Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2017 ; 10054: . doi:10.1117/12.2252295.

Novel diffuse optics system for continuous tissue viability monitoring – extended recovery *in vivo* testing in a porcine flap model

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Abstract

In reconstructive surgery, tissue perfusion/vessel patency is critical to the success of microvascular free tissue flaps. Early detection of flap failure secondary to compromise of vascular perfusion would significantly increase the chances of flap salvage. We have developed a compact, clinically-compatible monitoring system to enable automated, minimally-invasive, continuous, and quantitative assessment of flap viability/perfusion. We tested the system's continuous monitoring capability during extended non-recovery surgery using an *in vivo* porcine free flap model. Initial results indicated that the system could assess flap viability/perfusion in a quantitative and continuous manner. With proven performance, the compact form constructed with cost-effective components would make this system suitable for clinical translation

Keywords

diffuse correlation spectroscopy; diffuse reflectance spectroscopy; tissue flap monitoring; blood flow; tissue oxygenation; hemoglobin concentration

1. INTRODUCTION

In reconstructive surgery, tissue perfusion is critical to the success of flaps created via free tissue transfer. In case of impending failure (i.e., compromise in tissue perfusion), implementing earlier detection of any vascular compromise would significantly increase the chances of flap salvage. Current methods, which rely on infrequent and often subjective assessments such as flap color, temperature, and refill/reperfusion rate, do not address the

need for continuous objective monitoring and cannot assess instantaneous detection of failure.

Here we present a compact, low-cost, clinically-compatible monitoring system capable of automated, minimally-invasive, continuous, and quantitative assessment of tissue blood flow, hemoglobin concentration and tissue oxygenation. The system employs diffuse correlation spectroscopy (DCS) and diffuse reflectance spectroscopy (DRS) for tissue sensing via fiber-optic patches for light delivery and collection, and software for continuous monitoring. DCS is based on coherence-based technique, measuring intensity fluctuation induced by moving particles inside a medium [1, 2]. DCS has been employed as a non-invasive method to assess blood flow in brain, muscle and bone [3–5]. DRS is a well-established technique to analyze reflectance spectra determined by tissue scattering and absorption properties altering upon tissue morphology and physiology [1]. Due to its potential for clinical translation, DRS has been widely employed in many clinical applications [6–8]. Coupled with analytical models, combination of DCS and DRS can provide quantitative metrics relevant to tissue perfusion.

Using this system, we report the results of continuous microvascular free flap monitoring during an extended non-recovery surgery (for 5 monitoring hours after anastomosis) using an *in vivo* porcine flap model.

2. METHODS

2.1 Instrumentation

We have designed and developed a compact instrument with two DCS channels and one DRS channel (Figures 1 and 2). DCS is an optical technique assessing a relative blood flow (BF) change in a microvasculature. It detects speckle fluctuation in a scattered light induced by red blood cells inside the vessel. Due to its capability to sense a BF change in microvessels, DCS has been investigated as an alternative to the conventional Doppler flowmeter system. A detailed description of the instrument is noted in [9]. Briefly, DCS consists of an NIR (785 nm) diode laser, avalanche photodiode (APD) for single photon detection and a correlator. For cost-effective construction of the device, DCS employs an inexpensive laser diode (LD) with a long coherence length to cover a sample volume. Instead of the commercialized hardware correlator, the correlator is implemented using a custom PSoc time-tagger and USB logger [10]. DRS uses a white LED as a broadband source and a miniaturized spectrometer (Avantes) to acquire a reflectance spectrum (450 ~ 650 nm). The custom software written in C# controls a sequence of light sources (two LDs and one LED), and acquires, displays and saves DCS correlation (it can process up to four channels of DCS detection) and DRS spectrum data. Autocorrelation functions are calculated in the control software, further reducing hardware cost. The ultimate goal of the instrument design was to minimize size, weight, cost and complexity while enabling control via both an express and an expert software mode.

The heart of the system is a low-cost programmable system on a chip (PSOC) consisting of a microprocessor and a field programmable gate array (FPGA).

2.2 Instrument Construction

The DCS instrumentation consists of a coherent laser source, emission and collection fibers, a photon counter, and an autocorrelator. An LD785-SH300 785 nm laser diode (Thorlabs, Newton, NJ) is focused into a 200 μm multimode fiber optic which is positioned normal to the skin and held in an epoxy chuck surrounded by flexible PDMS base. The laser is run at a constant current and controlled by a WLD 3343 Laser driver (Wavelength Electronics, Bozeman, MT). Photons diffuse through the tissue and are reflected into the collection fiber. Time-dependent constructive and destructive interference based on the scattering and absorption of moving objects in the photon path (red blood cells) will change the intensity over time of the arriving photon stream at the collection fiber. A single mode collection fiber (780HP Thorlabs) is held in the epoxy at a fixed distance of 1mm, 2mm, 5mm, or 1 cm, depending on the experiment. The collection fiber is coupled to a single photon counting module such as Excelitas SPCM-AQRH. The pulses generated by the SPCM are time-tagged and converted to an autocorrelation function in a custom microprocessor-based correlator. Both the raw pulse train and the autocorrelation function are recorded to hard disk. The raw data can later be reprocessed at alternate integration times or timing resolutions via software.

