Assessment of Skin Microvascular Function and Dysfunction
With Laser Speckle Contrast Imaging

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In recent years, skin microcirculation has been considered an easily accessible and potentially representative vascular bed to evaluate and understand the mechanisms of microvascular function and dysfunction. Vascular dysfunction (including impaired endothelium-dependent vasodilation) induced by different pathologies is evident in the cutaneous circulation. It has been suggested that the skin microcirculation may mirror generalized systemic vascular dysfunction in magnitude and underlying mechanisms. Furthermore, minimally invasive skin-specific methodologies using laser systems make the cutaneous circulation a useful translational model for investigating mechanisms of skin physiology and skin pathophysiology induced either by skin disease itself or by other diseases such as vascular, rheumatologic, and pneumologic. To date, the skin has been used as a circulation model to investigate vascular mechanisms in a variety of diseased states, including hypercholesterolemia, Alzheimer disease, carpal tunnel syndrome, schizophrenia, hypertension, renal disease, type 2 diabetes, peripheral vascular disease, atherosclerotic coronary artery disease, heart failure, systemic sclerosis, obesity, primary aging, and sleep apnea.

Assessment of skin microvascular function can be done by both invasive and noninvasive techniques. Among noninvasive techniques, laser systems are mainly used. The recent development of the laser speckle contrast imaging (LSCI) technique for monitoring skin microvascular function enables its use as a surrogate end point in clinical trials. LSCI allows for noncontact, real-time, and noninvasive monitoring of cutaneous blood flow changes.

Recent evidence has shown that the LSCI technique dramatically reduces the variability of clinical measurements compared with laser Doppler flowmetry (LDF), making the technique a fascinating tool to facilitate microvascular studies in clinical routine. In this review, we describe the potential of LSCI to assess microvascular function and dysfunction. The way to use LSCI to understand skin physiology and physiopathology is also presented.

**LSCI Technique**

LSCI relies on the speckle phenomenon to obtain a perfusion map of the tissues; the speckles, which correspond to bright and dark areas, are generated by the backscattered light collected on a screen when an optically rough object (tissue region of interest [ROI]) is illuminated with a coherent laser light. Motions of particles in the illuminated tissue region lead to fluctuations in the speckle pattern on the detector. An image of the speckle is acquired with a camera (localized inside the LSCI imager head) during a certain time interval called exposure time. Because of the averaging of the speckle pattern over this time interval, which is in the order of milliseconds, a blurring of the speckle is obtained. The corresponding blurring in the image reduces its contrast. This reduction is a function of the moving particles’ speed: Without any or with little movements, the blurring is absent or very small. The speckle contrast is usually defined as the ratio of the standard deviation (SD) of the intensity \( I \) to the mean intensity \( \langle I \rangle \) of the speckle pattern, as follows:

\[
C = \frac{\sigma}{\langle I \rangle} = \frac{\sqrt{\langle I^2 \rangle - \langle I \rangle^2}}{\langle I \rangle}
\]

With movement, the speckle pattern is blurred, and the SD of the intensity is small compared with the unchanged mean intensity. The speckle contrast \( C \), therefore, is reduced. \( C \) can be computed with 2 ways: For pixel \( p_{i,j} \), \( C \) is determined (1) by calculating the ratio of the SD of the pixels in an \( n \times n \) pixel area around (spatially) \( p_{i,j} \) to the mean value of the pixels in this area and (2) by calculating the ratio of the SD of the intensity at \( p_{i,j} \) at different times and the mean intensity for \( p_{i,j} \). The advantage of the first method is that the temporal resolution is high, but the drawback is the lack of spatial resolution. For the second method, the spatial resolution is better. In what follows, the LSCI technique uses the first way of contrast value computation (high-frame laser speckle contrast analysis). The speckle contrast image leads to a 2D map of the perfusion.
Cutaneous Microcirculation, Pathologies, and Dysfunction

Study of the cutaneous microcirculation using LSCI can be easily performed in animal models as in humans. The level of cutaneous blood flow is subject to systemic, regional, local, and cellular mechanisms (eg, sympathetic activity, local axon reflexes, endothelial function, myogenic tone, metabolic responses). There are 2 distinct sympathetic pathways. The first releases norepinephrine through sympathetic vasoconstrictor nerves and causes cutaneous vasoconstriction. The second is a nonadrenergic sympathetic active vasodilator system. The endothelial pathway includes the release of NO, prostaglandins, and endothelium-derived hyperpolarizing factor, which act as vasodilator components.

