Skin trauma rapidly induces thermoregulatory plexus hyperemia, while an increased nutritive papillary capillary function can be detected after 24 h

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Abstract

Objective: Clinical assessments and laser Doppler perfusion measurements (LDPM) of skin microcirculation have limited value, as they fail to capture events regulated by local metabolic needs at a papillary capillary level. This study aimed to examine the ability of computer-assisted video microscopy (CAVM) and diffuse reflectance spectroscopy (DRS) to assess skin nutritive perfusion—compared to LDPM.

Methods: Healthy volunteers (n = 10) were examined after (≈1 and ≈24 h) an incision (5 × 1 mm) on the forearm, at 0.1 mm (only with CAVM), 2–3 mm, and 30 mm from the trauma.

Results: No changes were detected by CAVM after ≈1 h. After ≈24 h, 0–1 mm from the trauma, both CAVM parameters were increased: functional capillary density (capillary crossings/mm, 11.8 ± 1.4 vs. 7.3 ± 1.2, p < .01) and capillary flow velocities (CFV, %capillaries with brisk flow, 10 ± 6.8 vs. 1 ± 1, p < .01). At a distance of 2–3 mm, only CFV was increased (6.2 ± 6.1 vs. 1 ± 1, p < .05). DRS and LDPM measurements increased 2–3 mm from the trauma line in relation to baseline after both ≈1 and ≈24 h, that is, with DRS (%microvascular oxygen saturation): 45.8 ± 7.4% (baseline), 70.0 ± 12.5% (≈1 h), and 73.1 ± 10.4% (≈24 h), p < .01 and with LDPM (a.u.): 7.2 ± 2.5 (baseline), 28.3 ± 18.7 (≈1 h), and 45.9 ± 16.3 (≈24 h), p < .01.

Conclusions: ≈24 h after skin trauma, an increased function of the nutritive papillary capillaries can be detected by CAVM.

Keywords
computer-assisted video microscopy, skin microcirculation, skin trauma response

Abbreviations: a.u., arbitrary units; CAVM, computer-assisted video microscopy; DRS, diffuse reflectance spectroscopy; FCD, functional capillary density; ICC, intraclass correlation coefficient; LDPM, laser Doppler perfusion measurements; ROI, region of interest; SmvO2, microvascular oxygen saturation.

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1 | INTRODUCTION

Clinical assessment of skin circulation (temperature and color) in patients with potential systemic circulatory failure is imprecise and of limited value, because of the inability to differentiate between perfusion in papillary nutritive capillaries—regulated by local metabolic needs—and perfusion in the deep thermoregulatory plexus—regulated by autonomous nerve activity, Figure 1.

Laser Doppler perfusion measurements (LDPM), often used to assess skin microcirculation, also fail to merely assess perfusion in the non-innervated nutritive capillaries in the epidermis. For example at the volar aspect of the forearm, the LDPM volume of ≈1 mm$^3$ includes the deep plexus, Figure 1, at the dermal-hypodermal interface. Perfusion in this plexus, regulated by autonomous nerves, is mainly serving body temperature regulation but is also affected by emotions. LDPM-recorded skin perfusion, therefore, cannot be used for the assessment of perfusion in skin nutritive capillaries. Patients with erythromelalgia have, for example, hyper-perfusion in the deep plexus (detectable with LDPM) but at the same time reduced papillary nutritive perfusion and skin hypoxia (detectable with transcutaneous oxygen tension measurements and skin vital microscopy). There is increasing evidence that the effector cells for regulation of nutritive capillary blood flow are pericytes, containing contractile proteins (i.e., actin and myocin) and not smooth muscle cells acting as “pre-capillary sphincters”. Pericytes surrounding capillaries respond with dilatation or constriction not only to changes in pCO$_2$ and pH$^3$$^4$ but also to local humoral factors (i.e., endothelin and nitric oxide). Since we believe that pericytes regulate perfusion in skin papillary capillaries in the same way as nutritive perfusion in other vital organs (e.g., the brain), our hypothesis is that quantification of papillary capillary perfusion during systemic circulatory failure can be used as a surrogate for predicting changes in nutritive perfusion in other organs.

