Techniques for Measuring Perfusion during Reconstructive Surgery and Effects of Epinephrine in Local Anesthetics

Sheikh, Rafi

2018

Techniques for Measuring Perfusion during Reconstructive Surgery and Effects of Epinephrine in Local Anesthetics

RAFI SHEIKH
DEPARTMENT OF CLINICAL SCIENCES, LUND | LUND UNIVERSITY

Techniques for Measuring Perfusion during Reconstructive Surgery
and Effects of Epinephrine in Local Anesthetics

Techniques for Measuring Perfusion during Reconstructive Surgery and Effects of Epinephrine in Local Anesthetics

Rafi Sheikh

LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at the conference room of the Eye Clinic, SUS, Kioskgatan 1, Lund.
Friday 16 November 2018 at 12:30.

Faculty opponent

Associate Professor Peter Bjerre Toft
Department of Clinical Medicine, University of Copenhagen
Copenhagen, Denmark

Organization LUND UNIVERSITY Department of Clinical Sciences Lund, Ophthalmology, Lund, Sweden	Document name: DOCTORAL DISSERTATION
	Date of issue: 2018-11-16
Author(s)	Sponsoring organization

Title and subtitle: Techniques for Measuring Perfusion during Reconstructive Surgery and Effects of Epinephrine in Local Anesthetics

Abstract:

The aims of the work presented in this thesis were to investigate different techniques for the measurement of microvascular perfusion in skin, and to quantify the effect of epinephrine on skin perfusion and bleeding during surgery, in order to determine the optimal epinephrine concentration, and the optimal delay between injection and surgical incision.

Several techniques were used to quantify skin perfusion: laser Doppler velocimetry, laser speckle contrast imaging (LSCI), high-resolution thermography and diffuse reflectance spectroscopy. In one study, LSCI was combined with RGB image analysis to measure tissue blanching and perfusion. Quantitative measurements were also made of the amount of bleeding to investigate how this was affected by delayed incision following the administration of lidocaine in combination with epinephrine. The studies were carried out on pigs, human patients, and healthy volunteers.

The optimal concentration of epinephrine, in combination with a local anesthetic, to achieve adequate hypoperfusion prior to surgery was found to be about 10 μg/ml in random flaps on the pig flank, and in full-thickness eyelid flaps in pigs.
Based on the perfusion and bleeding studies, it was concluded that the optimal timing for skin incision following infiltration of lidocaine combined with epinephrine (12.5 μg/ml), is within 7 minutes. Waiting longer did not lead to a further decrease in bleeding.

All the techniques used, in this work, to measure blood flow have advantages and disadvantages, but at the present time, LSCI appears to be the best and the easiest to use for the measurement of tissue perfusion. However, there is still a need to improve techniques for the measurement of microvascular circulation.

Key words: microvascular, blood flow, perfusion, epinephrine, laser Doppler, Laser speckle contrast imaging

Classification system and/or index terms (if any)

Supplementary bibliographical information	Language: English

ISSN and key title: 1652-8220,	ISBN: 978-91-7619-703-5

Recipient's notes	Number of pages	Price
	Security classification	

Signature   Date 2018-10-02

Techniques for Measuring Perfusion during Reconstructive Surgery and Effects of Epinephrine in Local Anesthetics

Rafi Sheikh

LUND
UNIVERSITY

Coverphoto by Rafi Sheikh

"Wisdom is not a product of schooling but of the lifelong attempt to acquire it."

"Knowledge exists in two forms—lifeless, stored in books, and alive in the consciousness of men. The second form of existence is after all the essential one; the first, indispensable as it may be, occupies only an inferior position."

" I like neither new clothes nor new kinds of food."

Albert Einstein

Table of Contents

Abstract

The aims of the work presented in this thesis were to investigate different techniques for the measurement of microvascular perfusion in skin, and to quantify the effect of epinephrine on skin perfusion and bleeding during surgery, in order to determine the optimal epinephrine concentration, and the optimal delay between injection and surgical incision.

Several techniques were used to quantify skin perfusion: laser Doppler velocimetry, laser speckle contrast imaging (LSCI), high-resolution thermography and diffuse reflectance spectroscopy. In one study, LSCI was combined with RGB image analysis to measure tissue blanching and perfusion. Quantitative measurements were also made of the amount of bleeding to investigate how this was affected by delayed incision following the administration of lidocaine in combination with epinephrine. The studies were carried out on pigs, human patients, and healthy volunteers.

The optimal concentration of epinephrine, in combination with a local anesthetic, to achieve adequate hypoperfusion prior to surgery was found to be about 10 µg/ml in random flaps on the pig flank, and in full-thickness eyelid flaps in pigs.

Based on the perfusion and bleeding studies, it was concluded that the optimal timing for skin incision following infiltration of lidocaine combined with epinephrine (12.5 µg/ml), is within 7 minutes. Waiting longer did not lead to a further decrease in bleeding.

All the techniques used, in this work, to measure blood flow have advantages and disadvantages, but at the present time, LSCI appears to be the best and the easiest to use for the measurement of tissue perfusion. However, there is still a need to improve techniques for the measurement of microvascular circulation.

Papers included in this thesis

This thesis is based on the following five papers, which will be referred to in the text by their Roman numerals. The papers are reproduced with the permission of the respective publisher.

I.   **Sheikh R**, Memarzadeh K, Torbrand C, Blohme J, Malmsjo M. Hypoperfusion in response to epinephrine in local anaesthetics: *Investigation of dependence on epinephrine concentration, spread of hypoperfusion and time to maximal cutaneous vasoconstriction.* Journal of plastic, reconstructive & aesthetic surgery : JPRAS. 2017;70(3):322-9.

II. **Sheikh R**, Memarzadeh K, Torbrand C, Blohme J, Lindstedt S, Malmsjo M. *Blood Perfusion in a Full-Thickness Eyelid Flap, Investigated by Laser Doppler Velocimetry, Laser Speckle Contrast Imaging, and Thermography.* Eplasty. 2018;18:e9.

III. **Sheikh R**, Dahlstrand U, Memarzadeh K, Blohme J, Reistad N, Malmsjo M. *Optimal Epinephrine Concentration and Time Delay to Minimize Perfusion in Eyelid Surgery: Measured by Laser-Based Methods and a Novel Form of Extended-Wavelength Diffuse Reflectance Spectroscopy.* Ophthalmic Plast Reconstr Surg. 2018;34(2):123-9.

IV. Hult J, **Sheikh R**, Nguyen CD, Tenland K, Dahlstrand U, Malmsjo M. *A waiting time of 7 min is sufficient to reduce bleeding in oculoplastic surgery following the administration of epinephrine together with local anaesthesia.* Acta Ophthalmol. 2018;96(5):499-502.

V.   **Sheikh R**, Bunke J, Thorisdottir RL, Hult J, Tenland K, Gesslein B, et al. *Hypoperfusion following the injection of epinephrine in human forearm skin can be measured by RGB analysis but not with laser speckle contrast imaging.* Microvasc Res. 2018;121:7-13.

Introduction

Epinephrine is used in conjunction with local anesthetics to minimize bleeding during surgical procedures. Epinephrine has been shown to reduce bleeding (by vasoconstriction), prolong the analgesic effect, and reduce the systemic effects of the local anesthetic agents (lidocaine) (1, 2). Surgeons usually wait several minutes for epinephrine to act before commencing surgery in order to minimize bleeding. The optimal concentration of epinephrine to achieve vasoconstriction has been strenuously debated over the past 50 years (3), however, there is a lack of relevant data. As this hormone may have systemic effects, including hemodynamic disturbances such as hypertension and arrhythmia, it is important to determine the lowest concentration of epinephrine that produces local vasoconstriction (4, 5).

The optimal delay between the injection of local anesthetics containing epinephrine and skin incision, to ensure vasoconstriction and minimize bleeding, has recently also become the subject of debate. The delay commonly given in textbooks is around 7 to 10 min (6). However, in a study by McKee et al., who measured the relative hemoglobin concentration over time in the arm skin of healthy volunteers using oxygen spectroscopy, the lowest cutaneous hemoglobin level was not observed until 26 min after injection (7). In another study, McKee et al. measured the blood loss from the skin of patients undergoing carpal tunnel release surgery, and found a significant reduction in bleeding when skin incision was delayed by 30 minutes, compared to 7 minutes (8). This is in strong contrast to clinical experience from oculoplastic surgery, and if the findings of McKee et al. are truly representative, there would be a need to significantly extend the delay before commencing surgery.

In order to resolve this controversy, a reliable method of monitoring microvascular perfusion is required. It is well known that blood perfusion can be measured using laser-based techniques such as laser Doppler velocimetry (LDV) and laser speckle contrast imaging (LSCI) (9). LDV is an established technique for the measurement of blood flow during plastic surgery, in which the skin is illuminated by a beam of laser light using a fiber-optic probe. LDV is based on the change in frequency, i.e., the Doppler shift, that occurs when the laser light is scattered by moving objects (in this case, erythrocytes) (10). Light reflected by moving blood cells undergoes a change in wavelength, while light reflected from static objects is unchanged. The change in wavelength is interpreted as the velocity of blood cells, i.e. perfusion (11). However, invasive probes must be used to obtain reproducible results, and it is only possible to

study the blood flow in a very small region (~1 mm^3 surrounding the probe) (12). LDV is frequently used to measure blood flow in flaps during surgery (13), especially plastic surgery (Pietila et al., 1987), and after skin burns to assess burn wound outcome (14). The measurement depth of LDV, in forearm skin, is about 1 mm (10).

A more recently developed noninvasive technique is LSCI. The object is illuminated by laser light, and the backscattered light, which forms a random interference pattern called a speckle pattern is used to determine perfusion (15). Movement, such as the flow of red blood cells in a tissue, causes the speckle pattern to change, allowing the blood perfusion to be quantified. LSCI is a fast, full-field technique for the imaging of microvascular perfusion (12). Current LSCI equipment can produce representative images of the blood flow in the surface of tissue over a relatively large area (up to 24 x 24 cm). LSCI is now an established technique in, for example, experimental vascular brain research and plastic surgery, but has not been applied in the field of periorbital plastic surgery. However, these laser-based techniques are associated with drawbacks as they measure motion (interpreted as blood flow) in a specific tissue volume, and any movement of the patient, for example, due to breathing, will interfere with the measurements.

Thermography, employing a high-resolution infrared camera, is another non-invasive technique that can be used to measure the temperature of tissue, which can be used as a proxy for blood flow. This method is suitable for clinical use, but has not previously been applied to the study of periorbital skin.

Spectrometry has also been used to determine the degree of oxygenation by irradiating the tissue with light in the wavelength range of 500 nm to 650 nm, in which the absorption by hemoglobin changes upon oxygenation (16). The imaging depth in spectrometry is dependent on the wavelength, and varies from a few micrometers in the ultraviolet part of the spectrum to several millimeters in the near-infrared. In the red part of the visible spectrum the penetration depth in tissue is typically 3-5 mm (17).

Spectroscopy based methods, such as diffuse reflectance spectroscopy (DRS), can provide a wide range of information on tissue response, and DRS has been used to characterize tissue viability by measuring the degree of hemoglobin oxygenation (18), total hemoglobin concentration (19), and tissue hydration (20). In DRS, the tissue is illuminated with broad-spectrum light, and the diffuse reflected light is collected after interaction with the tissue. DRS is commonly used in the wavelength range 500 to 1000 nm, but in the technique developed in the present work the spectrum was extended to include wavelengths in both the visible and near-infrared regions, i.e., from 450 to 1550 nm. Tissue properties are determined based on the fact that different tissue components have different absorption characteristics. It has been shown in an initial study that it is possible to differentiate between normal and metastatic liver tissue in humans using this technique (21). The blood flow can be approximated using hemoglobin derivatives as a measure of perfusion. Traditional DRS measurements of

hemoglobin content are based on analysis at only a few wavelengths, at which the absorption of hemoglobin dominates (22).

In a previous report, Leahy described a method of visualizing red blood cell content in the microcirculation with a red, green and blue (RGB) camera, and reported a measurement depth of approximately 350 to 490 μm (23). Jakovels et al. have demonstrated that RGB analysis with a simple camera can be used to quantify the distribution of hemoglobin and perfusion dynamics (24).

Other techniques have been used for the measurement of microvascular perfusion. For example, radioisotope clearance was previously considered the gold standard for measuring skin perfusion, however, the method is no longer widely used due to the need to inject radionuclides, and the complexity of the procedure (25). To the best of our knowledge, no other method has replaced radioisotope clearance as the gold standard for skin perfusion measurements. There is thus a need to develop new techniques, preferably non-invasive ones, to quantify the response of tissue.

In this work studies have been performed on both an animal model (Papers I-III) and human subjects (Papers IV & V). The pig is considered to be a suitable animal for the study of full-thickness wounds, as the epidermis, dermis and subcutaneous fat resemble those of humans (26, 27), in contrast to small animals, which have a thick layer of fur, and a thinner epidermis and dermis. Moreover, porcine dermal collagen is similar to that in humans (28), and the skin is adherent to the deep fascia (29). However, the porcine skin on the flank is generally thicker than human skin, and is more comparable to thick skin (e.g. on the human back) than with thin skin (e.g. on the human face).

As a complement to the perfusion studies we measured the amount of bleeding (Paper IV), where we evaluated the relationship between delay before incision and the amount of bleeding, after an injection of lidocaine in combination with epinephrine.

Thesis at a glance

The studies described in this thesis are summarized in the table below.

Study	Aim	Subjects	Site	Methods
I	To study the dose-response and time-response of hypoperfusion in skin following the administration of a local anesthetic and epinephrine	Pigs	Random flaps and intact skin, in the flank of the pig	LDV and LSCI
II	To investigate the perfusion in a porcine full-thickness eyelid flap on a pedicle.	Pigs	Full-thickness eyelid flap	LDV, LSCI and high-resolution thermography
III	To study the dose-response and time-response of hypoperfusion in eyelid flaps following the administration of a local anesthetic and epinephrine	Pigs	Full-thickness eyelid flap	LDV, LSCI and DRS
IV	To determine the optimal delay, before incision following the administration of a local anesthetic with epinephrine, to minimize bleeding in oculoplastic surgery	Human patients	Eyelids in patients undergoing blepharoplasty	Bleeding quantified by collection on non-woven ocular sticks and weighing
V	To compare the ability of RGB image analysis to LSCI, for monitoring skin perfusion	Human volunteers	Volar side of the forearm	LSCI and RGB image analysis.

Aims

The aims of the work presented in this thesis were to investigate different techniques of measuring microvascular perfusion in skin, and to quantify the effect of epinephrine on perfusion and bleeding during surgery.

- The aim of Study I was to use LDV and LSCI to evaluate hypoperfusion in response to the injection of epinephrine following the administration of a local anesthetic in porcine skin. The *concentration* of epinephrine causing maximal hypoperfusion, the *spread* of hypoperfusion in the tissue, and the *time* required to reach stable hypoperfusion were investigated.

- The aim of Study II was to use LDV, LSCI and thermography to investigate the perfusion gradient in a porcine full-thickness eyelid flap on a pedicle.

- The aim of Study III was to investigate the use of LDV, LSCI and a novel form of extended-wavelength DRS to measure the effects of epinephrine in a local anesthetic on perfusion in eyelid surgery on pigs.

- The aim of Study IV was to determine the optimal delay between the administration of local anesthesia with epinephrine and skin incision, to minimize bleeding in oculoplastic surgery.

- The aim of Study V was to compare RGB image analysis to LSCI in monitoring of skin perfusion. The techniques were used to investigate the change in skin perfusion upon the administration of epinephrine in local anesthetics, which is known to cause vasoconstriction in the subcutaneous vascular plexus. The time to maximum response was evaluated.

Methods

Perfusion measurements

Laser Doppler velocimetry

LDV is based on measurements of a beam of laser light that is scattered and partly absorbed in the tissue being studied. Light that is reflected by moving blood cells undergoes a change in wavelength (Doppler shift), while light reflected from static tissue is unchanged. The magnitude and frequency distribution of these changes are related to the number and velocity of blood cells, and thus reflect perfusion (12). A filament probe (MT A500-0, straight microtip with slanted tip) was inserted 15 mm from the base of a random-pattern advancement flap in the flank of the pig, using a 22 G Venflon infusion cannula. This technique allows measurement of the perfusion in a 1 mm^3 volume surrounding the tip of the probe (13). The filament probe was attached to a master probe (Probe 418), which was connected to the main LDV unit (PF5010).

Laser speckle contrast imaging

A LSCI system (PeriCam PSI NR System) was used to obtain images of perfusion over a surface area of the tissue. The system uses a 785 nm infrared laser beam that is dispersed over the skin surface by a diffuser. A speckle pattern consisting of dark and bright areas is created by random interference of the light backscattered from the area illuminated by the laser. Blood perfusion is then determined by analyzing the variations in the speckle pattern caused by movement of the red blood cells. The speckle pattern would be stationary if there were no movements in the tissue. The changes in the speckle pattern is correlated to perfusion, and measurements are given in arbitrary units. The speckle pattern is recorded in real time at a rate of up to 100 images per second, on an area of up to 24 x 24 cm, with a resolution of up to 100 µm/pixel.

Thermography

Thermography is based on the thermal energy (infrared (IR) radiation) radiated by all objects at temperatures above absolute zero. Tissue temperature was measured using a high-resolution IR camera (FLIR A655sc), a focal plane array, an uncooled microbolometer with 640 × 480 pixel resolution, and a thermal sensitivity (noise equivalent temperature difference) of < 0.05 °C at +30 °C. In order to prevent the conduction of heat from underlying tissue, an insulating pad was placed under the flap.

Diffuse reflectance spectroscopy

Diffuse reflectance spectral signatures were collected using a portable spectroscopic system comprising a contact fiber-optical probe, a tungsten halogen light source, and two miniature spectrometers. The two spectrometers resolve light in the visible wavelength range from 350 nm to 1100 nm, and in the near infrared region from 900 nm to 1700 nm. The broader wavelength range used provides more detailed information on the response of the tissue, making it easier to measure components such as lipids, cholesterol and collagen (30), as well as the tissue water content, which is likely to change after vasoconstriction. Different parts of the diffuse reflectance spectrum represent different penetration depths and depend on the absorption of the tissue and the source-detector distance of the fiber-optic probe (31). Multivariate analysis was used to take full advantage of the spectra obtained from tissues with different levels of perfusion.] Measurements were made on full-thickness eyelid flaps in the pig.

