A compact fluorescence sensor for low-cost non-invasive monitoring of gut permeability in undernutrition

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ABSTRACT

Undernutrition is associated with approximately 45% of deaths among children under the age of 5. Furthermore, in 2020, around 149 million children suffered impaired physical/cognitive development due to lack of adequate nutrition. Environmental enteropathy (EE) is associated with undernutrition and is characterized by a multifaceted breakdown in gut function, including an increase in intestinal permeability that can lead to inflammatory responses. However, the role and mechanisms associated with EE (particularly gut permeability) are not well understood. This is partly because current techniques to assess changes in gut permeability, such as endoscopic biopsies, histopathology and chemical tests such as Lactulose:Mannitol assays, are either highly invasive, unreliable or difficult to perform on specific groups of patients (such as infants and patients with urine retention problems). Therefore, low-cost, non-invasive and reliable diagnostic tools are urgently needed for better evaluation of intestinal permeability. Here, we present a compact transcutaneous fluorescence spectroscopy sensor for non-invasive evaluation of gut permeability and report the first in vivo data collected from volunteers in an undernutrition trial. Using this technique and device, fluorescence signals are detected transcutaneously after oral ingestion of a fluorescent solution. Preliminary results demonstrate the potential use of the presented sensor for clinical assessment of gut permeability in low-income settings.

1. INTRODUCTION

As reported by the World Health Organization (WHO), undernutrition is associated with approximately 45% of deaths among children under the age of 5. In addition, in 2020, around 149 million children under the age of 5 were estimated to be stunted, a condition linked to lack of nutrition. Undernutrition is associated with a condition called Environmental Enteropathy (EE), which is characterized by complex changes in gut function including inflammatory responses (both gut-specific and systemic) and an increase in intestinal permeability (i.e., a breakdown in gut barrier function). Moreover, increases in gut permeability have also been linked to other gastrointestinal (GI) conditions – including coeliac disease, Crohn's disease and inflammatory bowel disease (IBD) – as well as conditions outside the GI tract such as Parkinson's disease and HIV [1-3].

Current techniques to assess gut permeability include endoscopic biopsies, histopathology and chemical tests such as Lactulose:Mannitol (L:M) assays. However, these techniques are either highly invasive, unreliable and/or difficult to perform on specific groups of patients (such as infants and patients with urine retention problems) [4]. Furthermore, the specialized facilities required to perform the above-mentioned techniques and to analyze the collected samples are often unavailable in low- and middle-income countries (LMICs). As a result, improved diagnostic tools that provide non-invasive and reliable measurements of intestinal permeability (and other aspects of gut function) have the potential to introduce considerable clinical benefits [5, 6].

Recent studies both in humans and animals have demonstrated the potential use of transcutaneous fluorescence spectroscopy, together with oral ingestion of fluorescent contrast agents, to non-invasively provide information about GI function, including gut permeability. This approach uses a spectroscopic probe/sensor to non-invasively measure the permeation of an orally administered fluorescent contrast agent from the gut into the bloodstream, thereby facilitating measurements of gut permeability (and other clinically relevant GI functions) [7-10]. However, the use of expensive benchtop systems (e.g. like the system reported in [11]) introduce limitations in certain populations (such as in children and in LMICs) and may be unsuitable for large-scale clinical deployment. Therefore, developing a compact, low-cost technology is vital to satisfy the demand to non-invasively assess gut permeability in undernutrition.

To address the limitations discussed above, we have developed an optical sensor that implements the fluorescence spectroscopy technique presented in previous studies (e.g. [11]) in a compact, low-cost format. The device is capable of detecting fluorescence signals emitted from an orally ingested contrast agent (fluorescein) as it permeates from the gut into the bloodstream. The size and cost of this device were designed in order to enable deployment in low-income settings. Here, we report the first data collected from volunteers in an undernutrition trial. Preliminary results from measurements in 4 participants indicate the potential of this technology for non-invasive assessment of gut permeability in undernutrition.

2. METHODS

Compact transcutaneous fluorescence sensor

In this paper, we present the first data collected in a clinical trial of undernutrition using a compact, low-cost fluorescence spectroscopy sensor. The approach consists of the oral administration of a fluorescent solution containing a clinically approved contrast agent (fluorescein) and the transcutaneous assessment of the dye permeation from the gut into the bloodstream. The fluorescence signal is non-invasively measured on the fingertip of the participant using a custom fiber-optic probe, thereby facilitating measurements of gut permeability. Figure 1A shows the setup of the transcutaneous sensor in a clinical environment.