Oxygen delivery to human tissue requires proximity to a perfused capillary. The Krogh diameter,$^{10,11}$ the maximal O$_2$ diffusion distance from a perfused capillary before chronic hypoxia develops, has been estimated to be in the range of 0.04–0.14 mm.$^{12}$ These distances can be visualized by computer-assisted video microscopy (CAVM), used to record films of skin papillary nutritive capillaries. Film analyses can be used to quantify the functional capillary density (FCD) and capillary flow velocities (CFV).

Diffuse reflectance spectroscopy (DRS) can be used to measure microvascular oxygen saturation (SmvO$_2$) in subepidermal capillaries.$^{13}$ In a previous study in healthy volunteers, we used LDPM, CAVM, and DRS to assess microcirculatory changes 30–60 min after a small skin incision at the volar aspect of the forearm (1 mm deep and 5 mm long) (T1 study). Lack of changes in FCD and CFV near to the trauma line indicated that the increase in the local metabolism needed (O$_2$ consumption and local pO$_2$) for the healing process has not yet taken place (i.e., pericyte activity was unchanged), although a significant increase in LDPM was found (deep plexus perfusion was increased because of the axon reflex).

Our group has developed the non-invasive oxygen delivery index (ODIN) concept including technologies (CAVM and DRS) and protocols for data acquisition and a technology platform for file analyses. The concept did not demonstrate changes in papillary capillary perfusion in the T1 study (≈1 h after trauma induction). In this study, our aim was to validate whether the ODIN technologies have sufficient sensitivity to detect and characterize changes in papillary capillary perfusion ≈24 h after the trauma, caused by increased metabolism and O$_2$ consumption for wound healing.

2 | MATERIALS AND METHODS

CAVM, DRS, and LDPM were used to examine skin microcirculation of healthy volunteers before and after (30–60 min and ±24 h) a standardized incision (5 × 1 mm) on the forearm, at 0.1 mm (only with CAVM) and 2–3 mm from the trauma line. The baseline measurements were used for comparison, but a control data set after ±24 h was also collected at 30 mm distal to the trauma.

2.1 Subject selection and ethics

Our selection criteria were the same as for the first trauma study (T1 study). We included healthy volunteers with Caucasian skin type, who did not use medication, nicotine, or alcohol regularly, and who abstained from physical exercise on the day of the examination and from tea and coffee for 5 h before the examination. All participants gave informed written consent. The study was approved by the Norwegian regional committee for medical and...
2.2 Techniques, measures, and procedures

For LDPM, we used the same procedures and equipment as in the T1 study, that is, monitor (PeriFlux 4001 Master Laser Doppler; Perimed, Järfalla, Sweden), measuring probe (Probe 408 Large Straight Probe), and LabView7 software (National Instruments Corporation, Austin, Texas, USA).

We used an improved version of the DRS technology; the algorithms used for analyses of the DRS spectra were an improved version from the T1 study (version $\alpha=2$: ODI Medical AS, Oslo, Norway). The spectroscope (AvaSpec-2048-2: Avantes, Apeldoorn, Netherlands), halogen light source (AvaLight-Hal-S: Apeldoorn, Netherlands), the custom-built fiber optic probe, and white polytetrafluoroethylene tile (WS2: Apeldoorn, Netherlands) for calibration, analyses, and decomposition of the DRS spectra were the same as in the T1 study. If the penalty functions for decomposition showed the goodness of fit lower than 90% and with residuals lower than 10% over the complete spectral range, the measurements were excluded from the data.

We used an improved version of the CAVM technology; the digital handheld video microscope (Mediscope, D1; Optilia, Sollentuna, Sweden) was equipped with a $\times 300$ lens. The field of view with the current setup was 1.3 mm $\times$ 0.7 mm, image format 1600 $\times$ 1200 pixels, and frame rate of seven frames/s. The instruments were built into a mobile microvascular laboratory (mLab, version $\alpha=2$: ODI Medical AS). CAVM analyses were performed offline by an operator on the analyzing platform cLab (ODI Medical AS).