RGB image analysis

Images of human forearm skin were obtained with a commercial digital camera recorded with a resolution of 1280 x 720 pixels, at 30 frames per second, with the white balance set to daylight mode. The light source was a professional grade LED panel (with daylight color-tint and CRI >95). Each color component, red (R), green (G), and blue (B), was analyzed separately in every frame over an area of 20 x 20 pixels. A total Euclidean color distance was calculated for each combination of RGB.

Bleeding measurements

Blood was collected from the site of skin excision on eyelids, using viscose nonwoven surgical swabs (Pro-Ophta®, ocular sticks). The ocular sticks containing the blood were weighed on calibrated scales, then the ocular sticks dry weight was subtracted to obtain the amount of blood.

Animal studies

A porcine model was used to study skin perfusion in the flank and eyelid. The pigs were a crossbred mixture of Landrace, Hampshire, Yorkshire, and Durocand pigs. They did not have pigmented skin, and had a body weight of approximately 70 kg. They underwent general anesthesia as described in Papers I-III.

The experimental protocol for these studies were approved by the Ethics Committee for Animal Research at Lund University, Sweden. All animals received humane care in compliance with the European Convention on Animal Care.

Perfusion was measured in porcine skin with LSCI and LDV, in intact skin and random flaps (Figure 1). The skin flaps were dissected superficially to the panniculus carnosus, and were thus random pattern flaps (32). Different amounts of epinephrine were used in combination with lidocaine to study the change in perfusion in order to determine the lowest concentration of epinephrine leading to maximal hypoperfusion, the spread of hypoperfusion in the tissue from the injection site, and the time to maximal cutaneous vasoconstriction (Paper I). To study the perfusion over time a single dose of lidocaine combined with epinephrine was injected into either intact skin on the pig's flank (LSCI), or in random flaps on the pig's flank (LDV and LSCI). To study the dose-response relationship, baseline measurements were made before the experiments were started, then the saline was injected as a negative control, followed by a single dose of lidocaine, and by epinephrine at increasing concentrations (0.1, 1.0, 10 and 100 µg/ml), was injected into random flaps on the pig's flank, and measurements were made with LDV and LSCI. To get a reference value equivalent to zero perfusion, a final measurement, when the blood flow had been occluded by a ligature in the base of the flap, was performed.
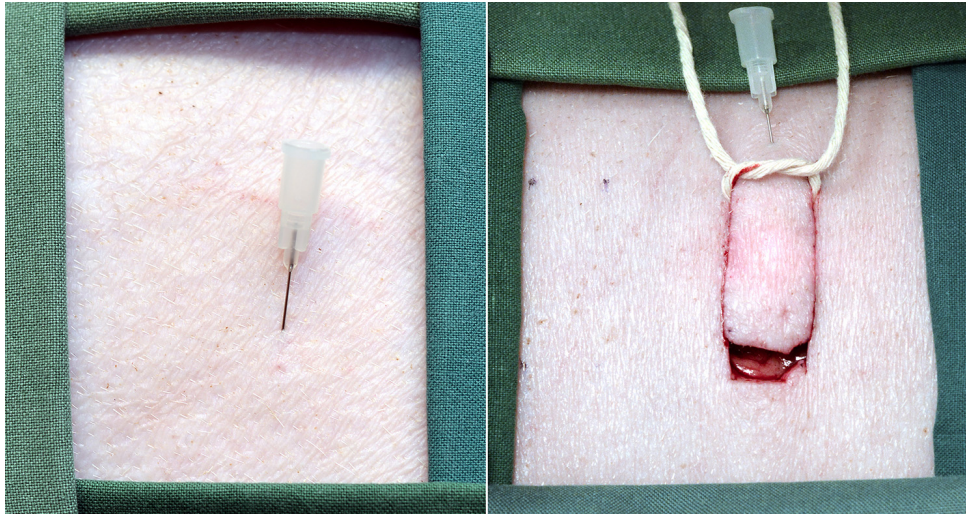
Figure 1:
The pharmacological agents were injected into intact skin (left) and the base of a 1 x 4 cm random-pattern advancement flap (right image) on the flank of the pig. (From Paper I.)

Clinically, surgical reconstruction using flaps in the periorbital area is commonly performed following the removal of a tumor from the eyelid, or to rectify entropion. The second animal study (Paper II) was therefore performed on an eyelid flap using the healthy tissue of a pig. The eyelid of the pigs was divided to produce a full-thickness axial eyelid flap on a pedicle. It was divided in a similar way to the common procedure in human blepharotomy for entropion repair, e.g. with the Quickert procedure (33), and reconstructive procedures after tumor surgery, e.g. the "switch flap", which is a full-thickness eyelid flap on a pedicle (34). Perfusion was measured with LDV, LSCI and thermography at several points along the flap (Paper II).

A full-thickness axial eyelid flap was also used to study the dose-response relationship and the time required to reach maximum hypoperfusion (Paper III). Measurements to study the dose-response were made with LDV, LSCI and DRS, and for the time-response measurements only LSCI was used. A needle was inserted into the subcutaneous tissue of the flap base, for the injection of the pharmacological agents. Saline was administered before each series of pharmacological agents, as a negative control, then a single dose of lidocaine was administered, followed by epinephrine at cumulatively increasing concentrations (0.1, 1.0, 10 and 100 μg/ml). Each series was ended with occlusion of blood flow by a ligature at the base of the flap, to get a reference value equivalent to zero perfusion.

The time to maximal hypoperfusion was measured by injecting a single dose of epinephrine (10 μg/ml) while continuously monitoring the perfusion with LSCI.

Human studies

The protocol for these experimental studies was approved by the Ethics Committee at Lund University, Sweden. The research adhered to the tenets of the Declaration of Helsinki as amended in 2008. All the patients participating in the study were given information about the study and were informed of the voluntary nature of participation. All patients gave their fully informed written consent.

Subjects were recruited from among patients admitted to the Department of Ophthalmology, Skåne University Hospital, for upper eyelid blepharoplasty for the correction of dermatochalasis during March 2017 (Paper IV). A local anesthetic was injected subcutaneously in the upper eyelid; either 1.0 ml lidocaine, or 1.0 ml lidocaine combined with 12.5 µg/ml epinephrine. In the latter case, incision was delayed by either 7, 15 or 30 minutes. After incision the amount of bleeding during the first 5 minutes was quantified by collecting blood with surgical swabs (also referred to as ocular sticks), which were weighed to determine the amount of bleeding.

The subjects in the final study (Paper V) were adult volunteers, who were classified according to the Fitzpatrick scale for skin pigmentation (35). A controlled light environment was set up before perfusion measurements were started. Perfusion was measured after the injection of a local anesthetic using LSCI or a digital camera for RGB image analysis. Three different commercially available local anesthetics were used: plain lidocaine, and lidocaine with either a low (5 µg/ml) or a high (12.5 µg/ml) concentration of epinephrine. The anesthetic solutions were injected into the subcutaneous tissue on the volar side of the forearm, at least 8 cm apart. LSCI image frames were analyzed every 30 seconds during the study period of 25 min.

Calculations and statistics

When perfusion was measured with LDV and LSCI (Papers I-III and V) the output was expressed in the arbitrary units, perfusion units (PU). To be able to compare values obtained from different series of measurements, they must be normalized. Baseline values were therefore recorded before injection of the pharmacological agents, which were defined as 100% perfusion and, when possible, 0% perfusion was defined as the value recorded when the blood flow had been occluded by a ligature in the base of the flap. After normalization, LDV and LSCI measurements was combined. The time from the injection of epinephrine until perfusion reached a plateau was calculated using nonlinear regression analysis (one-phase decay). Since the sample size was quite small, non-parametric tests were used to compare groups; i.e., the Mann-Whitney test (Paper I), the Wilcoxon matched-pair test and Friedman matched-pair test with Dunn's post-hoc test (Paper II) and the Kruskal-Wallis test (Paper III). When using thermography

(Paper II), the tissue temperature (°C) was normalized by calculating percentages of the baseline value measured at the flap base. The DRS data were analyzed by partial least-squares regression to calculate the relationship between the diffuse reflectance measurements and tissue perfusion (Paper III). Data were post-processed using the MATLAB software package. The amount of bleeding was quantified in milligrams and expressed as a percentage compared to the bleeding in the control site in each subject (Paper IV). Outliers were identified using the ROUT (Q = 1%) test, and were not included in the analysis. Statistical analysis was performed using the Kruskal-Wallis test. In Studies I-IV the calculations and statistical analysis were performed using GraphPad Prism. In Study V the RGB image analysis was performed by calculating the total Euclidean color distance for the region of interest, followed by two-phase nonlinear regression analysis to calculate the time required to reach steady state. The RGB data were post-processed using the MATLAB software package. More detailed information can be found in the appended papers.

Results

The optimal epinephrine concentration for hypoperfusion

Neither saline nor lidocaine alone resulted in any change in perfusion when measured with LDV or LSCI in skin flaps or full-thickness eyelid flaps on the pig (Papers I & III). The injection of increasing concentrations of epinephrine resulted in a gradual decrease in skin perfusion, approaching a minimum after the injection of 10 µg/ml with no further decrease at higher concentrations (figure 2). The same results were observed with a single dose of a frequently used commercially available preparation containing lidocaine combined with 12.5 µg/ml epinephrine. The DRS measurements showed a similar tissue response (Paper III).

The extent of hypoperfusion

The area of hypoperfusion following a single subcutaneous dose of 0.3 ml lidocaine with 12.5 µg/ml epinephrine, in intact skin had a radius of 12 mm from the injection site (Paper I).

The time to maximal cutaneous hypoperfusion

The time required to reach a plateau of maximum cutaneous hypoperfusion after subcutaneous injection of a single dose of lidocaine combined with 12.5 µg/ml epinephrine was 92 s in intact skin and 121 s in random flaps in pigs (figure 3)(Paper I). In full-thickness eyelid flaps, hypoperfusion reached a plateau after 75 s (Paper III).

The effect of epinephrine on perfusion was also studied in the human forearm with both LSCI and RGB image analysis (Paper V). In contrast to the findings on pigs, LSCI showed a paradoxical increase in signal after the injection of lidocaine containing epinephrine (figure 4). This was attributed to a change in the laser penetration depth resulting from blanching of the skin. However, the RGB image analysis of digital photographs gave more reliable results, showing skin blanching that corresponded to

the expected effect of epinephrine. The time required to reach maximum effect was found to be 7 minutes for 12.5 µg/ml epinephrine in lidocaine, and 9 minutes for 5 µg/ml epinephrine in lidocaine. No blanching was observed following the injection of lidocaine alone, indicating that it had no effect on perfusion.

The perfusion gradient in pig eyelid

The perfusion along a full-thickness eyelid flap was measured with LSCI, showing that the perfusion decreased gradually from the base to the tip of the eyelid flap, with most of the decrease being seen in the first 10 mm (83% 10 mm from the flap base, 79% 20 mm from the base, and 80% 30 mm from the base) (Paper II). Perfusion reached a plateau and stabilized 20 mm from the base, and no further decrease in perfusion was seen beyond this point. LDV measurements confirmed these results, showing a decrease in perfusion 15 mm from the base of the flap.

Thermographic measurements showed a corresponding decrease in temperature from the flap base to the tip of the full-thickness eyelid flap, but major difficulties associated with insulation from surrounding heat sources allowed only a few measurements to be obtained with good signal quality, and no statistical analysis was possible.

Bleeding during eyelid surgery in humans

Profuse bleeding was observed in the eyelid region during the first 5 minutes after skin incision when only lidocaine (control) was injected (Paper IV). When using lidocaine combined with epinephrine and a delay in skin incision of 7 min after injection, bleeding was markedly reduced, by 73%, during the 5 minutes after incision. No further decrease in bleeding was seen upon extending the delay of skin incision to 15 or 30 minutes (figure 5).
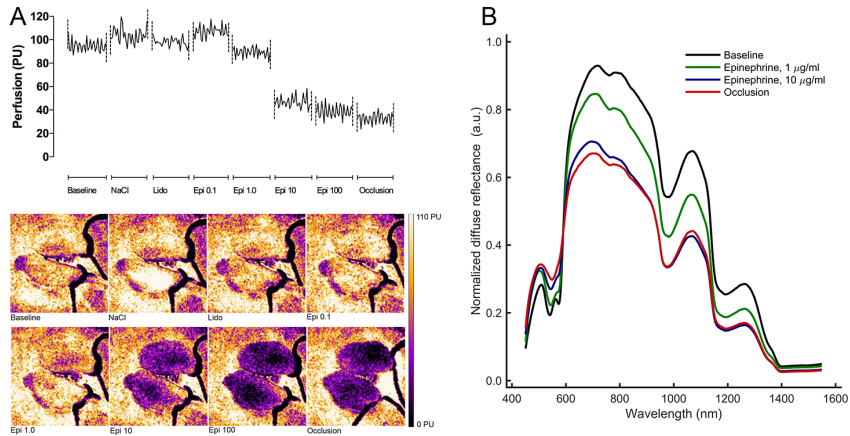
Figure 2. A.
The (upper) graph shows raw LSCI data recordings (30 s sample periods), and the (lower) images show images of the eyelid flaps obtained with LSCI, at baseline and after the injection of saline, lidocaine, and increasing concentrations of epinephrine, and after occlusion of the eyelid flap (the ligature is visible in black). It can be seen in both the graph and the images that perfusion decreases considerably after the injection of 10 μg epinephrine/ml. **B**. shows the normalized diffuse reflectance as a function of wavelength in a pig eyelid flap before (baseline) and after injecting lidocaine, followed by 1 and 10 μg/ml epinephrine, and occlusion (by ligature) of the blood flow. The curves differ, especially in the region of 550 nm, where the absorption of hemoglobin is greatest. (From Paper III.)
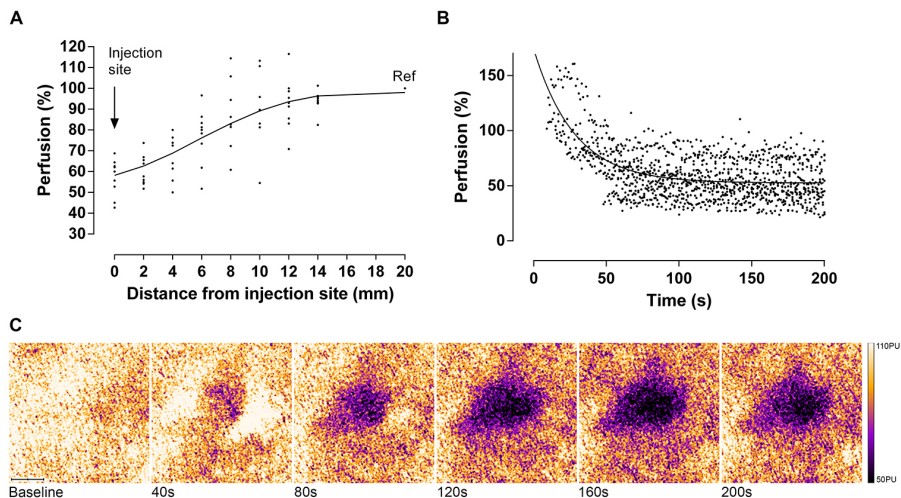

Figure 3:
Blood perfusion after a single injection of 20 mg/ml lidocaine + 12.5 μg/ml epinephrine in intact skin in pigs. A shows the distance that hypoperfusion has spread from the injection site (n=9). Perfusion was measured every 2 mm from the injection site, 120 s after injection, using LSCI. The results are expressed as a percentage compared to the signal at a reference point located 20 mm away from the injection site. It can be seen that hypoperfusion spread to a radius of 12 mm from the injection site. B shows the response in perfusion over time (n=7). Perfusion was measured in random skin flaps in the pig flank, using LSCI. Perfusion is expressed as a percentage of the baseline value before the experiments were started (defined as 100%), showing a maximum after 121 s. Data are shown for the first 200 seconds, beyond which no further change was seen. Panel C shows representative results from measurements of the spread of hypoperfusion over time and area in intact skin on the pig flank using LSCI. The results are expressed in perfusion units (PU). The scale bar (bottom left) is 10 mm. (From Paper I.)

25
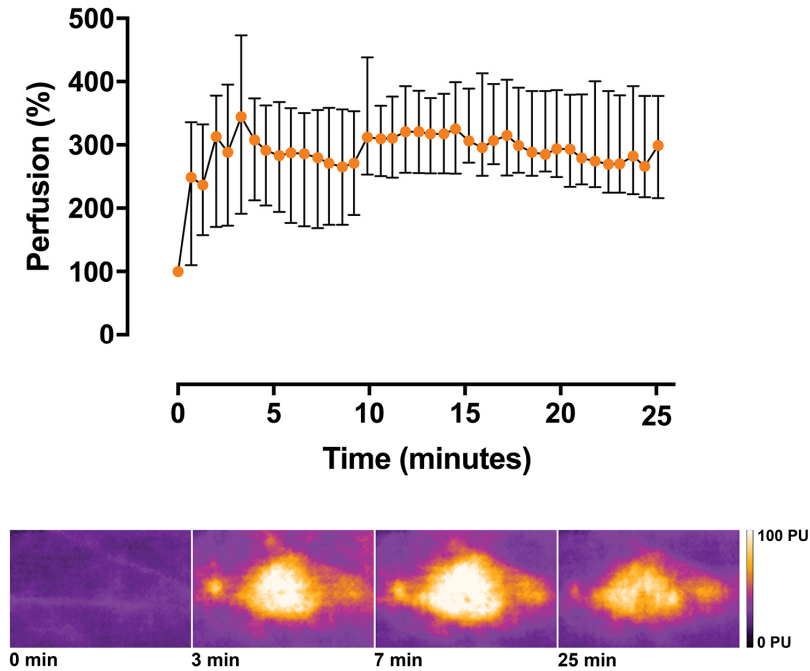
Figure 4.
Results of LSCI, showing perfusion after the injection of 20 mg/ml lidocaine +12.5 µg/ml epinephrine. Representative images are shown before injection and 3, 7, and 25 min after injection. Note the paradoxical increase in signal intensity despite the fact that epinephrine is known to induce vasoconstriction and subsequent hypoperfusion. Data are presented as median values and interquartile ranges (% of baseline values). (From Paper V.)
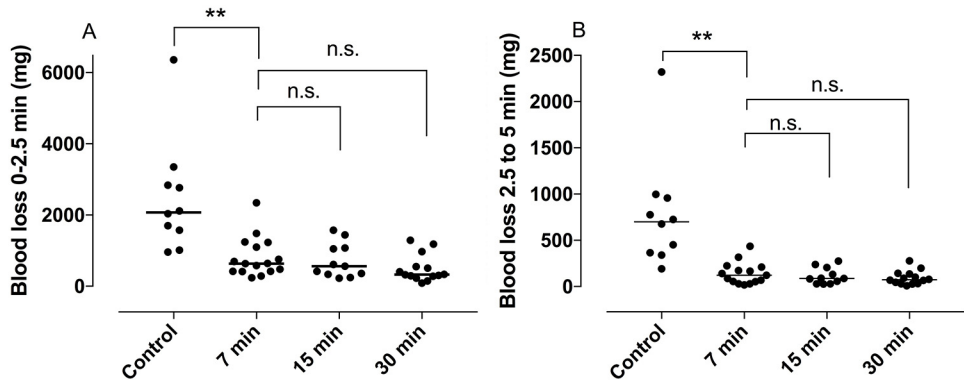
Figure 5.
The amount of blood lost after the injection of lidocaine only (control), and after the injection of lidocaine + epinephrine (12.5 µg/ml), and waiting 7, 15, or 30 minutes before incision. (A) Bleeding during the first 2.5 minutes after incision (one point for control at 6257 mg is included in the calculations, but not shown in the graph), and (B) during the second 2.5 minutes after incision (one point for control at 2320 mg is included in the calculations, but not shown in the graph). (From Paper IV.)