This global vasomotor system can be affected by numerous pathologies or conditions that possibly lead to microvascular dysfunction and affect the physiological functions associated with skin, such as thermoregulatory function. For example, patients with chronic heart failure exhibit attenuated endothelium-dependent vasodilation and impaired thermoregulatory response of the peripheral circulation, including the skin. It is also well-known that abnormalities of the microcirculation play a major role in the complications of diabetes and skin grafts. A close link as been reported between central microvascular dysfunction, such as coronary and skin microvessel dysfunction. On the other hand, exercise training improves thermoregulatory responses and enhances endothelial function. Consequently, numerous procedures based on pharmacological treatments coupled with imaging techniques, such as LSCI, have been developed to allow the study of cutaneous microcirculation. Recordings performed with or without pharmacological intervention have to be done in the best conditions. Measurement recommendations to obtain LSCI recordings are presented thereafter.

Factors Possibly Affecting LSCI Recordings

Distance Between Laser Head and Skin

For acquisitions, the operator has to choose a distance between the laser head and the skin under study. It has been shown that this distance (when chosen between 10 and 30 cm) has no influence on skin blood flow recordings at rest or at peak postocclusive hyperemia.

Air Movement and Lighting Conditions

Skin microcirculation is under the influence of environment temperature. To have a controlled temperature during LSCI recordings, fans and acclimatizers often are switched on in the acquisition rooms. However, it has recently been suggested that air movement (≥2.8 m/s) may increase the perfusion value given by the LSCI technique, specifically when low perfusion values are recorded. Therefore, users should prevent high air movement throughout the LSCI measurements, using, for example, a dedicated room. Moreover, LSCI imagers are designed to be used in normal ambient-lighting conditions. Thus, during measurements, ambient light can be continuously measured and compensated for. However, the background light should always be kept as stable as possible during acquisitions.

Region of Interest and Time of Interest

Skin blood flow presents high spatial and temporal variability. To reduce these variabilities, the LSCI operator can define an ROI and average the blood flow on a given time of interest (TOI). The ROI is defined as the average of all the pixel perfusion values in the skin area of interest. The TOI can range from 1 mm² to 400 mm² or even more. The time of interest is defined as the duration over which the perfusion is averaged. Some authors have shown that when the forearm is studied, an ROI size of >10 mm² and a time of interest >1 s reduce the variability of cutaneous blood flow measurements both at rest and during the peak of reactive hyperemia.

Expression of the Perfusion Values

Data expression is a key issue when assessing skin blood flow. As for LDF and laser Doppler perfusion imaging (LDPI), LSCI results cannot be expressed in absolute perfusion values (eg, cutaneous blood flow in milliliters per minute relative to the volume or weight of tissue). Therefore, data can be expressed as arbitrary perfusion units (ie, laser speckle perfusion units [LSPU]), percentage of baseline, or percentage of a maximal vasodilation. During an ischemic event, the recorded value with LSCI does not reach 0; this phenomenon is called the biological zero. The biological zero is also observed with LDF and LDPI. However, LSCI biological zero may differ from that of LDPI. It might be that LSCI biological zero is intrinsic to the LSCI measure; that is, unlike LDPI, the contrast of the speckle pattern is inversely correlated with the skin blood flow, with a potential problem when determining the biological zero. Biological zero subtraction could be performed when results are expressed in LSPU but should not be done in the case of expressing results as a percentage of baseline. Values can also be expressed in cutaneous vascular conductance (which is the flux divided by the mean arterial pressure [LSPU/mm Hg]). Using cutaneous vascular conductance has the advantage of taking into account the possible variations in blood pressure that may affect local blood flow. Some authors have also suggested taking into account the skin resistance when expressing results of iontophoresis coupled with laser.