Our most recent instrument design consists of a two channel DCS and single channel DRS system as seen in Figure 2. Each laser has a separate laser driver PCB attached to a signal bus in a compact board stack in the center back of the box. The signal bus brings power, ground and control signals for the laser drivers. The laser drivers are controlled by logic and DAC signals on the PSOC via the software interface. Two SPCM modules detect the light pulses from the collection fibers in the external probes. Beneath the laser drivers is the PSOC board that processes both data channels simultaneously. Data is transferred from the PSOC memory via USB, which has a limit of near 500 kB/s on the PSOC, due to the transfer method used. The instrument contains both infinite software control of laser current as well as a filter wheel to provide orders of magnitude attenuation of laser light output.

The SPCM or photon detector signals are fed into an autocorrelator. The autocorrelator consists of an analog conditioning circuit, a Cypress PSOC 5 based time tagging circuit, and a software correlator. The PSOC 5 is a combination FPGA and microprocessor that contains many standardized communication and calculation blocks making rapid prototyping possible. The analog input stage consists of a pulse detector, or comparator, and a pulse stretching circuit that stretched the initial 25 ns SPCM signal to 250 ns. A block diagram of the PSOC operating code is shown in Figure 3. Further details on the construction, design and operation of the DCS instrument is provided in [10].

The final dimensions of the box are 20 cm $\times$ 20 cm $\times$ 6 cm with an external power supply. The instrument also includes an Avantes Minispec spectrometer and a white LED source for performing diffuse reflectance spectrometry measurements (DRS).

2.3 Animal procedure

All animal experiments were performed in accordance with the University of Michigan Institutional Animal Care and Use Committee (IACUC) guidelines and approval. Pigs were anesthetized using Telazol, followed by a tracheostomy used to establish a secure airway. A

neck vein was accessed and an IV established. Animals were maintained on isoflurane general anesthesia and monitored for the duration of the experiment. A latissimus dorsi (LD) myocutaneous flap was raised using standard surgical procedures. The arterial and venous supplies were traced back to a single artery and vein. Following isolation of vessels, the artery and vein were transected and then anastomosed using standard microsurgical techniques. The first attempt was successful in all cases. The monitoring probes were then applied and sutured into place. Animals were continuously monitored for five hours at which time, venous congestion was simulated by tying off the vein for 30 minutes with continuous monitoring. After allowing for recovery, arterial compromise was simulated with arterial clamping after which the experiment was complete.

2.4 Data acquisition

After anastomosis, the skin patch was fixed onto the skin side of the raised flap [11]. The skin patch enabled monitoring of one DRS channel and one DCS channel. (Figure 5) The distance between source and detection fiber was 1.2 mm for DRS and 5 mm for DCS. For continuous flap recovery monitoring, DCS and DRS data was acquired every five minutes for five hours. In all cases the surgeon did not find any signs of flap failure such as poor capillary refill or “dusky” color indicative of venous compromised, during the 5-hour monitoring period. The vein was then tied securely to simulate a venous failure which is known as the most common type of complication during a free tissue transfer. After venous occlusion, the 30-minute monitoring was initiated, in which both DCS and DRS data was acquired every 30 seconds. After recovery from venous occlusion an arterial occlusion was completed and DCS/DRS monitoring was continued for 5 minutes until study end.

2.5 Data analysis

For blood flow index, the autocorrelation plots calculated by the custom software using multi-tau approach [10] were analyzed by a simple algorithm to obtain a tau half value. Analysis using complex fitting algorithms incorporating the photon diffusion equations to arrive upon blood flow index (BFI) is not incorporated in real-time. For this experiment, the tau half value was assigned by first normalizing the correlation plot, and then set to $\frac{1}{2}$ the time at which the integrated area above the curve equals that below. If that method fails due to noise or fast correlation curves without a normalizable “shelf”, the data is boxcar-averaged, and a three-consecutive point minimum is found in the derivative. If three consecutive points cannot be found, the absolute minimum is used.

To estimate total hemoglobin concentration and tissue oxygen saturation, a Monte Carlo lookup table (MCLUT)-based inverse method [12, 13] was employed to fit the measured reflectance spectra. Technical details on algorithm implementation are described in [9], and phantom verification of the method is found in [14]

3. RESULTS

3.1 5-hour monitoring

During 5-hour monitoring, the surgeons did not find any signs of flap failure from their physical exams, which confirmed successful anastomosis, resulting in normal tissue

perfusion. DCS and DRS data were acquired without any technical issues and are presented in Figure 6. The calculated autocorrelation plots from DCS shows $g_2(\tau)$ drops to half from the peak within a time delay of several tens of milliseconds, indicative of fast blood flow from our previous phantom experiments. The acquired plots and their tau half values stay consistent during the entire period of monitoring (Figure 6(A)), serving as evidence of recovered blood supply in a flap.