Discussion

Perfusion vs. dose of epinephrine

The dose-response relationship was studied to determine the optimal concentration of epinephrine in combination with lidocaine. Skin perfusion was measured in both the porcine model and in humans using several different techniques to quantify perfusion or tissue response to an injection of lidocaine combined with epinephrine. This combination is well known to cause vasoconstriction (and hypoperfusion).

LDV and LSCI were used to study perfusion in random skin flaps on the pig flank (Paper I), while LDV, LSCI and DRS were used to study perfusion in axial full-thickness eyelid flaps in the pig (Paper III). It was found in both studies that injecting increasing concentrations of epinephrine resulted in the maximal hypoperfusion in skin at a concentration of 10 µg/ml (1:100 000), with no further decrease at higher concentrations. Similar results were obtained for both the random flaps and the axial full-thickness eyelid flaps in the pig. The concentration of 10 µg/ml epinephrine is close to 12.5 µg/ml used in the common commercial preparation, Xylocaine Dental Adrenalin, which contains 12.5 µg/ml epinephrine (1:80 000). These findings are similar to those reported in previous human studies on the effect of epinephrine, where maximal local cutaneous hypoperfusion was observed at concentrations between 20 µg/ml (1:50 000) and 10 µg/ml (1:100 000) (1, 36). Similar results were obtained in the present measurements with LDV, LSCI, and with DRS tissue response. There are claims in the literature that "plain lidocaine" (i.e., without epinephrine) causes hyperperfusion (37, 38). However, this was not observed in any of the present studies (Papers I, III and V).

However, other local anesthetics with lower concentrations of epinephrine are used clinically, e.g. Xylocain® adrenalin containing 5 µg epinephrine/ml. In the clinical setting, achieving maximal vasoconstriction of the peripheral circulation must be weighed against the possibility of side effects. The use of epinephrine in local anesthetics has both advantages (minimizing bleeding during surgery) and potentially some disadvantages. There is reluctance among surgeons to inject epinephrine into a flap with a small pedicle, or into a finger due to the risk of ischemia and necrosis (39). Interestingly, hypoperfusion was observed to occur quickly in the present work (Paper III), and a plateau was reached at about 20% of the initial baseline perfusion in a full-

thickness eyelid flap, when epinephrine had been injected into the flap base, therefore the risk of ischemia and necrosis is thus minimal at least in an axial flap or tissue with similar blood supply. In accordance with these results, recent reviews suggest a more liberal attitude to the use of epinephrine in local anesthetics in surgery on the base of the finger and the hand (40, 41). Interestingly, over 100 cases of accidental injection of adrenaline into the finger have been reported in the literature, without a single case of digital necrosis (42).

In the clinical situation, achieving maximal vasoconstriction of the peripheral circulation must be weighed against the possibility of systemic effects. Nonetheless, an appropriate concentration of epinephrine should be given with the local anesthetic to minimize bleeding and to guarantee sufficient depth and duration of local anesthesia to avoid patient pain and stress. Several studies on local anesthesia with epinephrine have confirmed that although the blood pressure and heart rate may change significantly, the hemodynamic response, defined by the mean arterial blood pressure, is unchanged. No indications were found in these studies of any deleterious effects of local anesthetic formulations with epinephrine in dental patients with hypertension or cardiovascular disease (43-48). Interestingly, Knoll-Kohler et al. demonstrated that systemic norepinephrine levels (used as a measure of stress and pain) were significantly elevated following the administration of 4% articaine containing 5 µg/ml (1:200,000) epinephrine compared to 4% articaine with 10 µg/ml (1:100,000) epinephrine (49), supporting the higher dose of epinephrine in local anesthetics. In a recent study, our group has shown that local anesthetics such as lidocaine containing epinephrine have a longer duration than plain lidocaine; approximately 5-6 hours compared with 1 hour with plain lidocaine. It has been suggested that this is because it takes longer for the anesthetic to be removed from the tissue as a result of the reduced blood flow in the hypoperfused area (50). Taken together, these studies indicate that there is probably little risk of systemic toxicity when using epinephrine in local anesthetics.

Perfusion response to epinephrine over time

The change in perfusion over time following the injection of epinephrine in combination with lidocaine was evaluated in both the porcine model an in humans to determine the optimal delay before skin incision, to reduce bleeding during surgery to a minimum. The time required to reach maximal cutaneous hypoperfusion using epinephrine was evaluated in intact skin, random flaps, and in full-thickness eyelid flaps in the pig (paper I and III). Blood perfusion reached a plateau level of hypoperfusion before approximately two minutes after the injection in skin of pigs, which was quicker, and in contrast, to those obtained in recent human studies. The delay between the injection of a local anesthetic combined with epinephrine and surgical incision has recently become the subject of debate. McKee et al. claimed that, according to

spectroscopic measurements, a delay of 26 minutes was required to reach maximal vasoconstriction in the arm skin of healthy volunteers after the administration of lidocaine with 10 µg/ml epinephrine, and therefore recommended a delay of 25 minutes before commencing hand surgery (7). In a later study, they measured the quantity of blood lost after 7 min and 30 min from the time of injection of lidocaine + epinephrine to the time of incision, and found less blood loss after 30 min (8). In Study V, the results of the RGB-analysis indicated that maximum hypoperfusion of the skin was reached after 7 to 9 minutes. LSCI measurements in the same study gave paradoxical results with increased signal. We interpret this paradox as a result of *window effects*, due to bleaching where we in fact measure signals passing through the superficial skin from the deeper well perfused tissue rather than a sign of increased perfusion. The results obtained in human eyelids showed a 75% reduction in bleeding when surgical incision was delayed by 7 minutes after the injection of lidocaine in combination with epinephrine, compared to the use of plain lidocaine and waiting for 15 or 30 minutes had no further effect on bleeding (paper IV). These findings are in agreement with those in previous studies. Larrabee et al. injected epinephrine combined with lidocaine or saline into the skin of piglet trunks and measured vasoconstriction using LDV (1). They found that maximal vasoconstriction occurred at about 5 - 7 minutes in most cases, and in all cases by 10 minutes. O'Malley et al. found maximal vasoconstriction to occur after 3 - 4 minutes in the neck of patients undergoing head and neck surgery, also using LDV (51). Ghali et al. used LDV to measure blood flow in the forearm and face of healthy volunteers, and found the maximal decrease in blood flow to occur after 8 minutes in the face and 10 minutes in the forearm (38). The findings of the perfusion studies, carried out on both the porcine model and humans in the present work, showed that 7 minutes was sufficient to reach maximum vasoconstriction following the injection of lidocaine combined with epinephrine. These findings are in line with the delay traditionally applied to ensure minimal bleeding in reconstructive surgery, and the time often prescribed in textbooks (6), indicating that there is no reason to change the clinical practice of waiting 7 to 10 minutes between injection and commencing surgery.

The measurement of microvascular perfusion

Studies on microvascular blood perfusion using LDV and LSCI are difficult as they are hampered by motion artifacts resulting from breathing and other movements of the patient. LDV measures the total blood perfusion which includes capillaries, arterioles, venules and shunting vessels. LDV measurements are usually expressed as Perfusion Units, an arbitrary unit, and no current LDV instrument can provide values of the absolute perfusion, e.g. ml/min/100 g tissue. Another problem associated with LDV is that it only measures the perfusion in a very small sampling volume (~ 1 mm^3) (12),

and the blood flow in the surrounding tissue may be higher or lower than that in the volume being investigated due to variations in vascular density. It is therefore important to study perfusion with more than one technique.

Farnebo et al. showed that LDV is not a technique as reliable as microdialysis, which is a precise but invasive technique, for measuring perfusion in humans (52). It has previously been shown that measurements of cutaneous perfusion can be complicated even in baseline conditions. In skin, the microvascular bed only contains relatively few and slowly moving red blood cells and the normal blood flow is low (53). In this work it was found that, with LSCI, it was difficult to measure profound hypoperfusion in human forearm skin. The initial results obtained with LSCI were not correlated with the visible blanching of the skin, seen after the injection of local anesthetics with epinephrine, and are in direct contrast to surgical observations that epinephrine causes hypoperfusion. (Paper V). The results of the present work indicate that blanching affects the measurement depth of LSCI, leading to *a window effect,* were perfusion is actually measured deeper in the tissue, where the signal may be unchanged or even increased (paper V). Fredriksson et al. performed Monte Carlo simulations of light propagation in tissue and showed that the measurement depth in skin using LDV is dependent on the optical properties of the tissue (e.g. the red blood cell concentration and blood oxygen saturation) (10). Another, less likely, explanation of the paradoxical LSCI results could be constriction of the blood vessels by epinephrine, reducing their diameter, and thus leading to a higher velocity of blood flow. This would lead to a transient increase in the signal intensity and thus higher LSCI values. However, such a compensatory increase in blood flow is usually seen only as a short-term spike (54). LSCI thus appears not to be a suitable method for measuring severe hypoperfusion in the relatively thin skin of human forearm. Never the less, while taking these drawbacks into account, LSCI is an overall superb technique for perfusion measurements in superficial tissue.

Thermography has been used previously in plastic reconstructive procedures on other parts on the body (55-58). The change in skin temperature is believed to be proportional to changes in microcirculation, but may also be due to other metabolic processes in the cells, such as inflammatory responses and thermoregulatory enzymes. The change in temperature following the sudden obstruction of blood flow following surgical dissection will therefore be delayed, compared with the change in perfusion seen when using LDV or LSCI. This delay was observed during the measurements on eyelid flaps in pigs (Paper II). The change in temperature was therefore deemed not to be as reliable as the change in perfusion measured with the laser-based methods.

Full-thickness eyelid flaps include all layers (including the anterior and posterior lamellae with skin, the orbicularis muscle, the tarsal plate and conjunctiva) and vascular structures (including the tarsal plexus). The results in this work showed that perfusion is only decreased to approximately 80% over the length of the full-thickness eyelid flaps,

indicating good perfusion in the entire flap. The reason for this well-maintained perfusion is presumably that the flap is well vascularized, with blood supply through the base attachment, in this case at the lateral canthus, where a branch of the lateral palpebral artery supplies the tarsal arcades (59-61). The rich vascular supply of the periorbital region is probably the reason why full-thickness eyelid flap are so permissive, and that ischemia and necrosis are seldom seen post-surgically (62). This allows the use of surgical reconstructions whose design would be inappropriate in other areas of the body (6, 59-63). It is probable that the dissection of a skin flap (non full-thickness) on the eyelid, will have less perfusion as a result of less vasculature entering it. It is well known that there is a steep decrease in skin perfusion along the length of flaps that are not composite grafts. In a previous study on random advancement flaps on the flank of the pig, perfusion was found to be only 40% 20 mm from the flap base (64).

In addition to LDV and LSCI, the tissue response after local anesthesia in oculoplastic surgery was measured with DRS employing an extended wavelength range, and the results were compared to those obtained with conventional laser-based techniques (Paper III). The results showed a clear change in the reflectance spectra after the decrease in perfusion resulting from the injection of epinephrine, indicating that DRS can be used to monitor the tissue response upon changes in blood perfusion. Similar results regarding the dose-response relationship between epinephrine and hypoperfusion were obtained to those with LDV and LSCI.

A study carried out on the hand of 14 subjects using a RGB camera during upper limb occlusion at 50 and 250 mm Hg, has indicated the possibility of visualizing the hemodynamics of subsurface skin tissue (65). A novel method of monitoring the change in oxygen saturation has also been presented using RGB analysis. The change in oxygen saturation in five healthy volunteers was accurately quantified and compared to the values given by a conventional pulse oximeter, indicating that it is possible to use an RGB camera to determine changes in oxygen saturation (66). Farnebo et al., described a technique for monitoring the hyperemic response in the skin upon reperfusion after occlusion of the forearm (53), while Zhai et al. used 'colorimetry' to measure blanching after the administration of topical steroids (67). RGB image analysis was used in the present work to measure the effect of epinephrine on perfusion (Paper V). One limitation of this study is that it is not known whether the change in color is directly related to a change in perfusion or the velocity of the blood cells, vasoconstriction, or a change in red blood cell concentration. However, the observed changes in color corresponded well with previous work done on perfusion measurements. This indicates that the change in color was the result of hypoperfusion, i.e. a reduction in blood volume and flow, and thus the number of erythrocytes. Still, skin color changes were only monitored using a digital camera. One way of ensuring that hypoperfusion had been attained would have been to confirm the lack of bleeding surgically at various times. However, this is not ethically justified in healthy subjects. Another limitation of the present work is the lack of a gold standard technique, available for microvascular

perfusion measurements, for validating the RGB image analysis technique. The only non-invasive and non-contact measurement technique available is LSCI, which is hampered by motion artifacts and a non-linear relationship between signal intensity and skin blood flow at low perfusion rates (Briers et al., 2013). LSCI is based on both blood velocity and the concentration of red blood cells, but the signal is more sensitive to changes in blood velocity than red blood cell concentration (68). Another technique commonly used for validation is a pulse oximeter, which is based on spectroscopy at wavelengths of 660 and 940 nm. It is non-invasive, but depends on skin contact (69). The drawback of this technique is that it only measures the level of oxygenation of the tissue, and thus provides only an indirect measurement of perfusion. Monitoring the blanching of the skin by color analysis appears to be a simple and reliable technique for measuring hypoperfusion. Commercial digital cameras are compact, cheap, and easy to use, making them suitable for use in the clinical situation. They also allow non-invasive measurements, and do not need to be in direct contact with the skin. This constitutes a significant advantage over equipment in which contact probes are used.

Conclusions and Future Outlook

The optimal concentration of epinephrine in local anesthetics

Studies on the dose-response relationship between epinephrine and hypoperfusion showed that the optimal concentration of epinephrine in combination with local anesthetics to achieve adequate vasoconstriction prior to surgery is about 10 μg/ml.

The timing of skin incision after injection of local anesthetics combined with epinephrine

Based on the perfusion and bleeding studies, it was concluded that the optimal timing for skin incision following infiltration of lidocaine combined with 12.5 μg/ml epinephrine is 7 minutes. Waiting longer does not lead to a further decrease in bleeding.

Methods of measuring microvascular perfusion

- LSCI is a non-invasive and easy method of measuring superficial microvascular perfusion in skin, but motion artifacts must be considered.

- LDV is a reliable technique but it can only measure perfusion in a very small tissue volume, and is also sensitive to motion artifacts.

- DRS is a promising technique for measuring the characteristics of tissue, but it only provides information on the indirect effects of changes in perfusion.

- Thermography has the potential to quantify changes in perfusion in tissue, but is impractical as it is very sensitive to radiant heat from surrounding sources.

- RGB image analysis provides a simple way of measuring changes in superficial perfusion of the skin but, like DRS, it only measures the effect of changes, and not the actual blood flow.

In conclusion, all the techniques used to quantify changes in blood flow in the present work have drawbacks, but, at the present time, LSCI appears to be the best and most easily applied technique for quantifying microvascular blood flow.

Future outlook

The work described in this thesis was focused on the measurement of tissue perfusion. However, in the clinical situation tissue viability is often of greater interest, especially in plastic surgery. Further research is thus required to develop a reliable technique for the measurement of microvascular circulation. DRS is a promising technique for the measurement of tissue characteristics, while photoacoustic imaging is another, relatively new technique which, in combination with high-frequency ultrasound enables detailed two- and three-dimensional imaging of tissue. Most previous work done on photoacoustic imaging is animal studies, but we have recently made a clinical translation of a novel photoacoustic imaging system for examining human subject. A pilot project showed promising results on measuring perfusion and oxygenation in the tissue of a human forearm while injecting lidocaine in combination with epinephrine. With this technique we hope that it will be possible to quantify both tissue perfusion and other qualities to estimate tissue viability.

Populärvetenskaplig sammanfattning
Popular scientific summary in Swedish

Syftet med detta arbete var att testa olika tekniker för att mäta mikrovaskulärt blodflöde i huden, men även att mäta de kärlsammandragande effekterna av adrenalin. Lidokain, som ofta kallas tandläkarbedövning, kombineras ofta just med adrenalin för att minska blödningsmängden under kirurgi, men även för att förlänga tiden med bedövningseffekt.

Adrenalin är en substans som även finns naturligt i kroppen och som har kärlsammandragande effekter, vilket minskar blodflödet i vävnaden och därmed minskas blödningsmängden under kirurgiska ingrepp. När vävnaden får ett mindre blodflöde så minskar även bortsköljningen av själva bedövningsmedlet och man får därmed en förlängd tid med god bedövningseffekt. Nyttan med att tillsätta adrenalin är således dubbel.

Det tar dock en viss tid, från att bedövningsmedlet med adrenalin injicerats, tills maximal blodflödesminskande effekt uppnås, och det krävs således en viss fördröjning innan operationen kan påbörjas. Man har sedan länge ansett att det är tillräckligt att vänta ca 7–10 minuter för att uppnå denna effekt. Nyligen har denna väntetid dock ifrågasatts av vissa forskare, som istället hävdat att man för att uppnå maximal blodflödesminskning bör vänta en avsevärt längre tid, upp emot en halvtimme, innan kirurgin bör påbörjas. Om detta är korrekt borde man inom sjukvården behöva ändra sina rutiner.

I detta arbete har det med hjälp av flera olika mätmetoder studerats hur det mikrovaskulära blodflödet i hud och ögonlock påverkas av adrenalin, som tillsatts till Lidokain. Det undersöktes vilken koncentration av adrenalin som ger maximal blodflödesminskning och hur lång tid det tar att uppnå detta efter injektion. Studierna är utförda dels på en djurmodell och dels på människa, och samtliga genomgick prövning och var godkända av en etisk prövningsnämnd. Den djurmodell som användes var grisar, som hela tiden hölls sövda och bevakades av narkoskunnig personal och veterinär. Direkt efter att försöken genomförts avlivades djuren utan dröjsmål.