Moving Skin

Study of skin microcirculation physiology during exercise is still an exciting challenge to date. Indeed, cutaneous shiverings and movements associated with sport practice can induce significant artifacts and extreme variability of the recorded signal whatever the imaging technique used. Recently, specific procedures for using an opaque surface placed on the forearm and for data treatment have been proposed, as associated with LSCI, to allow accurate recordings over moving skin surfaces, giving promising perspectives for the study of cutaneous physiology during exercise and in nonimmobile patients.

Processing the LSCI Data

The processing of the data derived from the LSCI technique can be performed through 2 main ways: The user either postprocesses the perfusion images (with image processing algorithms) (eg, Figure 1D) or averages the perfusion values on an ROI and a time of interest (eg, Figure 1C). In the latter case, processing tools from the signal processing field are
used. To the best of our knowledge, very few studies relate the postprocessing of perfusion images or the signal processing of time-dependent spatial-averaged perfusion values. Further works, therefore, are needed to extract information from the data with image and signal processing algorithms because this was very often published with LDF signals. 

**Comparison of LSCI, LDPI, and LDF**

**Theoretical Background**

The LDF technique relies on the Doppler effect. When photons of a laser light encounter moving particles (mainly red blood cells) in the tissues under study, the Doppler effect appears (modification of photon frequency). When the reemitted light is directed toward a photodetector, optical mixing of light frequency shifted and nonfrequency shifted leads to a stochastic photocurrent. The first moment of the power spectrum of this photocurrent gives the perfusion value, which is called the LDF signal. LDPI relies on the same principles. Basically, the image given by LDPI imagers is a collection of LDF samples placed together on an image. As for the LDF technique, the backscattered light is collected by a photodetector and converted into a signal, which is proportional to perfusion, and is displayed on a monitor as a color-coded image. In the first LDPI imagers, a laser beam scanned the tissues in a stepwise manner by means of a computer-controlled mirror system housed in a camera-like scanner head or by a continuously moving laser-beam. Recent research extends LDPI to a full-field area measurement, as does LSCI.

For LDF and LDPI, the theoretical background is well-known. In contrast, for LSCI, the link between the blurring of the speckles on the detector with the average velocity of moving blood cells necessitates assumptions about an appropriate velocity distribution and the fraction of moving blood.
cells, among others. Further works, therefore, are still needed in this area to settle the speckle theoretical background.

**Correlation Between LSCI and LDPI**

LSCI and LDPI are both well-known techniques for a noninvasive investigation of microcirculatory blood flow. It has been shown recently that LSCI and LDPI show an excellent correlation when data are expressed as arbitrary perfusion units with and without subtracting the biological zero value. This correlation is still acceptable when data are represented as a percentage increase from baseline (for local heating at 36°C, 39°C, 42°C, and 44°C on the forearm of healthy subjects) but decreases when the biological zero value is subtracted.

**Measurement Depth**

Another difference among LDF, LSCI, and LDPI is that the 3 techniques sample different compartments of tissues. The LDF and LDPI systems monitor perfusion in deeper vessels, whereas the measurement depth with LSCI is superficial and corresponds to nutritional supply. Therefore, LSCI probes a shallower depth than laser Doppler techniques (depending on the laser wavelength). O’Doherty et al reported that the depth explored with the laser Doppler technique is ~1 mm, whereas the depth explored with LSCI is ~300 μm when the laser wavelength is 780 nm. For the 3 techniques, the measurement depth finally depends on the laser wavelength and on the optical properties of the tissues (as well as on the probe geometry for the monopoint laser Doppler flowmeters).

**Reproducibility**

LDF has a good temporal resolution, a poor spatial resolution, and a poor reproducibility. To solve for the poor spatial resolution, LDPI has been proposed. However, the corresponding devices show a poor temporal resolution when a full-field area measurement is not used. Nevertheless, the reproducibility of local thermal hyperemia is good with LDPI.