The FCD was defined as the mean number of capillary crossings of a grid of lines, per mm line (c/mm). The grid consisted of three vertical and three horizontal lines as described in the T1 study but with one modification; if two capillaries cross each other at the gridline, they were counted as one. To quantify CFV, a 5-category scale was used, ranging from 0 (no flow) to 4 (brisk flow) as described in the T1 study Table 1. The DRS and CAVM files were recoded with a computer-generated code consisting of six random letters and numbers and then analyzed randomly.

<table>
<thead>
<tr>
<th>Flow category</th>
<th>Description of flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No flow</td>
</tr>
<tr>
<td>1</td>
<td>Sluggish flow</td>
</tr>
<tr>
<td>2</td>
<td>Slow flow</td>
</tr>
<tr>
<td>3</td>
<td>Continuous flow</td>
</tr>
<tr>
<td>4</td>
<td>Rapid flow</td>
</tr>
<tr>
<td>5</td>
<td>Brisk flow</td>
</tr>
<tr>
<td>6</td>
<td>Uncertain</td>
</tr>
</tbody>
</table>

Data collection

The preparations, the acclimatization procedure resting in a supine position, and the region of interest (ROI) on the volar surface of the left forearm 60 mm distal to the elbow joint were the same as in the T1 study. After 10 min, blood pressure, pulse oximetry estimate of $\text{SaO}_2$ (Carescape V100: GE Healthcare, Chicago, IL, USA), and heart rate were examined. Skin temperature was continuously monitored 10 cm distally to the ROI (Anritsu Digital Surface Thermometer HFT-80, Kanagawa, Japan). The collection of baseline LDPM, DRS, and CAVM data was carried out following the same protocol as in the T1 study.

For trauma induction, the same standardized disposable mechanical device was used (Surgicutt®; Technidyne Corporation) as used in the T1 study, creating a skin trauma of 5 mm length and 1 mm depth at the ROI horizontal to the elbow crease.

In the period 30–60 min after trauma induction, data were obtained according to the same protocol as in the T1 study. A plastic probe holder shaped like a “D” and designed with a central opening was made for standardized LDPM and DRS probe placement on the skin, Figure 2. The two probes have the same diameter and contain asymmetrically placed optical fibers to guide light to and from the probe tip.

A tape marker on the probes and a corresponding tape marker on the probe holder were used to control the rotation of the probes within the probe holder. A controlled rotation of the asymmetrical LDPM and DRS probes of approximately 30° first clockwise and then counterclockwise was used to collect data from adjacent measuring volumes to quantify spatial heterogeneity. First, the probe holder was placed with the trauma line visible through the central opening—parallel to the straight part of the probe holder—before placing the probe in the probe holder. The eight LDPM were obtained before switching to the DRS probe to collect the 12 measurements, without removing the probe hat. With this placing of the probe holder and the tape markers, we were able to secure data collection 2–3 (2.5 $\pm$ 0.5) mm (as controlled by a digital caliper) from the edge of the trauma. Twenty-four hours ($\pm$1 h) after the trauma induction, the same preparations, acclimatization procedure, examinations of blood pressure, $\text{SaO}_2$, heart, and continuous monitoring of skin temperature as for the baseline data were performed. The LDPM, DRS, and CAVM recordings were repeated according to the same data acquisition protocol, at the same distances from the trauma and at a control site at 30 mm distal to the trauma site.
Results are presented as mean ± SD. To compare results at baseline and later, the two-sided t-test for paired data was used. The level of significance was set to 5%. Statistical analyses were performed using Microsoft Excel for Mac (Version 14.4.8.; Microsoft Corporation, Redmond, WA, USA) and SPSS version 26 (SPSS Inc., Chicago, IL, USA).

