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Assessment of skin flap viability using visible diffuse reflectance spectroscopy and auto-fluorescence spectroscopy

Caigang Zhu^a, Shuo Chen^a, Christopher Hoe-Kong Chui^b, Quan Liu^{*a}

^aDivision of Bioengineering, School of Chemical and Biomedical Engineering,
Nanyang Technological University, Singapore 637457

^bDepartment of Plastic Surgery, Singapore General Hospital

ABSTRACT

The accurate assessment of skin flap viability is vitally important in reconstructive surgery. Early identification of vascular compromise increases the chance of successful flap salvage. The ability to determine tissue viability intra-operatively is also extremely useful when the reconstructive surgeon must decide how to inset the flap and whether any tissue must be discarded. Visible diffuse reflectance and auto-fluorescence spectroscopy, which yield different sets of biochemical information, have not been used in the characterization of skin flap viability simultaneously to our best knowledge. We performed both diffuse reflectance and fluorescence measurements on a reverse MacFarlane rat dorsal skin flap model to identify the additional value of auto-fluorescence spectroscopy to the assessment of flap viability. Our result suggests that auto-fluorescence spectroscopy appears to be more sensitive to early biochemical changes in a failed flap than diffuse reflectance spectroscopy, which could be a valuable complement to diffuse reflectance spectroscopy for the assessment of flap viability.

Keywords: skin flap viability, diffuse reflectance spectroscopy, auto-fluorescence spectroscopy

1. INTRODUCTION

Cutaneous flaps are frequently needed to replace skin defects and provide well vascularized tissue for wound coverage. However, 6% to 25% of flaps require surgical re-exploration for vascular compromise. Despite attempted salvage, approximately 10% of flaps are not salvageable (1, 2). Therefore it is critical to identify vascular compromise as early as possible to maximize the chance of flap salvage. The current gold standard for determining flap viability is clinical examination, in which capillary refill, flap color, temperature and bleeding patterns are examined periodically. However, this method relies heavily on the expertise and availability of hospital staff. To overcome these limitations, several adjunct instrumental methods have been introduced for the purpose of monitoring skin flaps.

Laser Doppler flowmetry and imaging have been widely used as an adjunct tool to assess flap viability noninvasively (1, 3-5). Laser Doppler technique measures blood flow, which is an indirect assessment of flap viability. Diffuse reflectance spectroscopy in both the visible and near infrared spectra (6-10) and auto-fluorescence spectroscopy (11, 12) provide complementary information about tissue status and each of them has shown excellent potential for assessing tissue viability independently; however, their simultaneous use to characterize the skin flap viability has not been studied to our best knowledge. In this study, we performed both diffuse reflectance spectroscopy and auto-fluorescence spectroscopy on a reverse MacFarlane rat dorsal skin flap model in an attempt to identify the additional value of auto-fluorescence spectroscopy to the assessment of flap viability. Diffuse reflectance and auto-fluorescence spectra (excited by 405 nm laser light) were measured once every four hours over 72 hours after the creation of the flap. Total hemoglobin concentrations and blood oxygenation at different sites over time were estimated from diffuse reflectance spectra and the general trend agreed with published data in the literature. Total hemoglobin concentrations over time were also estimated from auto-fluorescence spectra, whose trend was found to be consistent with that obtained from diffuse reflectance spectra. Qualitative analysis of auto-fluorescence spectra indicates that the relative fluorescence contribution from individual fluorophores such as NADH and FAD changes significantly over time, which may offer additional values to skin flap assessment.

*Quanliu@ntu.edu.sg

2. METHODS

2.1 Animal model and surgical procedure

All animal experiments were conducted in compliance with the SingHealth Institutional Animal Care and Use Committee (IACUC) animal welfare committee's requirement for the care and use of laboratory animals in research

In this study, four Sprague Dawley rats with an age of 16 weeks were acclimated three days prior to the flap surgery. All the procedures were done under 1.2-2% isoflurane inhalational anesthesia. Thirty minutes prior to the surgery, the dorsa of the rats were shaved and the sites at which optical measurements would be made were marked on the exposed skin. Meanwhile the baseline optical measurements were performed and these data were used as control values in the analysis. A 2 cm wide and 8 cm long cutaneous flap was raised with a caudal skin attachment. TegadermTM (3M, St Paul, Minnesota, US) was placed under the flap to prevent revascularization from the wound bed. During the 72 hours postoperative study period, the animals were housed separately. Final tissue viability was determined at the end of the study period prior to the euthanizing of the animals.