With a high frame rate, LSCI shows good spatial and temporal resolutions and excellent reproducibility. The interday (between day 0 and day 7) reproducibility (expressed as the coefficient of variation) for the local thermal hyperemia peak (for the test explanation, see the next section) assessed by either LDF, LDPI, or LSCI was reported to be 40%, 39%, and 15% respectively. Further, the coefficient of variation for the local thermal plateau was 15%, 17%, and 42% for LSCI, LDPI, and LDF, respectively. This reproducibility (expressed as the coefficient of variation) was ~8% when studying the peak of postocclusive reactive hyperemia (PORH) with the LSCI (for the test explanation, see the next section).

Furthermore, Millet et al recently showed that intersite variability at baseline is lower with LSCI than with LDPI.

**Tests of Microvascular Functions**

Many different microvascular tests have been developed to study the microvascular function and evaluate different physiological and pathophysiological pathways, which are involved in the control of the skin microvasculature (Tables 1 and 2). Two main types of microvascular tests can be differentiated: tests without any pharmacological intervention and tests with pharmacological intervention. The choice of test depends on the physiological pathway of the skin microvascular function studied.

**Nonpharmacological Microvascular Tests**

The main tests that do not use pharmacological drugs are the PORH test, transient hyperemic response test, local thermal hyperemia test, and local cooling test (Table 1).
<table>
<thead>
<tr>
<th>Drugs</th>
<th>Polarity</th>
<th>Possible Physiological Pathway</th>
<th>Receptors</th>
<th>Vascular Effect</th>
<th>Possible Mechanisms</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>Positive</td>
<td>Endothelium dependent</td>
<td>M₃ muscarinic receptor</td>
<td>Vasodilation</td>
<td>NO, prostaglandins, EDHF</td>
<td>Durand et al⁶², Gaubert et al⁶³, Morris and Shore⁶⁴, Newton et al⁶⁵</td>
</tr>
<tr>
<td>Adrenaline (epinephrine)</td>
<td>Positive</td>
<td>Endothelium independent</td>
<td>Alpha receptor on vascular smooth muscle cells</td>
<td>Vasoconstriction</td>
<td>Different intracellular pathways depending on alpha receptor types</td>
<td>Brown et al⁶⁶, Levick³⁰</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>Positive</td>
<td>Endothelium dependent</td>
<td>B² receptor</td>
<td>Vasodilation</td>
<td>NO and eicosanoid production</td>
<td>Newton et al⁶⁵, Kellogg et al⁶⁷</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>Negative</td>
<td>Endothelium dependent</td>
<td>B² receptor</td>
<td>Vasodilation</td>
<td>NO, prostaglandins, hyperpolarization of the vascular wall</td>
<td>Brown et al⁶⁶</td>
</tr>
<tr>
<td>Bretylium</td>
<td>Positive</td>
<td>Endothelium independent</td>
<td>. . .</td>
<td>Vasodilation</td>
<td>Blocks the release of transmitter from noradrenergic nerve endings</td>
<td>Saumet et al⁶⁸, Johnson et al⁶⁹</td>
</tr>
<tr>
<td>C-peptide</td>
<td>Negative</td>
<td>Probably endothelium dependent</td>
<td>Not yet studied</td>
<td>Vasodilation</td>
<td>Na/K-ATPase activity and NO</td>
<td>Delaney et al⁷⁰</td>
</tr>
<tr>
<td>CGRP</td>
<td>Positive</td>
<td>Probably endothelium independent</td>
<td>Not yet studied</td>
<td>Vasodilation</td>
<td>Direct or indirect vascular smooth muscle cell and sympathetic fiber stimulation</td>
<td>Rossi et al⁷¹, Edvinsson and Edvinsson⁷²</td>
</tr>
<tr>
<td>Deionized water</td>