De tekniker som användes för att mäta effekter av blodflödesförändringar var Laser Doppler Velocimetry (LDV), Laser Speckle Contrast Imaging (LSCI), högupplöst termografi, diffus reflektansspektroskopi (DRS) och RGB-analys. Som komplement till

dessa mättekniker gjordes det även mätningar av blödningsmängden i samband med ögonlockskirurgi.

LDV och LSCI är båda laserbaserade mättekniker som med hjälp av infraröd laser mäter rörelse i vävnaden, vilket till största delen utgörs av de röda blodkropparnas rörelser i blodet, och det ger således ett mått av blodflödet i vävnaden. LDV-utrustningen är oftast konstruerad så att det krävs att en optisk fiber förs in i vävnaden, genom vilken laserstrålarna sedan går. Detta ger då en genomsnittlig mätning i en mycket liten vävnadsvolym. LSCI är konstruerad lite annorlunda även om tekniken till stor del baseras på samma sorts signaler som LDV. Vid användning av LSCI belyses en större yta, upp till 24x24 cm, med infrarött laserljus, som reflekteras av vävnaden. Den reflekterade signalen innehåller alltid en speciell sorts brus vars karaktär förändras beroende av rörelser, blodflödet, i vävnaden. En fördel med LSCI är att mätning sker från ett visst avstånd och inte kräver direktkontakt med vävnaden och att det går att mäta blodflöde över ett relativt stort område samtidigt. Både LDV och LSCI är dock mycket känsliga för rörelsestörningar som t ex vibrationer, andningsrörelser eller annat som påverkar mätobjektet.

I det här arbetet har även högupplöst termografi testats, där en värmekamera detekterar de mycket små temperaturskillnader som uppstår i samband med att blodflödet i huden ändras. Tekniken är dock mycket känslig för störande strålningsvärme från omgivningen, och det visade sig svårt att få tillförlitliga mätningar.

Ytterligare en mätteknik som användes var DRS, som fungerar genom att vävnaden belyses med en lampa med vitt ljus av mycket hög kvalitet. Ljuset leds till vävnaden genom en optisk fiber som sätts mot huden och sedan fångas det reflekterade ljuset upp genom andra fibrer och leds till en spektrometer (en slags avancerad färgmätare). Tekniken bygger, till skillnad från de tidigare nämnda laserbaserade teknikerna, på vitt ljus som är en blandning av färger. Varje färg kan även beskrivas som en viss våglängd. När ljus bryts genom ett prisma, eller när en regnbåge bildas på himmlen, så delas ljuset upp i de olika våglängderna (färgerna) som ljuset varit en blandning av och bildar ett spektrum av våglängder från violett till rött. Vid spektroskopi mäts egentligen bara med vilken relativ styrka varje våglängd (färg) lyser. DRS-tekniken utnyttjar att olika ämnen reflekterar ljus av olika våglängder olika mycket och det skapas således ett specifikt reflektansspektrum för varje vävnad (med våglängder inom det omfång som spektrometern klarar av att detektera). I detta arbete har det använts ett extra brett spektrum, d v s med ett extra stort omfång, för att mer noggrant kunna detektera förändringar som sker i vävnaden. En fördel, men även en nackdel, med DRS är att när de spektroskopiska egenskaperna i vävnaden mäts vid en viss tidpunkt, så kan spektrumet ge mycket annan information än enbart det som är relaterat till blodflödesförändringar. Nackdelen är framför allt att det inte är själva flödet, d v s rörelsen, i vävnaden som mäts utan snarare den indirekta effekt som blodflödesförändringarna medför i vävnaden.

En sista teknik som använts i det här arbetet är analys av RGB-bilder, inspelade med en helt vanlig digitalkamera. Det som spelades in var förloppet av förändringar i huden, efter att lokalbedövning med adrenalin injicerats. Genom att sedan analysera färgförändringarna i ett specifikt område av pixlar i bilderna, så kunde effekten av den blodflödesförändring som injektionen medförde mätas.

En av slutsatserna från studierna på gris var att 10 µg/ml är den optimala koncentrationen av adrenalin att använda i samband med kirurgi i huden eller på ögonlock, för att åstadkomma maximal blodflödesminskning. Blodflödesmätningar (på både gris och människa) och blödningsmätningar (på människa) visade att maximal blodflödesminskning fås inom sju minuter efter injektion av adrenalin (12,5 µg/ml) och ingen ytterligare minskning av blödningsmängden sker om man väntar ännu längre tid med att påbörja kirurgi.

Alla tekniker som användes för blodflödesmätningar i detta arbete har sina för- och nackdelar, men sammantaget är det LSCI som just nu är det smidigaste sättet att mäta ytligt blodflöde i vävnad med. Det finns dock ännu stor förbättringspotential inom området mikrovaskulär blodflödesmätning.

Acknowledgements

Nåväl. Jag får väl bjuda på lite ödmjukhet också. Som vanligt så hade inte mycket blivit av om det inte vore för alla ni som på ett eller annat sätt bidragit med olika former av pusselbitar som slutligen lett fram till skapandet av denna avhandling. Och här skippar vi utrikiskan till förmån för modersmål. Tyvärr blir det bitvis lite sentimentalt, men det får man ha överseende med.

Malin Malmsjö, professor. Min huvudhandledare. Utan din optimism och extremt framåtdrivande anda, så hade jag kanske ännu funderat på om jag skulle hoppa på det där mer forskning, eller om jag bara istället skulle satsa på att bli den bästa kliniker som gått i ett par skor. Jag inspireras av din förmåga att hela tiden se lösningar istället för problem. Du har en ovanligt förfinad talang att förverkliga det som för dom flesta skulle verka omöjligt.

Jonas Blohmé, min biträdande handledare. Du är okuloplastik- och strabismkirurgins motsvarighet till Ingemar Stenmark, dvs man får ändra spelreglerna om de andra ska ha en chans att hänga med. Du är kanske den mest allmänbildade filur jag stött på. ”Tystnad tagning” är inte riktigt ditt måtto.

Ulf Dahlstrand, min forskningsmässiga wingman. Tekniskt bevandrad som få andra kollegor. Du har en förmåga att alltid se de små men avgörande detaljerna. Lite av en motvillig optimist.

Josefine Bunke, forskarkollega och doktorand i gruppen. Du är målinriktad som få och har en enorm framåtanda som ger energi till alla projekt du är delaktig i.

Cu Dybelius Ansson, forskarkollega och doktorand i gruppen. Mer pålitlig forskarkollega får man nog leta länge efter. Han är ordning och reda personifierad. Får se hur det går nu när han lämnat över regeringsmakten i sitt tidigare herravälde av tid.

Jenny Hult, forskarkollega och doktorand i gruppen. Ihärdig och målmedveten som få. En av våra grafiker i gruppen, dock den med lite mer estetisk talang.

Kajsa Tenland, forskarkollega och doktorand i gruppen. Bidrar med positiv laganda till gruppen. Har en precis lagom balans avseende detaljer.

Johanna Berggren, forskarkollega och doktorand i gruppen. En nitisk optimist som egentligen är alldeles för vänlig och tillmötesgående.

Khashayar Memarzadeh, forskarkollega och doktorand i gruppen. Mannen som stakade ut vägen för oss andra som hoppade på tåget senare. Han har nog störst hjärta av alla!

Magdalena Naumovska, forskarkollega och doktorand i gruppen. Kliniskt även min adept. Envis och mycket målmedveten. Mycket välorganiserad, men behöver ännu vänja sig lite vid att millennieskiftet skedde för snart 20 år sedan, pdf, pdf, pdf!

Rannveig Linda Thorisdottir, forskarkollega och doktorand i gruppen. Har en positiv attityd och ett sinne för detaljer.

Tove Faxén, forskarkollega i gruppen. Bidrar med positiv och vänlig stämning i gruppen.

Sandra Lindstedt Ingemansson, docent och forskarkollega. Forskningsmässigt en senior kollega som vi har ett mycket gott och avgörande samarbete med.

Karl Engelsberg, forskarkollega. Möjliggöraren som gör mer än det som märks. Alltid behjälplig och positiv. Har ofta goda tankar som han delar med sig av.

Björn Hammar, forskarkollega. Troligen för evigt en "ivrig tonårspojke" när han ska kasta sig in i ett nytt projekt, men även en kameleont som anpassar sig efter miljö. Språknörd som få, har bl a pluggat främmande språk och är mycket allmänbildad. Har pedagogiska ambitioner som få andra!

Nina Reistad, docent och forskarkollega på atomfysik i Lund. Kan allt om spektrum och troligen lite mer! Har varit en enorm resurs och bidragit till bryggan mellan medicin och fysik.

Christian Torbrand, forskarkollega. Bidragit med många goda råd, ff a på stordjurs-avdelningen.

Bodil Gesslein, forskarkollega i gruppen. Bidrar med ovärderligt stöd till alla i gruppen och ovärderlig organisatorisk förmåga.

Poya Hård af Segerstad, forskarkollega. En filosof av rang! En mycket god vän som i viss mån är min motsats. Vi kommer ha många roliga år framöver i teamet.

Boel Bengtsson, docent och forskarkollega. En förebild och forskningsmässigt en superstar! Få andra har bedrivit forskning som haft så stor betydelse i vardagen för ögonsjukvården. Dessutom mycket trevlig!

Anders Heilj, professor. En förebild! En forskningsmässig superstar precis som Boel. Det är dessutom ett privilegium att ha haft honom som klinisk handledare på glaukommottagningen. Jag gillar även hans humor.

Magnus Cinthio, docent och forskarkollega. En man som får saker att ske! En ovärderlig resurs i de projekt vi har framför oss.

References

1. Larrabee WF, Jr., Lanier BJ, Miekle D. Effect of epinephrine on local cutaneous blood flow. Head & neck surgery. 1987;9(5):287-9.

2. Fink BR, Aasheim GM, Levy BA. Neural pharmacokinetics of epinephrine. Anesthesiology. 1978;48(4):263-6.

3. Brown RS, Rhodus NL. Epinephrine and local anesthesia revisited. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2005;100(4):401-8.

4. Bhatia N, Ghai B, Mangal K, Wig J, Mukherjee KK. Effect of intramucosal infiltration of different concentrations of adrenaline on hemodynamics during transsphenoidal surgery. Journal of anaesthesiology, clinical pharmacology. 2014;30(4):520-5.

5. Bader JD, Bonito AJ, Shugars DA. A systematic review of cardiovascular effects of epinephrine on hypertensive dental patients. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2002;93(6):647-53.

6. Collin R, Rose GA. Plastic and orbital surgery. London: BMJ Books; 2001. viii, 192 s. p.

7. McKee DE, Lalonde DH, Thoma A, Glennie DL, Hayward JE. Optimal time delay between epinephrine injection and incision to minimize bleeding. Plast Reconstr Surg. 2013;131(4):811-4.

8. McKee DE, Lalonde DH, Thoma A, Dickson L. Achieving the optimal epinephrine effect in wide awake hand surgery using local anesthesia without a tourniquet. Hand (N Y). 2015;10(4):613-5.

9. Zotterman J, Bergkvist M, Iredahl F, Tesselaar E, Farnebo S. Monitoring of partial and full venous outflow obstruction in a porcine flap model using laser speckle contrast imaging. Journal of plastic, reconstructive & aesthetic surgery : JPRAS. 2016;69(7):936-43.

10. Fredriksson I, Larsson M, Stromberg T. Measurement depth and volume in laser Doppler flowmetry. Microvasc Res. 2009;78(1):4-13.

11. Leahy MJ, de Mul FF, Nilsson GE, Maniewski R. Principles and practice of the laser-Doppler perfusion technique. Technology and health care : official journal of the European Society for Engineering and Medicine. 1999;7(2-3):143-62.

12. Allen J, Howell K. Microvascular imaging: techniques and opportunities for clinical physiological measurements. Physiol Meas. 2014;35(7):R91-R141.

13.   Zografos GC, Martis K, Morris DL. Laser Doppler flowmetry in evaluation of cutaneous wound blood flow using various suturing techniques. Annals of surgery. 1992;215(3):266-8.

14.   Jaskille AD, Ramella-Roman JC, Shupp JW, Jordan MH, Jeng JC. Critical review of burn depth assessment techniques: part II. Review of laser doppler technology. Journal of burn care & research : official publication of the American Burn Association. 2010;31(1):151-7.

15.   Yamamoto Y, Ohura T, Nohira K, Sugihara T, Minakawa H, Igawa H, et al. Laserflowgraphy: a new visual blood flow meter utilizing a dynamic laser speckle effect. Plast Reconstr Surg. 1993;91(5):884-94.

16.   Kasler M, von Glass W, Albrecht HP, Lang T. [Noninvasive intraoperative measurement of intracapillary hemoglobin oxygenation and relative hemoglobin concentration in surgical skin flaps]. HNO. 1990;38(10):375-8.

17.   Randeberg LL. Diagnostic applications of diffuse reflectance spectroscopy [Doctoral Theses at NTNU]. Trondheim: Norwegian University of Science and Technology; 2005.

18.   Myers DE, Anderson LD, Seifert RP, Ortner JP, Cooper CE, Beilman GJ, et al. Noninvasive method for measuring local hemoglobin oxygen saturation in tissue using wide gap second derivative near-infrared spectroscopy. J Biomed Opt. 2005;10(3):034017.

19.   Glennie DL, Hayward JE, Farrell TJ. Modeling changes in the hemoglobin concentration of skin with total diffuse reflectance spectroscopy. J Biomed Opt. 2015;20(3):035002.

20.   Egawa M, Arimoto H, Hirao T, Takahashi M, Ozaki Y. Regional difference of water content in human skin studied by diffuse-reflectance near-infrared spectroscopy: consideration of measurement depth. Applied spectroscopy. 2006;60(1):24-8.

21.   Reistad N, Nilsson J, Timmermand OV, Sturesson C, Andersson-Engels S. Diffuse reflectance spectroscopy of liver tissue. Proc Spie. 2015;9531.

22.   Phelps JE, Vishwanath K, Chang VT, Ramanujam N. Rapid ratiometric determination of hemoglobin concentration using UV-VIS diffuse reflectance at isosbestic wavelengths. Optics express. 2010;18(18):18779-92.

23.   Leahy M, ODoherty J, Nilsson G, Henricson J, Sjöberg F. A new method for visualizing red blood cell content in the microcirculation. SPIE newsroom. The International Society for Optical Engineering. 2006;2006.

24.   Jakovels D, Spigulis J, Rogule L, editors. RGB mapping of hemoglobin distribution in skin. European Conferences on Biomedical Optics; 2011: SPIE.

25.   Pan X, Chen G, Wu P, Han C, Ho JK. Skin perfusion pressure as a predictor of ischemic wound healing potential. Biomed Rep. 2018;8(4):330-4.

26.   Winter GD. A Study of wound healing in the domestic pig: University of London; 1966.

27. Hartwell SW. A mechanism of healing in human wounds. Springfield, IL: Charles Thomas; 1955.

28. Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society. 2001;9(2):66-76.

29. PATTERSON TJS. THE SURVIVAL OF SKIN FLAPS IN THE PIG. British Journal of Plastic Surgery. 1969;21(2):113-7.

30. Wilson RH, Nadeau KP, Jaworski FB, Tromberg BJ, Durkin AJ. Review of short-wave infrared spectroscopy and imaging methods for biological tissue characterization. J Biomed Opt. 2015;20(3):030901.

31. Hennessy R, Goth W, Sharma M, Markey MK, Tunnell JW. Effect of probe geometry and optical properties on the sampling depth for diffuse reflectance spectroscopy. J Biomed Opt. 2014;19(10):107002.

32. Stell PM. The pig as an experimental model for skin flap behaviour: a reappraisal of previous studies. British journal of plastic surgery. 1977;30(1):1-8.

33. Quickert M. The eyelids. Malmposition of the lid. In: Sorby A, editor Modern Ophthalmology, 2nd ed London: Butterworth, v 4A, sec II. 1972:937-54.

34. Stafanous SN. The switch flap in eyelid reconstruction. Orbit. 2007;26(4):255-62.

35. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol. 1988;124(6):869-71.

36. Liu S, Carpenter RL, Chiu AA, McGill TJ, Mantell SA. Epinephrine prolongs duration of subcutaneous infiltration of local anesthesia in a dose-related manner. Correlation with magnitude of vasoconstriction. Regional anesthesia. 1995;20(5):378-84.

37. Gessler EM, Hart AK, Dunlevy TM, Greinwald JH, Jr. Optimal concentration of epinephrine for vasoconstriction in ear surgery. The Laryngoscope. 2001;111(10):1687-90.

38. Ghali S, Knox KR, Verbesey J, Scarpidis U, Izadi K, Ganchi PA. Effects of lidocaine and epinephrine on cutaneous blood flow. Journal of plastic, reconstructive & aesthetic surgery : JPRAS. 2008;61(10):1226-31.

39. Bunnell S, Howard LD. Surgery of the hand. Philadelphia: Lippincott. 1944.

40. Thomson CJ, Lalonde DH, Denkler KA, Feicht AJ. A critical look at the evidence for and against elective epinephrine use in the finger. Plast Reconstr Surg. 2007;119(1):260-6.

41. Hagert E, Lalonde D. [Time to bury the adrenaline-myth!--Safe use of adrenaline anesthesia in hand surgery and orthopedics]. Lakartidningen. 2015;112.

42. Muck AE, Bebarta VS, Borys DJ, Morgan DL. Six years of epinephrine digital injections: absence of significant local or systemic effects. Annals of emergency medicine. 2010;56(3):270-4.

43. Chernow B, Balestrieri F, Ferguson CD, Terezhalmy GT, Fletcher JR, Lake CR. Local dental anesthesia with epinephrine. Minimal effects on the sympathetic nervous system or on hemodynamic variables. Archives of internal medicine. 1983;143(11):2141-3.

44. Niwa H, Satoh Y, Matsuura H. Cardiovascular responses to epinephrine-containing local anesthetics for dental use: a comparison of hemodynamic responses to infiltration anesthesia and ergometer-stress testing. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2000;90(2):171-81.

45. Meyer FU. Haemodynamic changes under emotional stress following a minor surgical procedure under local anaesthesia. International journal of oral and maxillofacial surgery. 1987;16(6):688-94.

46. Cioffi GA, Chernow B, Glahn RP, Terezhalmy GT, Lake CR. The hemodynamic and plasma catecholamine responses to routine restorative dental care. J Am Dent Assoc. 1985;111(1):67-70.

