopy (BOS) as a noninvasive method to characterize NEC.
Methods: NEC was induced in 7-day old mice by gavage feeding with formula supplemented with enteric bacteria plus hypoxia. Transabdominal spectroscopy was performed daily using a broad-spectrum halogen light source coupled with a spectroradiometer capable of detection from 400 to 1800 nm.
Results: A feature extraction algorithm was developed based on the spectral waveforms from mice with NEC. When subsequently tested on cohorts of diseased and control mice by a blinded examiner, noninvasive BOS was able to detect disease with 100% specificity and sensitivity.
Conclusions: We reveal that the use of BOS is able to accurately and noninvasively discriminate the presence of NEC in a mouse model, thus introducing a noninvasive early diagnostic modality for this devastating disease.

Necrotizing Enterocolitis (NEC) is among the most devastating of neonatal diseases, [1] with mortality rates as high as 30% with modern treatment. [2] The pathophysiology of NEC is not fully understood, while its clinical presentation is varied and nonspecific, making early diagnosis challenging. Previous approaches to NEC diagnosis have included risk factor identification, biomarkers, and noninvasive imaging such as ultrasound or near-infrared spectroscopy (NIRS). [3] The use of NIRS in this context typically refers to commercially available oximeters that detect light absorbance or reflectance in 2–5 wavelengths, generally between 700 and 850 nm, where there is minimal overlap of the absorption spectra of oxygenated and deoxygenated hemoglobin. [4] Though this approach is useful to measure tissue oxygen saturation, it has not yet been demonstrated sufficiently sensitive or specific for NEC to gain traction in clinical practice.

In this study we introduce the concept of broadband optical spectroscopy (BOS) as a diagnostic tool for NEC, demonstrated in a murine model. Utilizing a spectroradiometer capable of detection of wavelengths ranging from 400 to 1800 nm with 1 nm resolution, we have constructed a system that, in addition to measuring tissue oxygenation, captures other potential biologic chromophores present during disease progression. The breadth and increased granularity of this methodology require advanced machine-learning statistics to decipher, but it has obvious advantages over commercial NIRS probes.

1. Methods

All animal experiments were approved by an institutional animal care and use committee (Johns Hopkins ACUC MO14M362). As initial validation for the use of BOS for intraabdominal disease, a cohort of mice with dextran sulfate sodium (DSS) induced enteritis, which is a well-established model of intestinal inflammation, [5] was studied against normal controls. 4-week-old C57BL/6 mice were administered 4% ad libitum in drinking water for 9 days, and disease progression was assessed by recording body weight, stool consistency and the presence of fecal occult blood. Severity of the disease was confirmed by gross examination of colon at necropsy, with measurement of colon length. After establishment of BOS measurement protocols and techniques, NEC was induced in 7-day old C57BL/6 mice by gavage feeding with formula supplemented with enteric bacteria plus daily induction of transient hypoxia (5% oxygen, 10 min). Finally, a model

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of infectious sepsis was created to evaluate the specificity of BOS for NEC. A cecal slurry was created by harvesting the cecal contents of a sacrificed adult mouse and preparing an injectable solution according to a previously validated protocol. [6] This slurry was intraperitoneally injected into breastfed age-matched mice of the same strain, and BOS measurements were subsequently obtained.

Transabdominal spectroscopy was performed using a broad-spectrum halogen light source coupled with a spectroradiometer (Labspec 5000; ASD Inc.; Boulder, CO) capable of detection with 1 nm resolution from 400 to 1800 nm with a 100 ms acquisition time. A fiberoptic cable comprising channels for light source and detection was fashioned into a small-aperture probe and connected to the light source and detector (Fig. 1). This probe was gently placed onto the mouse abdomen in multiple locations for data acquisition (Fig. 2). Measurements were performed by an investigator blinded to the presence or absence of disease. At the completion of the multiday experiment or as clinical condition required, the mice were euthanized and disease severity was assessed with histologic confirmation.

Data were analyzed using linear discriminant analysis (LDA) to classify features of the reflectance waveforms. LDA plots all features of a spectrum in multidimensional space and subsequently tests and assigns a threshold that permits the most accurate distinction between groups of data. All data processing and analysis were conducting offline with algorithms developed using the Matlab 2016a platform (Mathworks Inc.; Natick, MA). Given the raw spectral signals with assigned class labels of breastfed, NEC, or sepsis; our analysis transformed spectra from a training dataset of advanced NEC versus normal into a single feature that allowed for the greatest degree of separation between the two classes. A feature value threshold was then assigned to generate a classifier for prospective day-by-day testing. For comparison of multiple groups such as NEC versus breastfed versus infectious sepsis, characteristics of the spectra in the near infrared (700–1100 nm) and shortwave infrared (>1100 nm) were analyzed separately. Nonparametric distributions were compared for statistical significance with a Mann–Whitney U test.