2.2 Optical measurements

Diffuse reflectance and fluorescence spectra were measured on the marked positions one by one approximately every four hours over the 72 hours after the surgery was performed. The spectra were collected with a spectrometer (USB4000, Ocean Optics, Dunedin, Florida, US) using a custom bifurcated fiber optic probe. The fiber probe consisted of one central illumination fiber with a core diameter of 600 μm and nine surrounding detection fibers with a core diameter of 100 μm . The source-detector separation was 410 μm . The diameter of stainless ferrule at the common end was about 2.5 mm. A DAQ card (USB-6501, National Instruments Corporation, Austin, Texas) provides transistor-transistor logic (TTL) control signals to synchronize the illumination and detection.

In fluorescence measurements, a diode laser which generates excitation light at 405nm was used for the illumination. The excitation light was delivered onto the skin through the excitation channel of the fiber-optics probe. The backscattered fluorescence light was collected and sent to the spectrometer by the emission channel of the fiber probe. An inline long pass filter, which was placed between the emission channel and the spectrometer, was used to block the excitation light. For diffuse reflectance measurements, a Tungsten Halogen white light source (HL-2000-FHSA, Ocean Optics, Dunedin, Florida, US) was used for illumination. The same probe was used for both illumination and detection. Different from fluorescence measurement, no filter was used between the emission channel and the spectrometer. A 99% reflectance standard (SRS-99-010, Labsphere, New Hampshire, US) was used as a reference to generate reflectance spectra for the purpose of calibration. A customer designed graphical user interface (GUI) software written in Labview (Labview 10.0, National Instruments, Austin, Texas) was used to control the entire measurement. The GUI software could generate TTL signals to synchronize the light sources and the spectrometer. Both the laser and white light source were modulated by sending TTL pulses to the light source controllers. The light source would be turned on if the TTL signal sent to the light source controller is high. In contrast, the light source would be turned off when the TTL signal was low. For every pulse sent to the light source, the GUI software sent two pulses to the spectrometer to collect two optical spectra, one for the situation when the light source was turned on and the other for the situation when the light source was turned off. The former spectrum serves as the original sample spectrum. The latter spectrum serves as the background. The true sample spectrum, in which the background spectrum was subtracted from the original sample spectrum, was likely not subject to the effect of ambient light. Six sample spectra were taken in each measurement and then averaged to improve the signal to noise (SNR) ratio. The integration time for each sample spectrum in both diffuse reflectance and fluorescence measurements was 200 ms.

Point measurements were taken once every 4 hours for 72 hours after the surgery. A total of around 20 sets of spectra at each site were acquired. Along with optical measurements, a corresponding color image of the rat's dorsum was taken at each time point which was used as a reference for the clinical examination. The total time to carry out a full set of diffuse reflectance and fluorescence measurements at all marked locations in one animal was around 30 minutes.

2.3 Data analysis

For diffuse reflectance spectra, the indicators of two biochemical parameters, i.e. total hemoglobin concentration (THB) and hemoglobin oxygen saturation (StO₂), were derived using a published method (13). For fluorescence spectra, the qualitative analysis about spectra shape over time was performed. In addition, the indicator of total hemoglobin concentration (THB) was also derived from fluorescence spectra by using a ratio metric method (14). The trends of THB over time obtained from fluorescence spectra were compared with those obtained from diffuse reflectance. It should be noted that although the flaps were monitored for 72 hours, the fluorescence spectra measured from those locations at which the tissue was necrotic after 16 hours were not reliable because the fluorescence signal was noisy, which is likely due to the physical changes in the tissues secondary to ischemia. Thus only the data obtained in the first 16 hours will be shown next.

3. RESULTS

3.1 Biochemical parameters derived from diffuse reflectance measurements

In this study, the regions close to the flap base (for example P7 shown in Fig.1) remained viable while the distal regions further away from the flap base did not survive. Fig.1 shows a sequence of images of a typical skin flap. Seven monitoring sites were located along the length of the flap.

Fig.1. A sequence of images in a reverse McFarlane dorsal skin flap taken immediately after the surgery ($t=15$ minutes) and at 12, 24 and 72 hours post elevation. Spectroscopic measurement sites were marked by blue circles on the flaps.

Figure 1 demonstrates the changes occurring in the viable and non-viable regions of the flap over the proceeding 72 hours after flap elevation. The skin color at sites P1 through P4 started to change soon after the surgery and their color became increasingly dark after 24 hours. The color at sites P5 and P6 also changed but the changes were more gradual. At 72 hours the skin flaps at P1 through P4 were necrotic as indicated by eschar formation and lack of perfusion. Only the skin adjacent to the flap base at P7 did not show significant color change at 72 hours. The flaps at P5 and P6 became slightly dark. The color changes correspond to the viability of the skin flap as explained next. The necrotic regions turned black and minimal color changes were seen in the viable regions. Moreover, ischemic but still viable regions were mottled and slightly darker. In order to clearly differentiate viable from non-viable flaps, ischemic regions were not taken into account in the following sections.