47. Davenport RE, Porcelli RJ, Iacono VJ, Bonura CF, Mallis GI, Baer PN. Effects of anesthetics containing epinephrine on catecholamine levels during periodontal surgery. Journal of periodontology. 1990;61(9):553-8.

48. Lipp M, Dick W, Daublander M, Fuder H, Stanton-Hicks M. Exogenous and endogenous plasma levels of epinephrine during dental treatment under local anesthesia. Regional anesthesia. 1993;18(1):6-12.

49. Knoll-Kohler E, Fortsch G. Pulpal anesthesia dependent on epinephrine dose in 2% lidocaine. A randomized controlled double-blind crossover study. Oral surgery, oral medicine, and oral pathology. 1992;73(5):537-40.

50. Bunke J, Sheikh R, Hult J, Malmsjo M. Buffered local anesthetics reduce injection pain and provide anesthesia for up to 5 hours. Journal of plastic, reconstructive & aesthetic surgery : JPRAS. 2018;71(8):1216-30.

51. O'Malley TP, Postma GN, Holtel M, Girod DA. Effect of local epinephrine on cutaneous bloodflow in the human neck. The Laryngoscope. 1995;105(2):140-3.

52. Farnebo S, Zettersten EK, Samuelsson A, Tesselaar E, Sjoberg F. Assessment of blood flow changes in human skin by microdialysis urea clearance. Microcirculation. 2011;18(3):198-204.

53. Farnebo S, Thorfinn J, Henricson J, Tesselaar E. Hyperaemic changes in forearm skin perfusion and RBC concentration after increasing occlusion times. Microvasc Res. 2010;80(3):412-6.

54. Kairinos N, Voogd AM, Botha PH, Kotze T, Kahn D, Hudson DA, et al. Negative-pressure wound therapy II: negative-pressure wound therapy and increased perfusion. Just an illusion? Plast Reconstr Surg. 2009;123(2):601-12.

55. de Weerd L, Mercer JB, Setsa LB. Intraoperative dynamic infrared thermography and free-flap surgery. Annals of plastic surgery. 2006;57(3):279-84.

56. de Weerd L, Miland AO, Mercer JB. Perfusion dynamics of free DIEP and SIEA flaps during the first postoperative week monitored with dynamic infrared thermography. Annals of plastic surgery. 2009;62(1):42-7.

57. de Weerd L, Weum S, Mercer JB. The value of dynamic infrared thermography (DIRT) in perforatorselection and planning of free DIEP flaps. Annals of plastic surgery. 2009;63(3):274-9.

58. de Weerd L, Weum S, Mercer JB. Dynamic Infrared Thermography (DIRT) in the preoperative, intraoperative and postoperative phase of DIEP flap surgery. Journal of plastic, reconstructive & aesthetic surgery : JPRAS. 2012;65(5):694-5; author reply 5-6.

59. Codner MA, McCord CD, Mejia JD, Lalonde D. Upper and lower eyelid reconstruction. Plast Reconstr Surg. 2010;126(5):231e-45e.

60. Erdogmus S, Govsa F. The arterial anatomy of the eyelid: importance for reconstructive and aesthetic surgery. Journal of plastic, reconstructive & aesthetic surgery : JPRAS. 2007;60(3):241-5.

61. Lopez R, Lauwers F, Paoli JR, Boutault F, Guitard J. The vascular system of the upper eyelid. Anatomical study and clinical interest. Surgical and radiologic anatomy : SRA. 2008;30(3):265-9.

62. Patrinely JR, Marines HM, Anderson RL. Skin flaps in periorbital reconstruction. Survey of ophthalmology. 1987;31(4):249-61.

63. McCord C, Nunnery W. Reconstructive procedures or the lower eyelid and outer canthus. Oculoplastic surgery. 1981;New York Raven Press:194-8.

64. Memarzadeh K, Sheikh R, Blohme J, Torbrand C, Malmsjo M. Perfusion and Oxygenation of Random Advancement Skin Flaps Depend More on the Length and Thickness of the Flap Than on the Width to Length Ratio. Eplasty. 2016;16:e12.

65. Nishidate I, Tanaka N, Kawase T, Maeda T, Yuasa T, Aizu Y, et al. Noninvasive imaging of human skin hemodynamics using a digital red-green-blue camera. J Biomed Opt. 2011;16(8):086012.

66. Guazzi AR, Villarroel M, Jorge J, Daly J, Frise MC, Robbins PA, et al. Non-contact measurement of oxygen saturation with an RGB camera. Biomed Opt Express. 2015;6(9):3320-38.

67. Zhai H, Chan HP, Farahmand S, Nilsson GE, Maibach HI. Comparison of tissue viability imaging and colorimetry: skin blanching. Skin Res Technol. 2009;15(1):20-3.

68. Tew GA, Klonizakis M, Crank H, Briers JD, Hodges GJ. Comparison of laser speckle contrast imaging with laser Doppler for assessing microvascular function. Microvasc Res. 2011;82(3):326-32.

69. Jubran A. Pulse oximetry. Crit Care. 2015;19:272.

Paper I

ELSEVIER

CrossMark

Hypoperfusion in response to epinephrine in local anaesthetics: Investigation of dependence on epinephrine concentration, spread of hypoperfusion and time to maximal cutaneous vasoconstriction

Rafi Sheikh [a], Khashayar Memarzadeh [a], Christian Torbrand [b], Jonas Blohmé [a], Malin Malmsjö [a],*

[a] *Lund University, Skane University Hospital, Department of Clinical Sciences Lund, Ophthalmology, Lund, Sweden*
[b] *Lund University, Skane University Hospital, Department of Clinical Sciences Lund, Urology, Lund, Sweden*

Summary   *Objectives:* The present study aimed to examine hypoperfusion in response to epinephrine following the administration of a local anaesthetic. The *concentration* of epinephrine that causes maximal hypoperfusion, the *spread* of hypoperfusion in the tissue and the *time* to the stabilization of hypoperfusion were investigated.
Methods: Blood perfusion was monitored using laser Doppler velocimetry and laser speckle contrast imaging of random-pattern advancement flaps (1 × 4 cm) or intact skin on the pig flank. Epinephrine was either injected cumulatively (0.1, 1.0, 10 or 100 μg/ml) after injecting 20 mg/ml lidocaine, to determine the concentration response, or given as a single dose (12.5 μg/ml epinephrine + 20 mg/ml lidocaine). Control experiments were performed with saline or lidocaine (without epinephrine).
Results: Increasing concentrations of epinephrine resulted in a gradual decrease in skin perfusion, approaching a minimum after injecting 10 μg/ml. The area of hypoperfusion was 12 mm in radius, and the time from the injection to the stabilization of hypoperfusion was approximately 120 s. After the administration of 10 μg/ml epinephrine in flaps with small pedicle, 25% blood perfusion still remained.
Conclusions: Local anaesthetic with an epinephrine concentration of approximately 10 μg/ml appears to be adequate for vasoconstriction before surgery. Incisions were required to be delayed only for 2 min following local anaesthetic with epinephrine in pigs. The remaining 25%

* Corresponding author. Skånes Universitetssjukhus, Ögonklinik A, Admin, 2nd Floor, Kioskgatan 1B, 221 85 Lund, Sweden.
  E-mail address: malin.malmsjo@med.lu.se (M. Malmsjö).

blood perfusion observed after the administration of epinephrine supports the use of epinephrine in flaps with a small pedicle. Obviously, these experimental findings must be clinically assessed before being considered for infiltration anaesthesia during plastic surgery procedures. © 2016 British Association of Plastic, Reconstructive and Aesthetic Surgeons. Published by Elsevier Ltd. All rights reserved.

Introduction

Epinephrine is used in conjunction with local anaesthetics to achieve vasoconstriction to minimize bleeding during surgical procedures. The optimal concentration of epinephrine to achieve vasoconstriction has been strenuously debated over the past 50 years[1]; however, there is a lack of relevant data. As this hormone may have systemic effects, including haemodynamic disturbances such as hypertension and arrhythmias, it is important to determine the lowest *concentration* of epinephrine that produces local vasoconstriction.[2,3]

The *spread* of hypoperfusion following the injection of epinephrine is also an important factor in the preparation for surgery. In infiltration anaesthesia, the area affected by epinephrine is the area in which the risk of bleeding has been minimized and is ready for surgery. Few studies have been conducted on the spread of hypoperfusion in tissue.

There has also been a debate concerning the *time* required to reach maximal cutaneous vasoconstriction following the administration of epinephrine. The time to maximal cutaneous vasoconstriction after the injection of lidocaine with epinephrine is often given as 7–10 min. In a previous study by Ghali et al., an immediate decrease in cutaneous blood flow was observed following the injection of epinephrine:lidocaine at a ratio of 1:100,000 in the forearm or face of healthy volunteers, reaching maximal value after 10 min for the forearm and 8 min for the face.[4] However, in a recent study by McKee et al., who measured the relative haemoglobin concentration over time using spectroscopy in the arm skin of healthy volunteers, the lowest cutaneous haemoglobin level was observed after a mean time of 26 min.[5] If this is truly representative, there is a need to significantly increase the preparation time before surgery.

Blood perfusion is commonly studied by laser Doppler velocimetry (LDV), which is a technique that is frequently used to measure blood flow in flaps during surgery[6] and after skin burns to assess burn wound outcome.[7] A beam of laser light is carried by a fibre optic probe. Light impinging on moving blood cells undergoes a change in wavelength (Doppler shift), whereas light reflected from static objects remains unchanged. Change in wavelength is interpreted as velocity of blood cells, that is, perfusion.[8] Laser speckle contrast imaging (LSCI) is a recently developed technique in which the object is illuminated by laser light, and the backscattered light will form a random interference pattern called a speckle pattern. Movement, such as the flow of red blood cells in a tissue, causes the speckle pattern to change, and the level of blurring is quantified. Information on blood perfusion in the tissue can thus be obtained by analysing the fluctuations in contrast.

The aim of the present study was to examine hypoperfusion in response to the administration of epinephrine in combination with local anaesthetics to minimize bleeding during surgical procedures. We studied three factors: (1) the lowest *concentration* of epinephrine that leads to maximal hypoperfusion, (2) the *spread* of hypoperfusion in the tissue from the injection site and (3) the *time* to maximal cutaneous vasoconstriction due to epinephrine. Two laser-based techniques were used to measure perfusion: LDV, to register perfusion by an invasive probe at a particular point in the tissue, and LSCI, to provide information on perfusion over a larger tissue area.

Methods

Animals and anaesthesia

Eight pigs with a mean body weight of 70 kg were fasted overnight with free access to water. The pigs were cross-bred and were a mixture of four breeds: Landrace, Hampshire, Yorkshire and Durocand. They did not have pigmented skin. An intramuscular injection of xylazine (Rompun® vet. 20 mg/ml; Bayer AG, Leverkusen, Germany; 2 mg/kg) mixed with ketamine (Ketaminol® vet. 100 mg/ml; Farmaceutici Gellini S.p.A., Aprilia, Italy; 20 mg/kg) was used for pre-medication. Anaesthesia was then induced with intravenous sodium thiopental (Pentothal®; Abbot Scandinavia, Stockholm, Sweden; 4 mg/kg) and fentanyl (Leptanal®; Lilly, France; 2 μg/kg) and maintained by continuous infusion of fentanyl in Ringer's acetate (3.5 μg/kg/h; Ringer-Acetat Baxter Viaflo, Baxter Medical AB, Kista, Sweden) in combination with sodium thiopental (~2.5 mg/kg). The animals were orally intubated with cuffed endotracheal tubes. Mechanical ventilation was established in a volume-controlled mode with 35% oxygen (Siemens-Elema AB, Solna, Sweden). The ventilation settings were identical for all animals: respiratory rate 15 breaths/min and minute ventilation 12 l/min. A positive end-expiratory pressure of 5 cm H_2O was applied. A Foley catheter was inserted into the urinary bladder through a suprapubic cystostomy.

Ethics

The study was approved by the Ethics Committee for Animal Research at Lund University, Sweden. All animals received humane care in compliance with the European Convention on Animal Care. The animals were also used for other experiments, but these were considered to not have any effect on this study.

Experimental procedure

Two injection sites were used (Figure 1). Pharmacological agents were either injected (1) in intact skin on the pig's flank, and perfusion was measured using LSCI, or (2) in flaps on the pig's flank, and perfusion was measured using LDV and LSCI. The flaps were dissected as rectangular random-pattern advancement flaps (1 cm wide and 4 cm long), extending all the way through the subcutaneous tissue down to the muscle fascia, giving a thickness of 8—9 mm. A small 27-gauge needle (BD medical, Franklin Lakes, NJ, USA) was inserted for the injection of pharmacological agents. Plastic cannulas 20 gauge BD Venflon™ Pro Peripheral IV Catheter with Injection Valve and OB (BD Medical, Franklin Lakes, NJ, USA) were inserted for LDV filament probes. One plastic cannula was inserted so that the tip of the filament could be positioned in subcutaneous tissue, in the flap, 1 cm from the flap base, and one plastic cannula was inserted in adjacent tissue for monitoring the peripheral circulatory status of the pig. After anaesthesia and the surgical procedure, the pig was allowed to stabilize for at least 1 h before the experiments were started. This was done because the surgical procedure and the initiation of anaesthesia affect both the central haemodynamic and peripheral perfusion. Allowing the pig to stabilize before microvascular perfusion experiments are started is essential to obtain accurate results with low variability.

Pharmacological agents

The pharmacological agents were diluted in saline and pre-heated to 38 °C before injection. The agents were injected into the subcutaneous tissue. The volume used was 0.3 ml and was chosen to represent a clinically significant amount without resulting in tissue compression that would cause immediate hypoperfusion and interfere with the perfusion measurements. Saline (Natriumklorid 9 mg/ml, Baxter Medical AB, Kista, Sweden) was administered before each series of active agents as a negative control.

There were three different experimental setups (Figure 2):

1. Flap that received a single dose of lidocaine (Lidokain Mylan 20 mg/ml; Mylan Hospital AS, Olso, Norway) without epinephrine, after which epinephrine was added cumulatively (increasing concentrations of epinephrine:
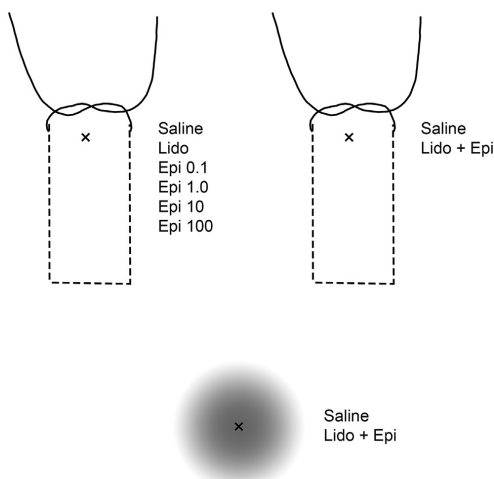
Figure 2 Schematic illustration of the three experimental setups in the study: (1) Flaps that received a single dose of lidocaine (without epinephrine), after which epinephrine was cumulatively added (0.1, 1.0, 10 and 100 μg/ml) to determine the concentration—response relationship. (2) Flaps that received a single dose of lidocaine + epinephrine (20 mg/ml lidocaine + 12.5 μg/ml epinephrine). (3) Intact skin that received a single dose of lidocaine + epinephrine. Saline was administered before each series of active agents as a negative control. The experimental setups were located at least 10 cm apart to eliminate the risk of interaction.
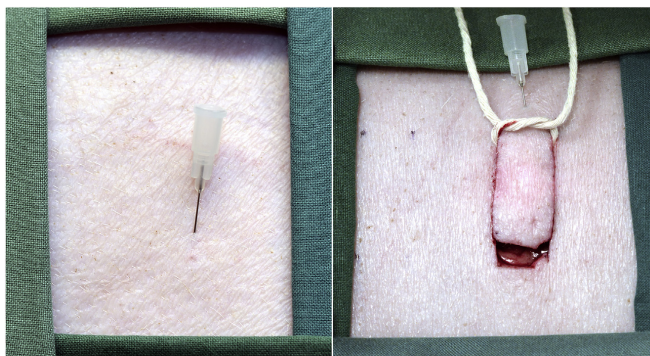
Figure 1 The two injection sites used in the study: the pharmacological agents injected in intact skin (left image) and in the base of a 1 × 4 cm random-pattern advancement flap (right image).

0.1, 1.0, 10 and 100 μg/ml)to determine the concentration−response relationship.

2. Flap that received a single dose of lidocaine + epinephrine (Xylocaine Dental Adrenalin, 20 mg/ml lidocaine + 12.5 microg/ml epinephrine, Dentsply Ltd. York, USA).

3. Intact skin that received a single dose of lidocaine + epinephrine.

The different series of pharmacological experiments were injected into different skin locations.

Perfusion measurement

Perfusion was measured using LDV or LSCI. Baseline measurements were performed before each series of injections of pharmacological agents. The perfusion response was allowed to stabilize between each injection, which typically occurred within 5 min. After the completion of the series of injections in the flaps, the blood supply to the pedicle of the flap was occluded using a ligature, after which perfusion was assumed to be zero.

Laser Doppler velocimetry (LDV) was used to measure tissue perfusion in flaps (not in intact tissue). The technique registers motion in the tissue, which reflects perfusion.[9] A filament probe was used for this study, which provides information about perfusion at a particular point in the tissue, i.e. 1 mm^3 region surrounding the probe (MT A500-0, straight microtip with slanted tip; Perimed, Stockholm, Sweden). The filament probe was inserted into the flap using a 22 G Venflon infusion cannula. The filament probe was attached to a master probe (Probe 418), which was then connected to a PF5010 LDV unit (Perimed). Recordings with baseline values between 150 and 10 perfusion units (PU) were analysed.

Laser speckle contrast imaging (LSCI) was used to measure tissue perfusion in both intact skin and flaps using a PeriCam PSI HR System (Perimed). This technique visualizes motion in a larger tissues volume compared to LDV (see above) by scanning an area of up to 24 × 24 cm. A camera records the changes in the speckle pattern at a rate of up to 100 images per second, with a spatial resolution of up to 10,000 pixels/cm^2 (100 μm/pixel), resulting in an instantaneous image of motion (interpreted as perfusion).

LDV was only used to measure perfusion in flaps and not in intact skin because LDV recordings of changes in perfusion in response to pharmacological agents could be made with high reproducibility only in flaps. This is because LDV only measures the blood flow in a limited volume of tissue around the tip of the probe (approximately 1 mm^3) and is therefore sensitive to the exact position of the probe, whereas LSCI provides a 'map' of the blood flow in a larger area (up to 24 × 24 mm).