2. Results

Measurements of 7 mice with DSS-colitis and 7 breastfed controls were taken at multiple daily intervals. Signal changes were detected using LDA feature extraction as early as 3 days after initiation of the experiment, concordant with the presence of fecal occult blood and days earlier than the onset of other clinical symptoms. Clinical symptoms of severe colitis were uniformly evident by 9 days with grossly bloody stools, diarrhea and significant weight loss compared to age-matched healthy controls (DSS final weight 12.8 ± 1.2 g vs controls 25.7 ± 1.4 g). Classification of spectral waveforms documented progressive alteration of tissue reflectance and could reliably differentiate disease on day 9 from lack of disease on day 1 with 100% sensitivity and specificity (Fig. 3). At necropsy, the colon of the diseased mice was significantly diseased as well as decreased in length compared to controls (DSS length 3.6 ± 0.5 cm vs 8.8 ± 0.2 cm).

Spectra were then obtained from 20 mice with advanced NEC (Day 5 of disease induction, immediately prior to sacrifice) and 20 age-matched breastfed controls. A representative sample is graphed in Fig. 4. BOS demonstrated predictable patterns among mice in each respective group, and the spectra were grossly different between control and diseased mice.

An LDA feature extraction algorithm was developed based on the spectral waveforms from mice with known NEC compared to breastfed. Following development of the LDA feature, when subsequently tested on cohorts of 35 diseased and 25 control mice by a blinded examiner, the mean (SD) unitless LDA value was −0.0068 (0.0026) for NEC mice, compared to 0.0117 (0.0066) in controls (Fig. 5). There was no overlap in values, allowing detection of disease with 100% specificity and sensitivity.

On a separate cohort of 14 mice undergoing induction of NEC, BOS measurements were obtained daily and the unitless LDA feature was calculated for each subject (Fig. 6). Statistically significant changes from baseline were first noted on Day 3, at which time the mice were noted to be hypoactive but not yet lethargic or otherwise clinically ill.

A cohort of mice with infectious abdominal sepsis was created via intraperitoneal injection of cecal slurry as described above. BOS readings were taken 48 h after injection and compared to previously unstudied groups of NEC and breastfed control. By plotting a unitless feature representing spectral traits in the known regions of blood proteins and water absorption, spectra from the mice with NEC were able to be grossly discriminated from both breastfed and sepsis groups (Fig. 7).

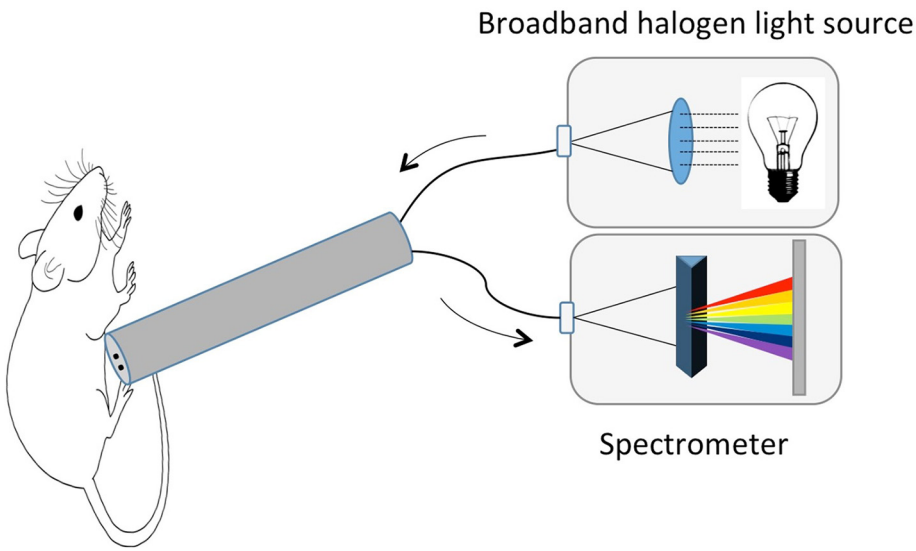

Fig. 1. Depiction of hardware setup: a fiberoptic probe was constructed with channels to transmit a broadband halogen light source as well as detect reflected spectra via a spectroradiometer.