Figure 6(B) displays representative diffuse reflectance spectra measured at three different time points during 5-hour monitoring (0, 2.5 and 5 hours). The overall reflectance spectral shapes of three plots are maintained while the intensity reduced about 15 % after 2.5 hours in the wavelength range of 460 – 580 nm, then keeps the same level until the end of monitoring. Figure 6 (C) and (D) show the estimated total hemoglobin concentration [THb] and tissue oxygen saturation (StO_2) by the inverse MCLUT algorithm applied on the corrected reflectance. [THb] increases slightly above 20 μM in an hour from the beginning ($\sim 15 \mu\text{M}$), then stabilized to 22 to 24 μM since one hour. StO_2 keeps around 80% for 5 hours (Figure 6(D)). The levels and consistency of both [THb] and StO_2 during 5 hours would indicate that tissue perfusion is recovered in the flap via successful anastomosis of the supplying artery and draining vein.

3.2 30-minute monitoring during vein occlusion

Since no spontaneous flap failure was observed, the surgeon occluded the vein for 30 minutes after 5-hour monitoring. Figure 7 shows the monitoring results of DCS and DRS after said occlusion. In DCS, the autocorrelation plots shift to the right side at 15 and 30 minutes. (Figure 7(A)), representing compromised blood flow. The calculated tau half values consistently increase over time during the vein occlusion. (Figure 7(B)). The decreased blood flow may be attributed to the blocked vein failing to drain the blood resulting in venous congestion.

Figure 7(C) shows representative diffuse reflectance spectra measured at three different time points during 30-minute monitoring after the vein occlusion (0, 15 and 30 minutes). In contrast to 5-hour monitoring, the 30-minute vein occlusion monitoring results show dramatic changes both in the spectral shape and intensity. The reflectance intensity in the entire wavelength range reduces more than 50% in 15 minutes, and the double dips at 540 nm and 575 nm characterized by β -band of the oxygenated hemoglobin disappear in the reflectance acquired at post-15 minutes. This indicates the contribution of deoxygenated hemoglobin dominates the measured reflectance given the fact oxy- and deoxy-hemoglobin are major absorbers in biological tissues.

Figure 7 (D) and (E) show the estimated [THb] and StO_2 during vein occlusion. Within 5 minutes after the vein occluded, [THb] increases up to 70 μM from 20 μM (Figure 7 (D)) and continuously increases. StO_2 drops to 0% (Figure 7 (E)) within 10 minutes and then have the same 0 % until the end of monitoring. The dramatic increase in [THb] and the sudden drop of StO_2 would be attributed to accumulation of deoxygenated hemoglobin in capillary due to blockage of draining vein.

The difference of changes over time in blood flow, concentration and oxygen saturation between normal perfusion and venous compromise is clearly identified by DCS/DRS monitoring directly on the skin side of the flap. The preliminary data obtained here suggests that our diffuse optical technologies coupled with quantification methods are capable of non-invasive, objective and continuous monitoring in real time of flap viability by providing complimentary biophysical parameters related to tissue perfusion.

4. CONCLUSION

Changes in physiological parameters related to tissue perfusion depending on flap status have been successfully monitored in real time by our perfusion monitoring device. These initial results suggest that our system could assess microvascular free flap viability in a non-invasive, objective and continuous manner. With proven performance, the compact form constructed with cost-effective components would make this system suitable for clinical translation.

Acknowledgments

This work was supported by National Institutes of Health (R44-DE021935).

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Figure 1. Block diagram of the perfusion monitoring device. (MMF: multi-mode fiber, SMF: single-mode fiber)

Figure 2.
Two versions of the 2-channel perfusion monitoring system containing various combinations of DCS and DRS capabilities

Figure 3.

Block diagram of PSOC operation. The laser and spectrometer control are not shown. The elements after the photon detector and before the host PC are all contained in the fabric of the FPGA, and are combinations of preprogrammed blocks and interfaced with the microprocessor with proprietary C# code.

Figure 4.

(A) The isolated Latissimus Dorsi (LD) Myocutaneous flap in which blood is supplied by a single artery and drained by a single vein. (B) Arterial anastomosis completed, prior to release of vascular clamps.

Figure 5.
Illustration showing how fiber patches on the raised flap are connected to the device

Figure 6.

5-hour monitoring results (A) Calculated tau half values from DCS autocorrelation at every hour (B) Representative DRS spectra acquired at 0, 2.5 and 5 hour. (C) Estimated total hemoglobin concentration ([THb], μM) and (D) Tissue oxygen saturation (StO_2 , %) using the inverse algorithm on the obtained reflectance spectra.

Figure 7.

30-minute monitoring during the vein occlusion (A) Representative DCS autocorrelation plots at 0, 15 and 30 minutes (B) Calculated tau half values (C) Representative DRS reflectance spectra at 0, 15 and 30 minutes (D) Estimated total hemoglobin [THb] concentration changes and (E) Oxygen saturation changes during 30 minutes