The hypoperfusion response to epinephrine was studied with regard to the following:

1. The lowest *concentration* of epinephrine that leads to maximal hypoperfusion: The hypoperfusion was studied after increasing the concentrations of epinephrine (0.1, 1.0, 10 or 100 μg/ml) in flaps on the pig flank. Perfusion was measured using LDV and LSCI.

2. The *spread* of hypoperfusion in the tissue from the injection site: The hypoperfusion response was studied after a single injection of epinephrine (20 mg/ml lidocaine + 12.5 μg/ml epinephrine) injected into intact skin. Perfusion was determined every 2 mm from the injection site 120 s after injection using LSCI.

3. The *time* to maximal cutaneous vasoconstriction due to epinephrine: Perfusion was measured over time in response to a single injection of lidocaine and epinephrine (20 mg/ml lidocaine + 12.5 μg/ml epinephrine). Perfusion was measured in flaps using LSCI.

Calculations and statistics

Blood perfusion was expressed in the arbitrary unit, PU. The values recorded before injection of the pharmacological agents were used as baseline values (set to 100%), and the value recorded when the base of the flap had been mechanically occluded by a ligature was set to 0%. In experiments where both LDV and LSCI were used, all values were used for the calculations. Calculations and statistical analysis were performed using GraphPad 6.0f software (San Diego, CA, USA). The time from the injection of epinephrine until the stabilization of hypoperfusion was determined using non-linear regression analysis (one-phase decay). The results are presented as median values and ranges for all analysis except for the non-linear regression analysis in which the 95% confidence intervals were calculated. Graphs show scatter plots or box-and-whisker plots in which the box is the 25th to 75th percentiles, the whiskers are the minimum and maximum values (range) and the line denotes the median. Eight pigs were used in this study. The number of repetitions of the experiments is denoted n. For practical reasons, not all experiments were performed on all pigs, and multiple experiments were performed on some pigs. Statistical analysis was performed using the Mann−Whitney U test. Significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****), and $p > 0.05$ (not significant, n.s.).

Results

Neither saline nor lidocaine resulted in any change in perfusion [52 PU (range 24−117) at baseline, 52 PU (27−125) for saline and 52 PU (25−102) for lidocaine, p = n.s., n = 10] according to LDV and LSCI measurements in flaps.

The epinephrine *concentration*

Increasing the concentration of epinephrine resulted in a gradual decrease in skin perfusion, as shown in Figure 3, approaching minimal perfusion at 10 μg/ml epinephrine [56 PU (range 24−117) at baseline, 25 PU (−2 to 60) after 10 μg/ml epinephrine and 24 PU (0−47) after 100 μg/ml epinephrine, p = n.s., n = 9] according to LDV and LSCI measurements in flaps. The response to epinephrine, when cumulatively injected to determine the concentration−response relationship [52 PU (range 24−117) at baseline and 25 PU (2−60) after epinephrine (10 μg/ml)],
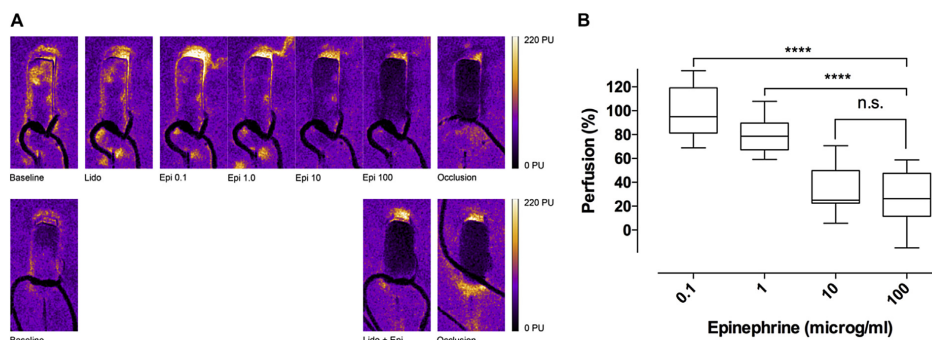
Figure 3 Blood perfusion in flaps after the injection of increasing concentrations of epinephrine. **Panel A** is a representative example of an experiment in which blood perfusion was measured using LSCI. Baseline measurements were performed before the experiments were started. Saline was injected after baseline recordings as a negative control, and the results showed no change in perfusion. The upper row shows the injection of lidocaine (Lido) followed by increasing concentrations of epinephrine (Epi 0.1, 1.0, 10 or 100 μg/ml). The lower row shows the injection of lidocaine + epinephrine (20 mg/ml lidocaine + 12.5 μg/ml epinephrine). The experiments were concluded by mechanically occluding the base of the flap using a ligature (occlusion). Perfusion is expressed in the arbitrary units, perfusion units (PU), as defined by the scale. **Panel B** is a box plot showing the results from the injection of increasing concentrations of epinephrine where perfusion was measured using LDV and LSCI (n = 9). The box denotes the 25–75th percentiles, the whiskers denote the minimum and maximum values (range) and the line denotes the median. Perfusion is expressed as percent of baseline values. Maximal response was reached at a concentration of 10 μg/ml epinephrine. Statistical analysis was performed using Mann–Whitney U test; $p < 0.0001$ (****) and $p > 0.05$ (not significant, n.s.).

measured by LDV and LSCI in flaps (n = 9), was similar to that following a single dose of epinephrine [52 PU (range 43–64) at baseline and 25 PU (21–39) after lidocaine + epinephrine 20 mg/ml lidocaine + 12.5 μg/ml epinephrine, n = 7] according to LSCI measurements on intact skin (p = n.s.).

The *spread* of hypoperfusion

The spread of hypoperfusion in response to epinephrine was measured after the single injection of epinephrine (20 mg/ml lidocaine + 12.5 μg/ml epinephrine) in intact skin using LSCI. The perfusion was 90 PU (range 72–111) 20 mm from the injection site and 52 PU (range 38–64) at the injection site. The hypoperfusion spread to a radius of 12 mm from the injection site where there was no effect on perfusion by epinephrine [78 PU (range 67–120), measured 120 s after injection, n = 9]. Refer to Figure 4 for detailed results.

The *time* to maximal cutaneous vasoconstriction

The time from the injection of lidocaine + epinephrine (20 mg/ml lidocaine + 12.5 epinephrine μg/ml) until the stabilization of hypoperfusion was measured in both intact skin and flaps using LSCI. Blood perfusion decreased over time and stabilized at a lower level after 92 s (95% confidence interval = 84–100) in intact skin (n = 9) and after 121 s (107–136) in flaps (n = 7) according to non-linear regression analysis (one-phase decay). Refer to Figure 4 for detailed results.

Discussion

The results of this study provide detailed information on the *concentration* of epinephrine that leads to maximal hypoperfusion, the *spread* of hypoperfusion in the tissue from the injection site and the *time* taken for maximal hypoperfusion to become established.

Injecting increasing concentrations of epinephrine (0.1, 1.0, 10 or 100 μg/ml) resulted in a gradual decrease in skin perfusion, the maximal effect being seen at 10 μg/ml. These results are similar to those reported in previous studies on the effect of epinephrine, where maximal local cutaneous hypoperfusion was observed at concentrations between 1:50,000 and 1:100,000.[10,11] These concentrations are similar to the concentration of epinephrine present in the commonly and clinically used Xylocaine Dental Adrenalin, which contains 12.5 μg/ml epinephrine. However, other local anaesthetics with lower concentrations of epinephrine are used clinically, e.g. Xylocaine adrenalin with 5 μg/ml epinephrine. This study showed that epinephrine concentrations lower than 10 μg/ml do not have a pronounced effect and that the degree of hypoperfusion is highly variable. In the clinical situation, achieving maximal vasoconstriction of the peripheral circulation must be weighed against the possibility of systemic effects. Nonetheless, an appropriate concentration of epinephrine should be given with the local anaesthetic to guarantee sufficient depth and duration of local anaesthesia to avoid pain and stress to patient.

A local anaesthetic such as lidocaine containing epinephrine will have a more profound effect in a hypoperfused area as it presumably will take a longer time for
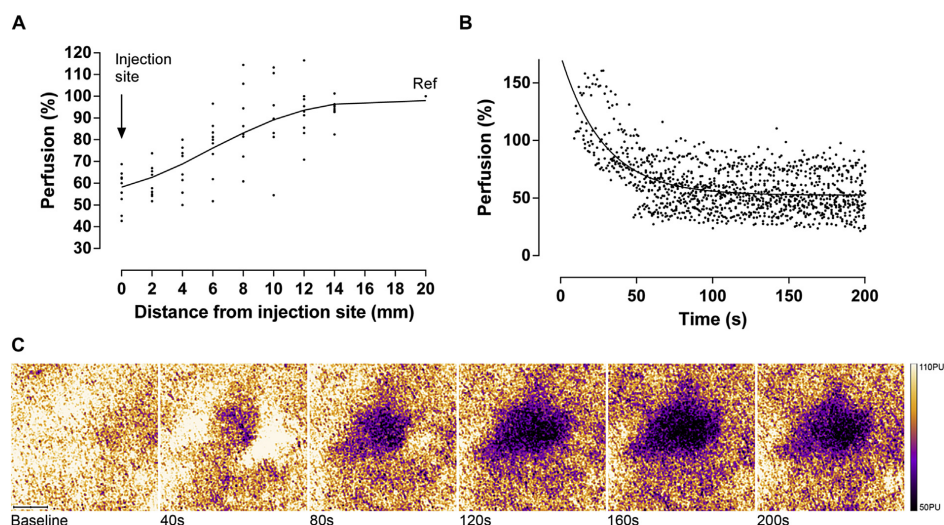
A

B

C

Baseline   40s   80s   120s   160s   200s

Figure 4   Blood perfusion after a single injection of lidocaine + epinephrine (20 mg/ml lidocaine + 12.5 μg/ml epinephrine). **Panel A** shows the distance the hypoperfusion spread from the injection site in intact skin (n = 9). Perfusion was measured every 2 mm from the injection site 120 s after injection using LSCI. The results are expressed as percent of a reference point (Ref) 20 mm from the injection site. Note that the hypoperfusion spread to a radius of 12 mm from the injection site. **Panel B** shows the drop in perfusion over time (n = 7). Perfusion was measured in flaps using LSCI. Perfusion is expressed as percent of the baseline value before the experiments were started (defined as 100%) and analysed using non-linear regression analysis (one-phase decay), reaching a maximum after 121 s (95% confidence interval of 107−136 s). The experiments went on for 5 min. The graph illustrates what happens in the first 200 s, and beyond this time-point, there was no further change. **Panel C** is a representative example of an experiment that shows the spread of the hypoperfusion over time and area. Saline was injected after baseline recordings as a negative control, and the results showed no change in perfusion. Perfusion was measured by LSCI of intact skin on the pig flank, and the results are expressed in the arbitrary units, perfusion units (PU). The scale bar (bottom left) is 10 mm.

the anaesthetic to be removed because of the reduced blood flow. Interestingly, Knoll-Kohler et al. demonstrated that systemic norepinephrine levels (used as a measure of stress and pain) were significantly elevated following the administration of 4% articaine containing 1:200,000 epinephrine compared to the levels when 4% articaine with 1:100,000 epinephrine was administered,[12] supporting the use of higher dose of epinephrine for local anaesthetics. Several studies on local anaesthesia with epinephrine have confirmed that although the blood pressure and heart rate may have changed significantly, the haemodynamic response, defined by the mean arterial blood pressure, is unchanged.[13−16] These studies could not demonstrate any deleterious effects of local anaesthetic formulations with epinephrine in dental patients with hypertension or cardiovascular disease.[13−18] Taken together, there is probably little risk of systemic toxicity of epinephrine when used in local anaesthetics.

One limitation of this study was that in the experiments where epinephrine was added cumulatively, desensitization of the adrenergic receptors may have occurred in the vasculature, resulting in depressed responses. However, adding the pharmacological agents cumulatively is a classic pharmacological approach to examine the

concentration−response relationship of epinephrine, and the logarithmic increase in the amount of epinephrine injected and letting only 5 min pass between the injections decreases the risk of receptor desensitization.

Lidocaine (without epinephrine) is known to cause hyperperfusion.[4,19] However, in the present study, lidocaine alone (10 mg/ml) was found to have no effect on perfusion. Moreover, the effect of 10 μg/ml epinephrine added to 10 mg/ml lidocaine was comparable to the effect of 20 mg/ml lidocaine with 12.5 μg/ml epinephrine, when given as a single dose. The reason why lidocaine appeared to have no effect on perfusion in the present study is unknown. Lidocaine was administered at a concentration of 10 mg/ml, which is the typical concentration in clinical infiltration anaesthesia. Every effort was made to ensure that the study was performed on otherwise well-perfused tissue, and we can only speculate that it was not possible to measure an increase in perfusion as no further increase was possible.

The time required to reach maximal cutaneous vasoconstriction using epinephrine was evaluated in both intact skin and flaps using laser speckle imaging. Blood perfusion decreased with time and stabilized at a lower plateau level approximately 2 min after the injection. This is in line with

our clinical experience, i.e. the time the surgeon must wait for reduced cutaneous bleeding in plastic surgical procedures is rather short, approximately 1–2 min. These results are reassuring and indicate that surgery should be started as soon as possible after infiltration anaesthesia has been administered. The results are also in accordance with the short times often given in textbooks, i.e. 7–10 min, and previous scientific studies,[4] showing that local anaesthetics with epinephrine resulted in an immediate decrease in cutaneous blood flow that was maximal at 10 min in the forearm and 8 min in the face. McKee et al. used spectroscopy to measure the mean time at which the lowest cutaneous haemoglobin level was obtained and found it to be 26 min in the arm skin of healthy volunteers after the administration of 1% lidocaine with epinephrine.[5] One possible reason for the difference in results between the present study and the study by McKee et al.[5] is that the present study was performed using LDV and LSCI techniques, which quantify motion in a specific volume of the tissue, and the results are then interpreted as blood perfusion. McKee et al. used spectroscopy in which the tissue is illuminated with broad-spectrum light, and the diffuse reflected light is collected after interaction with the tissue. The tissue characteristics are then determined on the basis of the fact that different tissue components have different absorption characteristics. Haemoglobin content is based on the analysis at only a few wavelengths at which the absorption of haemoglobin is dominating, typically wave lengths 510, 542, 560, 576 and 610 nm. Briefly, LDV measures the rate of blood flow, whereas spectroscopy measures blood quantity in the tissues. Interestingly, McKee et al. measured the quantity of blood loss after 7 or 30 min from the time of injection of lidocaine + epinephrine to the time of incision and found less blood loss after 30 min.[20] These results are somewhat different to the results from the present study. Future studies in humans combing these different techniques may be helpful in describing the true effect of epinephrine in local anaesthetics.

In the experiments in the present study, the skin was incised before the infiltration of local anaesthesia and epinephrine. In clinical practice, the local anaesthesia and epinephrine would be infiltrated before skin incision. The results show that maximum hypoperfusion was reached after 92 s in intact skin and after 121 s in flaps (incised before infiltration epinephrine). The reason for this discrepancy cannot be deduced from this study but may be a result of the wounding process provoking an inflammatory response, which affected local skin perfusion and may have prolonged the time taken for epinephrine to have optimal effect after the incision for creating flaps.

The use of epinephrine in local anaesthetics has both advantages (minimizing bleeding during surgery) and disadvantages (risk of ischaemia and systemic adrenergic effects). There is reluctance among surgeons to inject epinephrine into a flap with a small pedicle or into a finger because of the risk of ischaemia and necrosis.[21] Interestingly, the present study showed that 25% perfusion remained in the tip of the flaps injected with epinephrine, and the risk of ischaemia and necrosis in this case is thus minimal. In accordance with these results, recent reviews suggest a more liberal attitude to the use of epinephrine in local anaesthetics in surgery at the base of the finger

and hand.[22,23] Interestingly, over 100 cases of accidental injection of epinephrine into the finger have been reported in the literature, without a single case of digital necrosis.[24]

LDV and LSCI are used to quantify motion in a specific volume, and the results are then interpreted as blood perfusion. Thus, if the tissue under investigation moves, a much higher signal will be obtained than that caused by blood flow itself. This means that artefacts will be introduced by movements resulting, for example, from breathing, and care must be taken to eliminate all such sources of motion. Another limitation of LDV is that it only measures a small sampling volume (1 mm^3).[9] The blood flow in the surrounding tissue may be higher or lower than that in the volume being investigated because of variations in vascular density. It is therefore important to study perfusion with more than one technique, and we chose to use LSCI, which provides information over a larger area (24 × 24 cm). The other difference between LDV and LSCI is that probes are inserted into the tissue in the former, whereas the latter technique is non-invasive. Similar results were obtained with both techniques in this study, indicating that both LDV and LSCI can be used to measure the blood perfusion in this setting, supporting the conclusions of the study.

This study was performed on pigs and not on humans. The pig is considered to be a suitable animal for the study of full-thickness wounds as its epidermis, dermis and subcutaneous fat resemble those of humans[25,26] in contrast to small animals, which have a thick layer of fur and a thinner epidermis and dermis. Moreover, porcine dermal collagen is similar to that in humans,[27] and the skin is adherent to the deep fascia.[28] The blood supply to the pig flank consists of rows of segmental vessels, each vascular bundle containing a central artery with two concomitant venae. In the present study, the skin flaps were dissected superficially to the panniculus carnosus and were thus random-pattern flaps.[29] The vasoconstriction response to injected pharmacological effect will depend on regional variations in blood perfusion and the presence of receptors in the tissue (e.g. adrenergic receptors). To minimize the effect of this variability, the recorded blood flow after the injection of pharmacological agents was calculated as percent of baseline blood flow before the injection of pharmacological agents. In this way, each experiment had its own internal control, minimizing the effect of variability between the experiments and study sites. Porcine skin is generally thicker than human skin, and the results from the present study may be more comparable with thick skin (e.g. on the human back) than with thin skin (e.g. on the human face). Future studies are required to elucidate the effect of epinephrine in local anaesthetics in humans.

In conclusion, the results of the present study provide detailed information on the *concentration* of epinephrine that leads to maximal hypoperfusion, the *spread* of hypoperfusion in the tissue from the injection site, and the *time* taken for maximal hypoperfusion to become established. An epinephrine concentration of approximately 10 μg/ml appears to be adequate for vasoconstriction in tissue before surgery, and incisions should be delayed for 2 min following lidocaine + epinephrine injection to allow the maximal effect to be reached. The 25% remaining blood perfusion observed after the administration of epinephrine in the

present study supports the use of epinephrine in flaps with a small pedicle. The study was performed in pigs, and future detailed studies on perfusion following local anaesthetics with epinephrine in humans are required before clinical recommendations can be made. Furthermore, the status of the tissue must be assessed in each clinical situation, and if the patient is suffering from other vascular diseases such as diabetes, this may increase the risk of ischaemia.