<td>Negative</td>
<td>Endothelium dependent and endothelium independent</td>
<td>Not yet studied</td>
<td>Vasodilation</td>
<td>C fibers and prostaglandins</td>
<td>Tartas et al⁷³, Durand et al⁷⁴, Gohin et al⁷⁵</td>
</tr>
<tr>
<td>Deionized water</td>
<td>Positive</td>
<td>Endothelium dependent and endothelium independent</td>
<td>Not yet studied</td>
<td>Vasodilation</td>
<td>C fibers and prostaglandins</td>
<td>Rousseau et al⁸⁵, Tartas et al⁷⁶, Durand et al⁷⁴, Durand et al⁷⁷</td>
</tr>
<tr>
<td>Histamine</td>
<td>Positive</td>
<td>Endothelium dependent and endothelium independent</td>
<td>H¹ and H² receptors</td>
<td>Vasodilation</td>
<td>H¹: endothelial cells; H²: direct effect on smooth muscle cells</td>
<td>Leroy et al⁷⁸, Jones et al⁷⁹</td>
</tr>
<tr>
<td>Insulin</td>
<td>Negative</td>
<td>Endothelium dependent and endothelium independent</td>
<td>Insulin receptor</td>
<td>Vasodilation</td>
<td>NO, Na/K-ATPase</td>
<td>Delaney et al⁷⁰, Rossi et al⁷¹, Arnevig⁸⁶</td>
</tr>
<tr>
<td>Methacholine</td>
<td>Positive</td>
<td>Endothelium dependent</td>
<td>Muscarinic receptor</td>
<td>Vasodilation</td>
<td>NO, prostaglandins, EDHF</td>
<td>Newton et al⁶⁵, Sauvet et al⁸¹</td>
</tr>
<tr>
<td>Noradrenaline (norepinephrine)</td>
<td>Positive</td>
<td>Endothelium independent</td>
<td>Alpha receptor on vascular smooth muscle cells</td>
<td>Vasoconstriction</td>
<td>Different intracellular pathways depending on alpha receptor types</td>
<td>Brown et al⁶⁶, Levick³⁰</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>Positive</td>
<td>Endothelium independent</td>
<td>Alpha receptor on vascular smooth muscle cells</td>
<td>Vasoconstriction</td>
<td>Different intracellular pathways depending on alpha receptor types</td>
<td>Brown et al⁶⁶, Levick³⁰</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>Negative</td>
<td>Endothelium independent</td>
<td>. . .</td>
<td>Vasodilation</td>
<td>NO donor</td>
<td>Newton et al⁶⁵, Noon et al⁶⁵, Morris and Shore⁶⁴, Levick³⁰</td>
</tr>
<tr>
<td>Substance P</td>
<td>Positive</td>
<td>Endothelium dependent</td>
<td>NK1 receptor</td>
<td>Vasodilation</td>
<td>Possibly NO production</td>
<td>Newton et al⁶⁵, Levick³⁰</td>
</tr>
</tbody>
</table>

NK1 indicates neurokinin 1; NOS, nitric oxide synthase; CGRP, calcitonin gene-related peptide; EDHF, endothelium-derived hyperpolarizing factor.
PORH Test
The PORH test induces an increase in skin blood flow to tissue that follows the release of a brief arterial occlusion. Although to date no consensus exists about the optimal ischemia duration, an occlusion with the cuff inflated at a suprasystolic pressure (50 mm Hg above the systolic pressure) during at least 3 minutes could be performed. The PORH test, which is believed to be endothelium dependent and involving both myogenic and metabolic factors, evaluates the reperfusion of the vascular beds and is characterized by a peak in the skin blood flow. PORH relies mainly on adenosine, ATP-sensitive potassium channels, partial pressure of oxygen, prostaglandins, and NO. The role of NO is controversial in PORH and appears not to be involved in either the development or the maintenance of the hyperemic response after short (3 minutes) or long (15 minutes) arterial occlusion. Sensory nerves and large-conductance calcium-activated potassium channels might play a major role in the endothelium-derived hyperpolarizing factor component of PORH and appear to work partly independent of each other.