Among the four animals in this study, there were 15 sites that were deemed non-viable and 5 sites that were deemed viable at the end of 72 hours. The StO₂ and THB indicators at every time point for each group calculated from diffuse reflectance spectra were averaged as shown in Fig 2 and 3, respectively, in which the standard deviations were used to create error bars in the figures.

Fig.2. StO2 indicator over time derived from diffuse reflectance spectra, in which the legends give the final status of the flaps at the end of 72 hours.

Fig.3. THB indicator over time derived from diffuse reflectance spectra, in which the legends give the final status of the flaps at the end of 72 hours.

Clear trends of StO2 and THB over time can be observed. From Fig.2, it can be seen that the StO2 indicator for those flaps that eventually died decreased significantly in 4 hours and kept at a low value after that. In contrast, the StO2 indicator for the viable flaps that were close to the blood supply and stayed viable did not show any significant change. This finding agrees very well with published data (7, 9). From Fig.3, it can be seen that the THB indicator for the regions that did not survive increased significantly after the surgery. In contrast, the THB indicator for those flaps that were close to the blood supply and remained viable did not change significantly. It has been reported that the THB would increase significantly in a failed flap on human patients (6). Our results reconfirmed this phenomenon in this animal study. Furthermore, the comparison in the trends of StO2 and THB suggests that the decrease of StO2 and increase of THB were happening at the same pace in non-viable flaps.

3.2 Biochemical parameters derived from fluorescence spectra and spectral shape changes

The THB indicators were also derived from fluorescence spectra as shown in Fig.4, in which error bars were created using the standard deviations calculated for results from the same group. From Fig.4, it can be seen that the THB indicator for those regions that did not survive increased significantly after the surgery. In contrast, the THB indicator for those regions that remained viable did not change. This trend agrees with that in THB derived from diffuse reflectance spectra (Fig. 3). The subtle difference between Fig. 4 and Fig. 3 is that the THB indicator derived from fluorescence spectra increased significantly in 4 hours after the surgery and decreased slowly after that, while the THB indicator derived from diffuse reflectance spectra kept increasing until 16 hours after the surgery.

Fig.4. THb indicators over time obtained from fluorescence spectra, in which the legends give the final status of the flaps at the end of 72 hours.

The typical fluorescence spectra of non-viable flaps and viable flaps were shown in Fig. 5. It can be seen that the shape of the fluorescence spectrum in a non-viable region changed dramatically as seen in Fig. 5(a). Considerable decreases can be seen in the wavelength region corresponding to FAD emission band (520 nm to 570 nm) and NADH emission band (450 nm to 470nm). The normalized intensity decreased significantly in the first four hours after the surgery but remained approximately unchanged after that. As a result, the peak between 450 nm and 600 nm was narrowed down in the first four hours. In contrast, the shape of fluorescence spectra for the viable site did not show any significant change throughout the process as shown in the Fig. 5(b). Moreover, there was a tiny blue shift in the fluorescence peak in the first four hours in non-viable skin. In contrast, a small red shift can be seen in control flaps.

Fig.5. Normalized fluorescence spectra for (a) non-viable flaps; and (b) viable flaps.

4. DISCUSSION AND CONCLUSION

Visible diffuse reflectance and auto-fluorescence spectroscopy were used to study changes in a reverse MacFarlane rat dorsal skin flap model. The trends in the indicators of total hemoglobin concentration and hemoglobin oxygenation were consistent with other reports. Based on our results shown in this report, it is clear that the visible diffuse reflectance spectroscopy is capable of monitoring tissue oxygenation and total hemoglobin concentration. Moreover, the indicator of total hemoglobin concentration derived from auto-fluorescence spectra shows a similar trend as that derived from diffuse reflectance spectra. Qualitative analysis of auto-fluorescence spectra indicates that the shape of the auto-fluorescence spectrum changes dramatically in the first four hours, which could be related to the variation in the relative fluorescence contribution from individual fluorophores such as NADH and FAD, which may be able to offer additional information to skin flap assessment for early diagnosis of vascular compromise. Quantitative analysis of the fluorescence spectra will be performed in the future. In short, while diffuse reflectance spectroscopy provides useful information for assessing flap viability, auto-fluorescence spectroscopy may be able to offer additional values to skin flap assessment. The combination of the two techniques may be able to improve the accuracy of optical assessment of skin flap viability considerably.

5. ACKNOWLEDGEMENT

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