Conflict of interest

None.

Funding

None.

Acknowledgements

References

1. Brown RS, Rhodus NL. Epinephrine and local anesthesia revisited. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005; **100**(4):401—8.

2. Bhatia N, Ghai B, Mangal K, Wig J, Mukherjee KK. Effect of intramucosal infiltration of different concentrations of adrenaline on hemodynamics during transsphenoidal surgery. *J Anaesthesiol Clin Pharmacol* 2014;**30**(4):520—5.

3. Bader JD, Bonito AJ, Shugars DA. A systematic review of cardiovascular effects of epinephrine on hypertensive dental patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **93**(6):647—53.

4. Ghali S, Knox KR, Verbesey J, Scarpidis U, Izadi K, Ganchi PA. Effects of lidocaine and epinephrine on cutaneous blood flow. *J Plast Reconstr Aesthet Surg* 2008;**61**(10):1226—31.

5. McKee DE, Lalonde DH, Thoma A, Glennie DL, Hayward JE. Optimal time delay between epinephrine injection and incision to minimize bleeding. *Plast Reconstr Surg* 2013;**131**(4):811—4.

6. Zografos GC, Martis K, Morris DL. Laser Doppler flowmetry in evaluation of cutaneous wound blood flow using various suturing techniques. *Ann Surg* 1992;**215**(3):266—8.

7. Jaskille AD, Ramella-Roman JC, Shupp JW, Jordan MH, Jeng JC. Critical review of burn depth assessment techniques: part II. Review of laser doppler technology. *J Burn Care Res* 2010; **31**(1):151—7.

8. Leahy MJ, de Mul FF, Nilsson GE, Maniewski R. Principles and practice of the laser-Doppler perfusion technique. *Technol Health Care* 1999;**7**(2—3):143—62.

9. Allen J, Howell K. Microvascular imaging: techniques and opportunities for clinical physiological measurements. *Physiol Meas* 2014;**35**(7):R91—141.

10. Liu S, Carpenter RL, Chiu AA, McGill TJ, Mantell SA. Epinephrine prolongs duration of subcutaneous infiltration of local anesthesia in a dose-related manner. Correlation with magnitude of vasoconstriction. *Reg Anesth* 1995;**20**(5):378—84.

11. Larrabee Jr WF, Lanier BJ, Miekle D. Effect of epinephrine on local cutaneous blood flow. *Head Neck Surg* 1987;**9**(5):287—9.

12. Knoll-Kohler E, Fortsch G. Pulpal anesthesia dependent on epinephrine dose in 2% lidocaine. A randomized controlled double-blind crossover study. *Oral Surg Oral Med Oral Pathol* 1992;**73**(5):537—40.

13. Chernow B, Balestrieri F, Ferguson CD, Terezhalmy GT, Fletcher JR, Lake CR. Local dental anesthesia with epinephrine. Minimal effects on the sympathetic nervous system or on hemodynamic variables. *Arch Intern Med* 1983;**143**(11): 2141—3.

14. Niwa H, Satoh Y, Matsuura H. Cardiovascular responses to epinephrine-containing local anesthetics for dental use: a comparison of hemodynamic responses to infiltration anesthesia and ergometer-stress testing. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000;**90**(2):171—81.

15. Meyer FU. Haemodynamic changes under emotional stress following a minor surgical procedure under local anaesthesia. *Int J Oral Maxillofac Surg* 1987;**16**(6):688—94.

16. Cioffi GA, Chernow B, Glahn RP, Terezhalmy GT, Lake CR. The hemodynamic and plasma catecholamine responses to routine restorative dental care. *J Am Dent Assoc* 1985;**111**(1):67—70.

17. Davenport RE, Porcelli RJ, Iacono VJ, Bonura CF, Mallis GI, Baer PN. Effects of anesthetics containing epinephrine on catecholamine levels during periodontal surgery. *J Periodontol* 1990;**61**(9):553—8.

18. Lipp M, Dick W, Daublander M, Fuder H, Stanton-Hicks M. Exogenous and endogenous plasma levels of epinephrine during dental treatment under local anesthesia. *Reg Anesth* 1993; **18**(1):6—12.

19. Gessler EM, Hart AK, Dunlevy TM, Greinwald Jr JH. Optimal concentration of epinephrine for vasoconstriction in ear surgery. *Laryngoscope* 2001;**111**(10):1687—90.

20. McKee DE, Lalonde DH, Thoma A, Dickson L. Achieving the optimal epinephrine effect in wide awake hand surgery using local anesthesia without a tourniquet. *Hand (NY)* 2015;**10**(4): 613—5.

21. Bunnell S, Howard LD. *Surgery of the hand*. Philadelphia: Lippincott; 1944.

22. Thomson CJ, Lalonde DH, Denkler KA, Feicht AJ. A critical look at the evidence for and against elective epinephrine use in the finger. *Plast Reconstr Surg* 2007;**119**(1):260—6.

23. Hagert E, Lalonde D. Time to bury the adrenaline-myth!-Safe use of adrenaline anesthesia in hand surgery and orthopedics. *Lakartidningen* 2015:112.

24. Muck AE, Bebarta VS, Borys DJ, Morgan DL. Six years of epinephrine digital injections: absence of significant local or systemic effects. *Ann Emerg Med* 2010;**56**(3):270—4.

25. Winter GD. *A Study of wound healing in the domestic pig*. University of London; 1966.

26. Hartwell SW. *A mechanism of healing in human wounds*. Springfield, IL: Charles Thomas; 1955.

27. Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. *Wound Repair Regen* 2001;**9**(2): 66—76.

28. Patterson TJS. The survival of skin flaps in the pig. *Br J Plast Surg* 1969;**21**(2):113—7.

29. Stell PM. The pig as an experimental model for skin flap behaviour: a reappraisal of previous studies. *Br J Plast Surg* 1977;**30**(1):1—8.

Paper II

Blood Perfusion in a Full-Thickness Eyelid Flap, Investigated by Laser Doppler Velocimetry, Laser Speckle Contrast Imaging, and Thermography

Rafi Sheikh, MD,[a] Khashayar Memarzadeh, MD,[a] Christian Torbrand, MD, PhD,[a,b] Jonas Blohmé, MD, PhD,[a] Sandra Lindstedt, MD, PhD,[c] and Malin Malmsjö, MD, PhD[a]

Lund University, Skane University Hospital, Department of Clinical Sciences Lund, [a]Ophthalmology; [b]Urology; and [c]Cardiothoracic Surgery, Lund, Sweden

Correspondence: malin.malmsjo@med.lu.se

Keywords: blood perfusion, eyelid, flap, laser Doppler, laser speckle

Purpose: The eyelid is commonly dissected and divided in the process of, for example, blepharotomy, entropion repair, or when preparing a full-thickness eyelid flap to reconstruct a tumor defect. No study has yet been conducted to examine how perfusion in an eyelid is affected by dissection, using modern imaging techniques. **Methods:** The eyelid was divided with a 10-mm vertical incision, 5 mm from the medial canthus, and the incision was extended horizontally by 30 mm to provide a full-thickness eyelid. Blood perfusion was measured along the length of the free dissected eyelid using both laser Doppler velocimetry and laser speckle contrast imaging. Tissue temperature was visualized using a high-resolution infrared camera (thermography). **Results:** Measurements using laser speckle contrast imaging showed that blood flow decreased gradually from the pedicel base to the tip of the free dissected eyelid: 83% at 10 mm, stabilizing at 80% at 20 mm from the pedicel base. These results were supported by laser Doppler velocimetry, showing a reduction in perfusion to 67%, 15 mm from the pedicel base. Thermographic imaging showed a corresponding decrease in temperature from the tip to the pedicel base compared with nondissected eyelids. **Conclusions:** Dissection of an eyelid, to provide a full-thickness eyelid flap, results in only a slight decrease in blood flow. The results support the view that plastic surgery of the eyelids is permissive, and

the rich vascularization of the eyelid due to the anastomotic network of vessels in the tarsal plate may increase the likelihood of flap survival and surgical success.

The eyelid is commonly dissected and divided in the process of, for example, (i) a blepharotomy procedure for thyroid-associated ophthalmopathy; (ii) a Quickert procedure for correction of involutional entropion in which the eyelid split transversally, to prevent the upward movement of the preseptal orbicularis, combined with horizontal lid shortening to correct excess lid laxity, and everting sutures to shorten the retractors[1]; (iii) a Tenzel semicircular rotational flap for reconstruction of a full-thickness lid defect by advancing a flap from the lateral canthus[2]; or (iv) a "switch flap" that involves switching a full-thickness eyelid flap on a pedicel (with tarsus, lid margin, and eyelashes), from lower lid to upper lid, or vice versa, in order to reconstruct a tumor defect.[3] No study has yet been conducted to examine how blood perfusion in an eyelid is affected by such dissection, using modern imaging techniques.

During recent decades, several imaging techniques have been developed providing high-resolution images of the structure and function of tissue. Laser Doppler velocimetry (LDV) is an established technique used for measuring blood flow in plastic surgery. However, to get reproducible results, the technique is best used with invasive probes and it is only possible to study the blood flow in a very small region (1 mm^3 surrounding the probe, with the technique used in this study).[4] A more recent technique is laser speckle contrast imaging (LSCI), which produces representative images of the blood flow in the surface of tissue over a larger area (24 $\times$ 24 cm^2, with the technique used in this study). Not only is this technique noninvasive but is also highly reproducible. LSCI is now an established technique in, for example, experimental vascular brain research and plastic surgery but has not been applied in the field of periorbital plastic surgery. Thermography, employing a high-resolution infrared (IR) camera, is another noninvasive technique that measures the temperature of the tissue, which can be used as a proxy for blood flow. This method is suitable for clinical use but has not previously been applied to the study of periorbital skin.

The aim of this study was to investigate perfusion in a full-thickness eyelid flap on a pedicel by using modern imaging techniques. The study was conducted on pigs, which are considered to be a suitable model for dermal and plastic surgery studies, as the epidermis, dermis, and subcutaneous fat resemble those in humans.[5,6] The microvascular perfusion was measured using both LDV and LSCI, and the tissue temperature was visualized using thermography.

METHODS

Animals and anesthesia

Eight pigs with a body weight of 70 kg were fasted overnight but had free access to water. An intramuscular injection of xylazine (Rompun vet. 20 mg/mL; Bayer AG, Leverkusen, Germany; 2 mg/kg) mixed with ketamine (Ketaminol vet. 100 mg/mL; Farmaceutici Gellini S.p.A., Aprilia, Italy; 20 mg/kg) was used for premedication. Anesthesia was then induced with intravenous sodium thiopental (Pentothal; Abbot Scandinavia,

Stockholm, Sweden; 4 mg/kg) and fentanyl (Leptanal; Lilly, France; 2 μg/kg) and maintained by continuous infusion of fentanyl in Ringer's acetate (3.5 μg/kg/h) in combination with sodium thiopental ($\sim$2.5 mg/kg). The animals were orally intubated with cuffed endotracheal tubes. Mechanical ventilation was established in the volume-controlled mode with 35% oxygen (Siemens-Elema AB, Solna, Sweden). The ventilation settings were identical for all animals: respiratory rate 15 breaths/min and minute ventilation 12 L/min. A positive end-expiratory pressure of 5 cm H_2O was applied. A Foley catheter was inserted into the urinary bladder through a suprapubic cystostomy. Following anesthesia and the surgical procedures, the pig was allowed to stabilize for 1 hour before the experiments were started.

Ethics

The experimental protocol for this study was approved by the Ethics Committee for Animal Research at Lund University, Lund, Sweden. The research adhered to the tenets of the Declaration of Helsinki as amended in 2008. The animals were also used for other experiments that were considered not to have an impact on the present study.

Experimental procedure

The eyelid was divided with a 10-mm vertical incision at 5 mm from the medial canthus, and the incision was extended 30 mm horizontally (Fig 1) to produce a full-thickness eyelid flap on a pedicel. Both upper and lower eyelids were used in the study and the results presented together. It could be argued that the results for the 2 eyelids should be considered separately. However, there were no difference in the results between the upper and lower eyelids and conclusions drawn are considered a true reflection of the actual situation. Perfusion was imaged by LDV, LSCI, and thermography at different measurement points as shown in Figure 1.

Perfusion measurements

Laser Doppler velocimetry

Perfusion was measured by LDV. The technique is based on the emission of a beam of laser light. The light is scattered and partly absorbed by the studied tissue. Light that hit moving blood cells undergoes a change in wavelength (Doppler shift), whereas light hitting static tissue is unchanged. The magnitude and frequency distribution of these changes in wavelength is presumed to be related to the number and velocity of blood cells. A filament probe (MT A500-0, straight microtip with slanted microtip; Perimed AB, Stockholm, Sweden) was inserted at measurement points in the flap base (0 mm) and 15 mm from the flap base (Fig 1), using a 22-G Venflon infusion cannula. The technique enables perfusion in a 1-mm^3 volume surrounding the tip of the probe.[4] The filament probe was attached to a master probe (Probe 418), which was then connected to the main LDV unit (Perimed PF5010 unit).

Laser speckle contrast imaging

A LSCI system (PeriCam PSI NR System, Perimed AB, Stockholm, Sweden) was used to obtain images of perfusion over the surface of a larger tissue area (24 $\times$ 24 cm). The

system uses a 785-nm invisible laser beam that is spread over the skin surface by a diffuser creating a speckle pattern. A speckle pattern is the pattern of dark and bright areas formed by random interference in the backscattered light of the area illuminated by laser. Blood perfusion is calculated by analyzing the variations in the speckle pattern. The speckle pattern is recorded in real time at a rate of up to 100 images per second, with a high resolution of up to 100 μm/pixel.
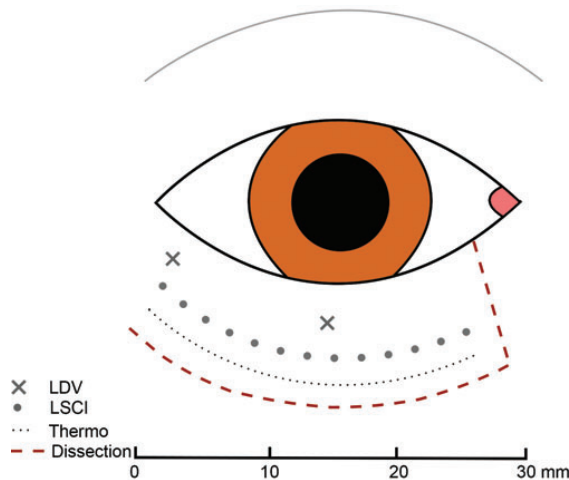

Figure 1. Schematic illustration of the location of the measurement points along the length of a full-thickness eyelid flap (dissection). Blood perfusion was measured using LDV and LSCI. Tissue temperature (Thermo) was measured with a high-resolution infrared camera. LDV indicates laser Doppler velocimetry; LSCI, laser speckle contrast imaging.

Thermography

Thermal energy (electromagnetic radiation) is emitted by all objects at temperatures above absolute zero, and the amount of radiation increases with temperature. Tissue temperature (thermal radiation) measurements were performed using a high-resolution IR camera (FLIR A655sc; FLIR Systems AB, Danderyd, Sweden). The IR camera was a focal plane array, uncooled microbolometer with 640×480 pixel resolution, and a thermal sensitivity/NETD (noise equivalent temperature difference) of less than $0.05°C$ at $+ 30°C/50$ mK. The IR camera was placed approximately 50 cm above the animal, on a Manfrotto 244 Variable Friction Magic Arm mounted on a Manfrotto 190 series tripod. The software ThermaCAM Researcher Pro 2.10 from FLIR Systems was installed in a PC laptop and used to capture the IR images and for data postprocessing. The IR camera was connected to the laptop through the Ethernet interface using a 2-m shielded Ethernet cable. To prevent the conductance of heat from the underlying tissue, an isolating pad was placed under the eyelid.

An Open Access Journal
...Committed to the free exchange of medical knowledge in a global community
www.eplasty.com

Plasty

SHEIKH ET AL

Calculations and statistics

Blood perfusion measured with LDV and LSCI is expressed in the arbitrary unit, perfusion units (PUs). Tissue temperature is expressed in degree Celsius (°C). Perfusion and temperature were also calculated as a percentage of the value in the pedicel base (0 mm). The results are presented as median values and ranges. The distance from the pedicel base at which the perfusion and temperature reached a plateau was analyzed using nonlinear regression analysis (1-phase decay) and expressed as median values and 95% confidence intervals. The results are given in scatter plots or box-and-whisker plots in which the box is the 25th to 75th percentiles, the whiskers are the minimum and maximum values (range), and the line indicates the median. Eight pigs were used in this study. For practical reasons, not all experiments were performed in all pigs or all eyelids. The number of experiments (n) is therefore given in the text and figures. Statistical analysis was performed using the Wilcoxon matched-pair test for single comparisons and the Friedman matched-pair test with Dunn's posttest for multiple comparisons. All P values in the interval .001 to .300 are written out, whereas other P values are given as $P < .001$ and $P > .30$. Calculations and statistics were performed using GraphPad PRISM 7.0a software.

RESULTS

Perfusion in the pedicel base was 45 PU (range, 36-52) according to LDV and 110 PU (range, 105-145) according to LSCI. LSCI measurements showed that the blood flow decreased gradually from the pedicel base to the tip of the full-thickness eyelid flap; most of the decrease being seen over the first 10 mm (95 PU at 10 mm; range, 87-116 PU; $P = .025$, compared with that in the pedicel base). Perfusion reached a plateau and stabilized at 20 mm from the base (85 PU at 20 mm; range, 73-117; $P < .001$, compared with the pedicel base). No further decrease in perfusion was seen beyond this point (90 PU at 30 mm; range, 72-116; n = 8; Fig 2). LDV measurements confirmed these results, showing a decrease in perfusion of 15 mm from the pedicel base (Fig 3).

Thermography showed a decrease in tissue temperature over the length of the full-thickness eyelid flaps. The tissue temperature was 34.34°C (range, 32.91-35.17) in the pedicel base, 33.16°C (range, 32.36-33.93) 10 mm from the pedicel base, 32.51°C (range, 31.16-33.26) 20 mm from the pedicel base, and 33.57°C (range, 29.53-32.37) 30 mm from the pedicel base (Fig 4). Thermography measurements were only performed in 3 pigs to illustrate the change in temperature, rather than to provide the basis for statistical assessment. The results of the thermography measurements are in accordance with, and support, the results obtained using LDV and LSCI.