Transient Hyperemic Response Test
To induce a transient hyperemic response, a brief (<20 s) occlusion of the brachial artery is performed. After occlusion release, a transient vasodilation occurs, mainly caused by a myogenic relaxation. Indeed, myogenic relaxation operates initially (up to 20 s). If circulation is arrested longer, as in the PORH test, vasodilation mechanisms involved are different. Nevertheless, to our knowledge, there is no publication of the use of the transient hyperemic response test with LSCI.

Local Cooling Test
The local cooling test could be of interest in the study of age-related modifications or Raynaud phenomenon. The typical response is characterized by an initial decrease in skin blood flow followed by a transient vasodilation and a secondary progressive vasoconstriction. This vasoconstriction is caused by an adrenergic nerve component involving norepinephrine-mediated vasoconstriction throughout the cooling stimulus. To date, the physiological pathways involved in the transient vasodilation are unknown. Unfortunately, this cooling test cannot be performed with LSCI because no probe is currently available.

Pharmacological Microvascular Tests
As for other laser systems, different pharmacological microvascular tests can be performed with LSCI, such as iontophoresis and microdialysis (Table 2). Iontophoresis is a noninvasive method that drives a pharmacologically charged drug by electrorepulsion through the interstitium surrounding the blood vessels (Figures 1 and 2). This method allows a very small amount of drug to be administered noninvasively and safely to a localized area. The quantity of drug delivered depends on the current intensity and on the duration of the current application. Several protocols exist to perform iontophoresis, including changing the duration of the current stimulation, the duration of rest time before stimulation, the intensity of the stimulation, the number of stimulations, and the duration between stimulations. The polarity of the pharmacological drug determines the polarity of the current administration. Unfortunately, with iontophoresis, the current on its own can cause a current-induced vasodilation. The cathodal current effect is more important than the anodal, and the
current effect increases with the increase of the charge density (intensity/duration).7,50 The vehicle used with the pharmacological drug can also participate in the response with iontophoresis, then inducing a confounding response. Further, it is important to note that among the most-used vehicles, deionized water provides a more important current-induced vasodilation than NaCl when a cathodal current is used.51 Different strategies have been evoked to assess and limit this vehicle effect, such as the administration of the vehicle alone by iontophoresis with the identical current application used to deliver the drug or the use of local anesthetics.7,50 Readers must keep in mind that the influence of the vehicle can depend on the added drug.52,53 Different drugs can be used to test different pathways, which induce vasodilatation or vasoconstriction (Table 2). When studied with iontophoresis, pathways involved in the microvascular reactivity can be different from those studied by intraarterial investigations.

Microdialysis is a semi-invasive method used to introduce or remove drugs or molecules of interest to the interstitial space in skin.3,8,54 This method is based on the use of fibers with a semipermeable membrane at the fiber tip. The fiber, which is implanted in the skin in the interstitial space, is continuously perfused with a physiological solution. Drugs or molecules of interest pass the membrane of the fiber by passive diffusion along their concentration gradient, resulting in a specific vessel response related to the molecule or the studied drug. A laser placed over the fiber allows the recording of blood flow modifications induced by different drugs.

### Practical Recommendations and Standardized Protocols for LSCI

Table 3 provides some recommendations for LSCI recordings, and Table 4 some examples of standardized protocols.
Conclusion

LSCI is a noncontact and real-time technique that is a promising new tool to assess skin microvascular function and dysfunction. Recordings can be performed with or without pharmacological intervention. In contrast to other techniques available, such as LDF or LDPI, one of the main advantages of LSCI is its high reproducibility. All included, these advantages of the LSCI technique offer strong possibilities to study and to understand skin physiology in healthy and in pathological states in many clinical fields, such as dermatology, vascular medicine, plastic surgery, diabetes, and aging.

Disclosures

None.

References


Key Words: endothelium ▪ lasers ▪ microcirculation ▪ iontophoresis ▪ physiology ▪ skin ▪ methods
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