Figure 1B shows the developed transcutaneous fluorescence sensor, which comprises one light source (LED with peak intensity at 465 nm) for excitation of the fluorescent contrast agent (fluorescein) and two photodiodes (sensitivity range: 350–1000 nm) to detect the fluorescence signal and the backscattered LED signal respectively. The use of a second photodiode to measure the backscattered LED signal allows for correction of the fluorescence intensity for variations due to the probe location, excitation power, skin tone, etc. The LED and photodiodes are coupled into a custom, trifurcated fiber-optic probe to permit measurement of the fluorescence signals at the fingertip (or other location on the body of the participant).

The fiber probe is attached to the participant's fingertip using a 3D-printed finger clip (Figure 1A and 1B). A custom written LabView interface (Figure 1C) controls the light source power and duty cycle (to keep the optical power and LED temperature constant and safe) as well as the collection and pre-processing of data (allowing automated data collection and analysis). The user-friendly interface displays the raw data from the sensor and the pre-processed fluorescence signal in real time, allowing the clinician/user to assess/interpret the data from the sensor without any additional processing steps (i.e. unlike L:M assays where samples must be sent to a laboratory for analysis).

Data collection

To assess the potential of transcutaneous spectroscopy for non-invasive monitoring of gut permeability in undernutrition, we are currently deploying the sensor presented in Figure 1 in a clinical trial in St Augustine clinic (Lusaka, Zambia). In our first clinical measurements, 4 volunteers (2 healthy, 2 undernutrition) were recruited. All volunteers gave informed consent prior to the experiments. Ethics approval for the clinical study was obtained for the University of Zambia Biomedical Research Ethics Committee, 2291-2021, dated 20th December 2021, and the clinical protocol was conducted in full concordance with Good Clinical Practice and the Declaration of Helsinki. For all participants, the sensor was attached to the forefinger of the participant before the experiments began. The fluorescence measurements started at the moment the participants were asked to begin drinking an aqueous fluorescein solution. To investigate both the effect of undernutrition and the optimal experimental protocol, in these preliminary experiments healthy volunteers ingested a solution containing 100mg of fluorescein diluted in 100ml of water whereas undernutrition volunteers ingested a solution containing 200mg of fluorescein diluted in 100ml of water. For healthy volunteers, fluorescence signals were collected until 10 minutes after the maximum value was detected in the data. For undernutrition patients, data was collected for 4

hours (as data was collected alongside other tests that required longer acquisitions). Following signal collection, the fluorescence data were plotted as functions of time (fluorescence vs. time curves) to allow comparison of the four datasets.

Figure 1. Compact, low-cost transcutaneous fluorescence sensor. (**A**) Clinical setup of the portable fluorescence sensor (main components indicated by annotations). The laptop contains the LabView interface developed to control and collect data from the sensor. Laptop and sensor are both easily accessible to the clinician/user. The participant is sat with the arm rested in a comfortable position. The 3D printed finger clip is attached to the forefinger of the participant. When the experiment starts, the participant is asked to drink the fluorescent solution (shown in the image). (**B**) Photograph of the developed fluorescence sensor and the 3D printed finger clip used to collect the fluorescence signals presented in this article. The total diameter of the tip of the fiber probe is 2.1mm. (**C**) LabView interface developed to control the power and duty cycle of the light source, and to collect and pre-process the fluorescence signal. The raw data collected from the sensor and the pre-processed data are displayed in real time as the sensor is running, allowing the clinician/user to briefly analyze/interpret the data without the need for any additional processing steps. Additional options (such as the buttons “Drink” and “Custom Event”) are also available to the clinician/user to record the time at which any event of interest happens.

3. RESULTS

The fluorescence vs. time curves recorded in all four participants are presented in Figure 2. As expected, fluorescence intensities increased over time in all participants, reaching peak values within approximately 30 to 60 minutes, before decreasing back towards zero. This represents the permeation of the fluorescent dye from the gut into the blood stream followed by the elimination of the contrast agent from the body. The maximum observed fluorescence values are in the range 0.4-0.5 (normalized units) for the healthy volunteers and 1.1-1.2 for the undernutrition patients. These differences can be attributed to the different fluorescein doses (100 mg vs. 200 mg) used during the experiment and potentially to differences in intestinal permeability (as the maximum values observed in the undernutrition patients are more than 2x higher than those in the healthy volunteers). While the small number of volunteers means that quantitative conclusions cannot be drawn at this stage, this nonetheless demonstrates the potential of this technique for non-invasive assessment of intestinal permeability in low-resource settings.