To determine the intra-observer and inter-observer reliability, a random selection of 20 recoded films containing an even distribution of films from baseline, =1, and =24 h post-trauma, and the control site was analyzed by observer 1 a second time, as well as by an experienced independent examiner (observer 2). The intraclass correlation coefficient (ICC) was used to measure reliability. To judge the ICC, we used the guidelines given by Koo and Li.15

3 RESULTS

Of the 10 healthy volunteers screened, all met the eligibility criteria. The subjects (five male and five female), age 31 ± 11 (mean ± SD) years, all had average blood pressure (BP), heart rate (HR), and arterial oxygen saturation (SaO₂) within reference levels: BP: 115/73 ±11/6; HR: 70 ± 9; and SaO₂: 98% ± 2%. The average room temperature, 23.5 ± 0.9°C, and the average skin temperature, 31 ± 0.8°C, did not change significantly during the experiment.

3.1 Computer-assisted video microscopy (CAVM)

After =24 h, capillary density (capillary crossings [cc]/mm) had increased 0–1 mm from the trauma line in relation to baseline, Figure 3, as had capillary flow velocities (see Figure 4A,B); no changes were detected after =1 h. More than 95% of all the assessed capillaries that were not in category 3 were in category 4.

3.2 Test-retest reliability of the CAVM parameters

Intra-observer reliability between the first and second analysis by observer 1 was good for FCD with an ICC of 0.86 and excellent for CFV with an ICC of 0.94.

Inter-observer reliability between the two independent examiners was good for both CAVM parameters, with an ICC of 0.87 for FCD and 0.84 for CFV.

3.3 Laser Doppler perfusion measurements and diffuse reflectance spectroscopy

Both the DRS measurements and the LDPM increased at 2–3 mm from the trauma line in relation to baseline after both =1 and =24 h, Figures 5 and 6.
were significant changes in the nutritive locally regulated papillary capillaries detectable by the CAVM technique.

At 30–60 min after the trauma, the perfusion in the deep plexus is increased—via the axon reflex—as shown in the LDPM results, Figure 5. The ≥50% increase in the DRS recordings after 30–60 min, Figure 6, indicates an increase in superficial (distributional) plexus perfusion via ascending arterioles from the deep plexus. A potential for increased nutritive perfusion is secured, but the pericytes regulating papillary nutritive perfusion have still not experienced signals of increased metabolic needs—both FCD and CFV are unchanged—confirming the results of the T1 study.14

After ≥24 h, the LDPM perfusion increased further, Figure 5, and the DRS values remained elevated, Figure 6. CAVM parameters showed increased FCD at 0–1 mm distance from the trauma line but...
not at 2–3 mm distance, Figure 3. The capillary flow velocities (CFV) increased at both 0–1 and 2–3 mm distances from the trauma line, Figure 4A,B.

This study was designed based on the results of the previous T1 study, using the same standardized method for trauma induction and techniques for DRS and LDPM examinations. The control site 30 mm distal to the trauma line was also the same and is based on a previous study. The DRS, the CAVM parameter, and CFV results from baseline measurements and from the first time interval for post-trauma data collection (30–60 min) confirmed the results of the T1 study. The trauma was followed by a fourfold increase in LDPM measured perfusion in the T1 study and a threefold increase in this study, but this is probably due to the huge spread, Figure 5—well-known limitations with LDPM measurements, which, in general, has poor reproducibility.

FCD was calculated as capillary crossings/mm gridline rather than the absolute number of capillaries in the field of view. This was chosen because approximately 50% of the capillaries are perpendicular and the other 50% horizontal at the ROI and, therefore, delivers oxygen to a variable area of the skin. A minor CAVM modification of determining FCD was introduced compared to the T1 study; capillaries that cross each other at the point of a gridline crossing were counted as one crossing instead of two—as two capillaries this close supply oxygen to the same tissue volume. This modification can explain the lower average FCD at baseline as compared to the T1 study. We are confident that the CAVM data were in the same location for both the ≈1 h and the ≈24 h trauma data, as the trauma line is easily visible with the microscope and the five films per subject and site were collected using the ≈1 mm field of view along the 5 mm long trauma line.

Both intra- and inter-observer reliability of the CAVM data were good in this study. Using the preset version of the cLab, analyzing platform requires experience, preferably in a certified quality-assured laboratory setting.

Oxygen delivery from capillaries is essential for all human cells. A complex set of physiological mechanisms regulate microvascular hemodynamics to optimize oxygen delivery with the least use of energy. In the skin, the critical function of this regulatory system is to maintain the delivery of $O_2$ for the proliferation of stem cells at the epidermal basement membrane. Flow and flow velocities in individual capillaries are regulated—like in other blood vessels—by perfusion pressure and vascular resistance. The diameter of the nutritive capillaries is controlled by pericytes surrounding the capillaries—containing actin and myosin—contracting when oxygen supply is sufficient and dilating in response to acidic extracellular pH and elevated $pCO_2$. The repair process with increased metabolic needs takes place along the trauma line. Based on the Krogh model and the maximal $O_2$ diffusion distance ($\approx 0.1$ mm), the CAVM data 0–1 mm from the trauma are from the repair zone with increased metabolic and $O_2$ needs, reflected by an increase in FCD and CFV, Figures 3 and 4A,B. Increased CFV means an increased number of oxygen-carrying erythrocytes pass through the area, and the increase in FCD corresponds to reduced diffusion distances. The smaller increase in CFV at 2–3 mm may represent a mechanism analogous to upstream flow-mediated dilatation increasing blood supply to the area with increased needs.

5 | IMPLICATIONS

The technologies, data acquisition protocol, and analyzing platform used in this study have sufficient sensitivity to characterize the microcirculatory changes in response to the repair process following a minor skin trauma.

This study suggests that the results of CAVM—in contrast to clinical and LDPM assessments of skin perfusion—can be used to assess the microcirculation in the locally regulated nutritive papillary capillaries separately. In this study, the DRS results are influenced by perfusion in the superficial distributional vascular plexus. Skin measurements of the nutritive papillary capillaries can be of great value for diagnosis and monitoring of patients with local skin circulatory disorders (e.g., lower limb atherosclerosis, assessments of amputation levels, diabetic and venous skin ulcers, and viability of skin flaps in plastic and reconstructive surgery). Measurements of skin nutritive papillary capillaries can also be of value for diagnosis and monitoring of patients with systemic circulatory failure (e.g., acute and chronic heart failure and sepsis) and for assessing the effect of therapeutic measures (e.g., volume replacement and vasoactive drugs or effectiveness of mechanical assist devices used in heart failure).

6 | PERSPECTIVES

Repair following a skin trauma requires increased oxygen delivery. One hour after a small trauma, an increased perfusion in the deep thermoregulatory plexus (laser Doppler technology) was found, but papillary capillary nutritive perfusion (digital microscopy) was not changed. After ≈24 h, papillary capillaries showed increased densities and flow velocities. Digital microscopy assesses the locally regulated nutritive microcirculation and has in combination with spectroscopy a potential for monitoring patients with systemic circulatory failure.

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CONFLICT OF INTEREST

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ODIN concept is patented in Japan, and in the USA, and a patent is pending in the Europe. Kvernebo, K, is the founder, shareholder, and CMO of ODIN Medical AS. ODIN Medical AS has provided the analysis algorithms and analysis platform used in this study. Kvernebo, AK, is family related to Kvernebo, K.

AUTHOR CONTRIBUTIONS
Knut Kvernebo developed the theoretical framework. Liv Kristin Wikslund and Knut Kvernebo planned and developed the idea of the study and planned the experiments with input from Vivian Shubira Amundsen. Liv Kristin Wikslund and Vivian Shubira Amundsen carried out the experiment and performed the measurements. Knut Kvernebo supervised the work. Liv Kristin Wikslund performed the LDPM and CAVM analysis with support from Knut Kvernebo. Anne Kari Kvernebo re-analyzed the CAVM data. Øyvind Kravel-Velle Standal performed the DRS analysis. All authors contributed to the interpretation of the results. Liv Kristin Wikslund wrote the manuscript and designed the figures with support from Knut Kvernebo, with input from all authors who all provided critical feedback and discussed the results and commented on the manuscript.

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REFERENCES


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