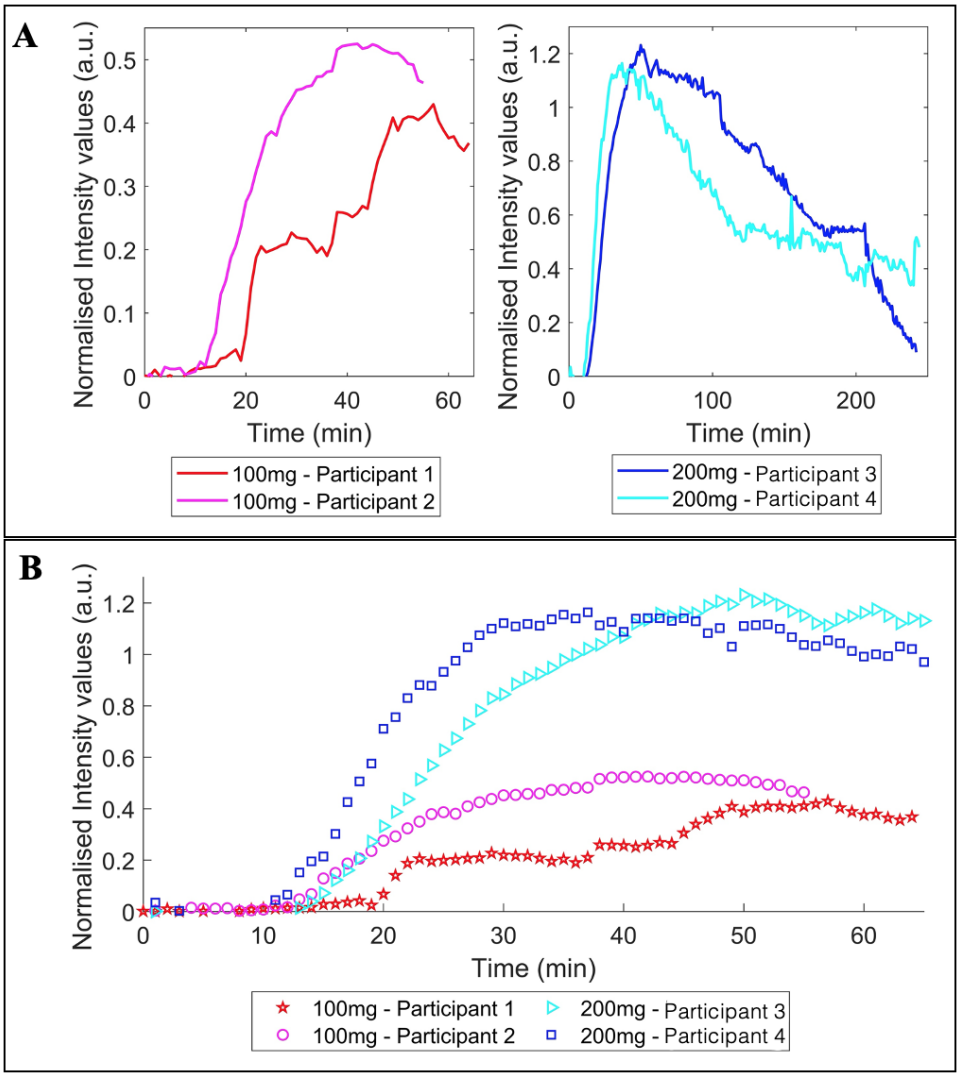

Figure 2. Fluorescence intensity vs. time curves collected using the compact fluorescence sensor for different fluorescein doses and participant types (healthy vs. undernutrition). (A) Left – signals collected in healthy volunteers (Participant 1 and 2, 100mg of fluorescein in 100ml of water); Right – signals collected in undernutrition patients (Participant 3 and 4, 200mg of fluorescein in 100ml of water). (B) Fluorescence vs. time curves for all participants showing the first hour after fluorescein ingestion. Approximately 1 hour of data is displayed to facilitate comparison across all datasets (as this is the minimum time for which data was collected in all participants).

Importantly, these results also demonstrate the ability to detect low doses of fluorescein (down to 100 mg) with good signal to-noise-ratio (SNR) even in healthy participants. Fluorescein is a clinically approved fluorescent contrast agent with a standard dose of 500 mg [12]. However, fluorescein can cause a number of adverse reactions including nausea, vomiting and (in very rare cases) anaphylaxis [13]. As such, it is beneficial to minimize the fluorescein dose where possible (both for use in transcutaneous spectroscopy and for any other purpose/procedure). The strong SNR observed at doses of both 100 mg and 200 mg (see Figure 2) demonstrate that transcutaneous fluorescence spectroscopy can be performed using fluorescein doses of 100 mg or lower.

4. CONCLUSIONS

In conclusion, the present study shows that fluorescence signals from an orally ingested fluorescent contrast agent (fluorescein) can be measured using a compact, fiber-optic, transcutaneous fluorescence sensor. Furthermore, our results demonstrate the capability to detect fluorescein doses as low as 100 mg and indicate the potential of this technology to assess gut function in low-income settings. Future work will now involve collection and analysis of data from a larger cohort of volunteers (both healthy volunteers and undernutrition patients) to allow more quantitative interpretation of the fluorescence signals.

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