DISCUSSION

Dividing the eyelid from its blood supply is common during blepharotomy procedures, entropion repair with, for example, a Quickert procedure,[1] or reconstructive procedures after tumor surgery, for example, Tenzel flap,[2] or "switch flap" that is a full-thickness eyelid flap on a pedicel.[3] The results of the present study show a decrease in perfusion

over the length of the full-thickness eyelid flaps. However, perfusion is as high as 83% at 10 mm from the pedicel base, 79% at 20 mm, and 80% at 30 mm, indicating good perfusion in the entire flap. The reason for this well-maintained perfusion is presumably that it is well vascularized with blood supply through the attachment at the lateral canthus, where a branch of the lateral palpebral artery supplies the tarsal arcades.[7-9]
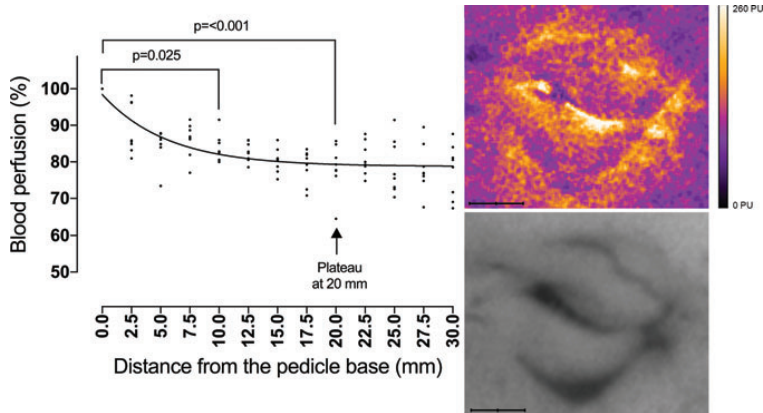

Figure 2. Laser speckle contrast imaging measurements showing a decrease in perfusion along the length of a full-thickness eyelid flap as the percent decrease in blood perfusion at increasing distance from the pedicel base. Nonlinear regression analysis showed that perfusion reached a plateau and stabilized at 20 mm from the base (95% CI, 16-23). Statistical analysis was performed using the Friedman matched-pair test with Dunn's posttest (n = 8). The images on the right are representative examples of the laser speckle pattern (top) and the corresponding grayscale image (bottom) of the upper and lower eyelids.

On the contrary, it is well known that in skin flaps that are not composite grafts, there is a steep drop in perfusion along the length of the flap. In a previous study on random advancement flaps on the flank of the pig, perfusion was found to be 40% at 20 mm from the flap base.[10] Full-thickness eyelid flaps include all layers (including the anterior and posterior lamellae with skin, the orbicularis muscle, tarsal plate, and conjunctiva) and vascular structures (including the tarsal plexus)[7] and may therefore be well perfused despite its length. It is most probable that the dissection of a skin flap on the eyelid will have less perfusion as a result of less vasculature entering it. The rich vascular supply of the periorbital region is probably why full-thickness eyelid flap are so permissive, and ischemia and necrosis seldom occur postsurgically.[11] It allows the use of surgical reconstructions whose design would be inappropriate in other areas of the body.[7-9,11-13]

The eyelid was divided so that it remained attached only at the lateral canthus. Indeed, most of the blood flow to the eyelids comes from the medial canthus.[7] It cannot be deduced from the present study whether this was responsible for the good perfusion in the eyelid or what the result would have been if the flap had been dissected in the opposite direction, that is, extending from the medial canthus.

Studies on microvascular blood perfusion are difficult, as they are hampered by artifacts. It is therefore of the utmost importance that studies are conducted using multiple

methods to confirm the results. In the present study, perfusion was measured using 2 laser-based methods, LDV and LSCI, and tissue temperature was visualized using thermography. Laser-based methods such as LDV and LSCI quantify the change in motion in a specific tissue volume, which is interpreted as tissue blood perfusion. This means that artifacts will be introduced by movement resulting from, for example, breathing, and care must be taken to eliminate all sources of motion error. Thermography has been used to study plastic reconstructive procedures performance on other parts on the body.[14-17] The change in skin temperature is believed to be proportional to changes in microcirculation, but it may also be due to other metabolic processes in the cells such as inflammatory responses and thermoregulatory enzymes. The change in temperature following a sudden obstruction of blood perfusion following surgical dissection will therefore be seen after a certain delay compared with the change in perfusion seen when using LDV or LSCI. This delay was obvious during the measurements in the present study. The change in temperature was not as reliable as the change in perfusion measured with the laser-based methods. However, the possible limitations of the techniques used in the present study are of less importance in the interpretation of the results since we were interested in changes in perfusion compared with a reference point in the same object and at the same point in time, rather than measuring the actual perfusion. Furthermore, comparison of the results obtained with the 3 methods supports the results and conclusion drawn from the study.
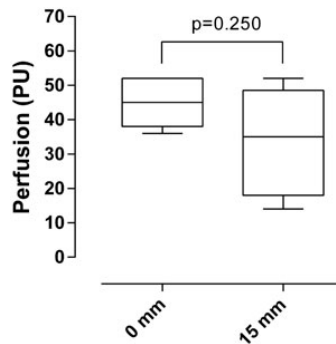

Figure 3. Laser Doppler velocimetry measurements of perfusion in a full-thickness eyelid flap on a pedicel, at 0 mm and 15 mm from the pedicel base (n = 5). Statistical analysis was performed using the Wilcoxon matched-pair test.

The current study was conducted in an eyelid flap model using the healthy tissue of a pig. Clinically, surgical reconstruction using flaps is commonly performed following the removal of a tumor from the eyelid or to rectify entropion. There is a risk of impaired wound healing in the tissues of the eyelids due to inflammation, infection, or edema or, in the case of the elderly, the skin may be very thin. Diabetes and cardiovascular disease may also affect the local vascular status of the eyelid, as will smoking. Other factors that may jeopardize postoperative healing include hematoma, tension or kinking of the tissue,

extensive previous surgery, and irradiation. Infection in the early postoperative period can destroy poorly vascularized tissues. Since the present study was conducted on healthy tissues in an experimental setting, the results should be interpreted with caution, as other local factors may affect flap survival in the clinical situation.
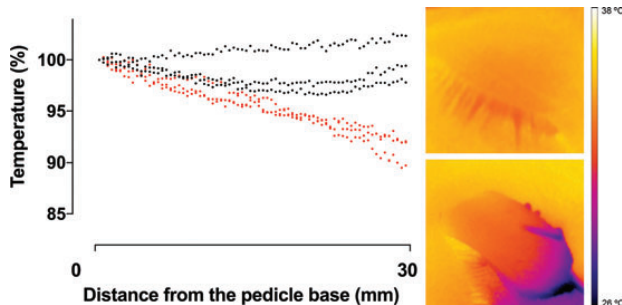

Figure 4. Thermographic measurements showing a decrease in temperature from the pedicel base to the tip of the full-thickness eyelid flap (red symbols), compared with that of intact eyelids (black symbols), calculated as the percentage of the temperature in the pedicel base (n = 3). The images on the right are representative examples of thermographic images of an intact eyelid (top) and a dissected eyelid (bottom).

In conclusion, perfusion in a full-thickness eyelid flap was measured using LDV, LSCI, and high-resolution thermography. The results show that there is only a slight drop in perfusion over the length of the full-thickness eyelid flaps. In total, 80% perfusion is maintained even if the flap was 3 cm long. The good perfusion in the eyelid flaps may be due to the rich vascularization by the anastomotic network of blood vessels in the tarsal plate, which may increase the likelihood of flap survival and surgical success.

REFERENCES

1. Quickert M, Sorby A. The eyelids. Malposition of the lid. In: *Modern Ophthalmology*. 2nd ed. London: Butterworth; 1972:937-54.
2. Tenzel RR, Stewart WB. Eyelid reconstruction by the semicircle flap technique. *Ophthalmology.* 1978;85(11):1164-9.
3. Stafanous SN. The switch flap in eyelid reconstruction. *Orbit.* 2007;26(4):255-62.
4. Allen J, Howell K. Microvascular imaging: techniques and opportunities for clinical physiological measurements. *Physiol Meas.* 2014;35(7):R91-141.
5. Winter GD. *A Study of Wound Healing in the Domestic Pig*. London: University of London; 1966.
6. Hartwell SW. *A Mechanism of Healing in Human Wounds*. Springfield, IL: Charles Thomas; 1955.
7. Codner MA, McCord CD, Mejia JD, Lalonde D. Upper and lower eyelid reconstruction. *Plast Reconstr Surg.* 2010;126(5):231e-45e.
8. Erdogmus S, Govsa F. The arterial anatomy of the eyelid: importance for reconstructive and aesthetic surgery. *J Plast Reconstr Aesthet Surg.* 2007;60(3):241-5.
9. Lopez R, Lauwers F, Paoli JR, Boutault F, Guitard J. The vascular system of the upper eyelid. Anatomical study and clinical interest. *Surg Radiol Anat.* 2008;30(3):265-9.

An Open Access Journal
...Committed to the free exchange of medical knowledge in a global community
www.eplasty.com

SHEIKH ET AL

10. Memarzadeh K, Sheikh R, Blohmé J, Torbrand C, Malmsjö M. Perfusion and oxygenation of random advancement skin flaps depend more on the length and thickness of the flap than on the width to length ratio. *Eplasty.* 2016;16:e12.

11. Patrinely JR, Marines HM, Anderson RL. Skin flaps in periorbital reconstruction. *Surv Ophthalmol.* 1987;31(4):249-61.

12. McCord C, Nunnery W. Reconstructive procedures or the lower eyelid and outer canthus. In: *Oculoplastic Surgery*. New York: Raven Press; 1981:194-8.

13. Collin JRO. *A Manual of Systematic Eyelid Surgery*. 2nd ed. New York: Churchill Living-stone; 1989:72-98.

14. de Weerd L Weum S, Mercer JB. Dynamic infrared thermography (DIRT) in the preoperative, intraoperative and postoperative phase of DIEP flap surgery. *J Plast Reconstr Aesthet Surg.* 2012;65(5):694-5, author reply 695-6.

15. de Weerd L Weum S, Mercer JB. The value of dynamic infrared thermography (DIRT) in perforator selection and planning of free DIEP flaps. *Ann Plast Surg.* 2009;63(3):274-9.

16. de Weerd L Miland AO, Mercer JB. Perfusion dynamics of free DIEP and SIEA flaps during the first postoperative week monitored with dynamic infrared thermography. *Ann Plast Surg.* 2009;62(1):42-7.

17. de Weerd L Mercer JB, Setsa LB. Intraoperative dynamic infrared thermography and free-flap surgery. *Ann Plast Surg.* 2006;57(3):279-84.

Paper III

Optimal Epinephrine Concentration and Time Delay to Minimize Perfusion in Eyelid Surgery: Measured by Laser-Based Methods and a Novel Form of Extended-Wavelength Diffuse Reflectance Spectroscopy

Rafi Sheikh, M.D.*, Ulf Dahlstrand, M.D.*, Khashayar Memarzadeh, M.D.*, Jonas Blohmé, M.D., Ph.D.*, Nina Reistad, Ph.D.†, and Malin Malmsjö, M.D., Ph.D.*

*Lund University, Skane University Hospital, Department of Clinical Sciences Lund, Ophthalmology, and †Department of Physics, Lund University, Lund, Sweden

Objective: This study investigates the hypoperfusion effects of epinephrine in local anesthesia in eyelid surgery. A novel form of extended-wavelength diffuse reflectance spectroscopy was evaluated.

Methods: Blood perfusion in porcine eyelid flaps was measured using laser Doppler velocimetry and laser speckle contrast imaging, whereas the tissue response was measured using diffuse reflectance spectroscopy with a broad spectrum (450–1550 nm). Epinephrine was either injected cumulatively, 0.1 (1:10,000,000), 1.0 (1:1,000,000), 10 (1:100 000), and 100 µg/ml (1:10 000), to determine the dose–response relation, or given as a single dose (10 µg/ml). Control experiments were performed with saline or lidocaine.

Results: Increasing concentrations of epinephrine resulted in a gradual decrease in tissue perfusion, measured by laser Doppler velocimetry and laser speckle contrast imaging, approaching a minimum after the injection of 10 µg/ml. Similar tissue response was observed with diffuse reflectance spectroscopy. The time from the injection of epinephrine (10 µg/ml) to the stabilization of hypoperfusion was 75 seconds. After administration of 10 µg/ml epinephrine, about 20% of the blood perfusion remained, supporting the use of epinephrine in eyelid flaps with a narrow pedicle.

Conclusions: 10 µg/ml epinephrine appears to be adequate for vasoconstriction before oculoplastic surgery. Incisions need only be delayed for about 1 minute. Extended-wavelength diffuse reflectance spectroscopy appears to be a promising technique for monitoring the tissue response following changes in blood perfusion in plastic surgery reconstructions. However, more rigorous validation of the technique is required before it can be implemented in clinical practice.

(*Ophthal Plast Reconstr Surg* 2017;XX:00–00)

Epinephrine is used in oculoplastic surgery to induce vaso-constriction to reduce bleeding and prolong anesthesia. The optimal concentration of epinephrine has been strenuously debated during the past 50 years[1]; however, there is a lack of relevant data. As this hormone may have systemic effects, including hemodynamic disturbances such as hypertension and arrhythmia, it is important to determine the lowest concentration of epinephrine that produces local vasoconstriction.[2,3] The time required to reach maximal cutaneous vasoconstriction following the administration of epinephrine has also been the subject of debate. The time to maximal cutaneous vasoconstriction after the injection of lidocaine with epinephrine is often given as 7 to 10 minutes. However, in a recent study by McKee et al.,[4] who measured the relative hemoglobin concentration over time in the arm skin of healthy volunteers, the lowest cutaneous hemoglobin level was observed after a mean time of 26 minutes. If this is truly representative, there is a need to significantly increase the preparation time for surgery.

It is well known that blood perfusion can be measured using laser-based techniques such as laser Doppler velocimetry (LDV) and laser speckle contrast imaging (LSCI).[5] However, drawbacks are associated with these techniques because they measure motion (interpreted as blood flow) in a specific tissue volume, and any movement of the patient, for example, due to breathing, will interfere with the measurements. The laser-based techniques do not provide detailed estimates of the cellular access to oxygen or the metabolic response of the tissue. There is thus a must to develop new techniques, preferably noninvasive ones, to quantify the response of the tissue. Spectroscopy-based methods, such as diffuse reflectance spectroscopy (DRS), can provide a wide range of information on the tissue response, and has so far been used to characterize tissue viability by measuring the degree of hemoglobin oxygenation,[6] total hemoglobin concentration[7] and tissue hydration.[8] In DRS, the tissue is illuminated with broad-spectrum light, and the diffuse reflected light is collected after interaction with the tissue. Diffuse reflectance spectroscopy is commonly used in the wavelength range 500 to 1000 nm, but the novelty of the technique is that the spectrum has been extended to include wavelengths in both the visible and near-infrared regions, that is, from 450 to 1550 nm.[9] The tissue characteristics are determined based on the fact that different tissue components have different absorption characteristics. It has been shown in an initial study that it is possible to differentiate between normal and metastatic liver tissue in humans using this technique.[9] The blood flow can be approximated using hemoglobin derivatives as a measure of perfusion. Traditionally, DRS measurements of

hemoglobin content are based on analysis at only a few wavelengths, at which the absorption of hemoglobin is dominating.[10] To make full use of the information contained in the extended spectrum, the authors used a standard multivariate statistical method, partial least squares regression. The authors hope that this will provide a clinically useful technique for simple, noninvasive measurements of the tissue response.

The aim of this study was to investigate the effects of epinephrine on the tissue and its perfusion in oculoplastic surgery. The concentration of epinephrine that provides maximal hypoperfusion and the time required to reach maximal hypoperfusion was studied in particular. Perfusion was measured using 2 laser-based techniques: LDV, in which perfusion is measured at a specific point in the tissue using an invasive probe, and LSCI, in which perfusion is measured over a larger tissue area. The results were compared with those from the extended-wavelength DRS technique.

METHODS

Animals and Anesthesia. Eight pigs with a bodyweight of 70 kg were fasted overnight, but had free access to water. An intramuscular injection of xylazine (Rompun vet. 20 mg/ml; Bayer AG, Leverkusen, Germany; 2 mg/kg) mixed with ketamine (Ketaminol vet. 100 mg/ml; Farmaceutici Gellini S.p.A., Aprilia, Italy; 20 mg/kg) was used for premedication. Anesthesia was then induced by intravenous sodium thiopental (Pentothal; Abbot Scandinavia, Stockholm, Sweden; 4 mg/kg) and fentanyl (Leptanal; Lilly, France; 2 μg/kg), and maintained by continuous infusion of fentanyl in Ringer's acetate (3.5 μg/kg/hour) in combination with sodium thiopental (~2.5 mg/kg). The animals were orally intubated with cuffed endotracheal tubes. Mechanical ventilation was established in the volume-controlled mode with 35% oxygen (Siemens-Elema AB, Solna, Sweden). The ventilation settings were identical for all animals: respiratory rate 15 breaths/min and minute ventilation 12 l/min. A positive end-expiratory pressure of 5 cmH$_2$O was applied. A Foley catheter was inserted into the urinary bladder through a suprapubic cystostomy. Following anesthesia and the surgical procedures, the pig was allowed to stabilize for 1 hour before the experiments were started.

Ethics. The experimental protocol for this study was approved by the Ethics Committee for Animal Research at Lund University, Sweden. All animals received humane care in compliance with the European Convention on Animal Care. The animals were also used for other experiments, which were considered not to have an impact on the present study.

Experimental Procedure. A vertical incision was made in the eyelid, and extended horizontally to obtain a full-thickness eyelid flap 10 mm high and 30 mm long (Fig. 1), extending from the lateral canthus. A 27-gauge needle was inserted in the flap base, for the injection of pharmacological agents. The pharmacological agents were diluted in saline and preheated to 38°C before injection. Pharmacological agents were injected into the subcutaneous tissue in the pedicle of the eyelid flap. The volume chosen was 0.3 ml so as to represent a clinically significant amount, without resulting in tissue compression that would cause immediate hypoperfusion and interfere with the perfusion measurements. Saline (Natriumklorid; Baxter Medical AB, Kista, Sweden; 9 mg/ml) was administered before each series of pharmacological agents, as a negative control. A single dose of lidocaine (Lidokain; Mylan Hospital AS, Oslo, Norway; 20 mg/ml) was administered, after which epinephrine (Adrenalin; Mylan Hospital AB, Oslo, Norway; 1 mg/ml) was diluted and injected at increasing concentrations of 0.1 (1:10,000,000), 1.0 (1:1,000,000), 10 (1:100,000), and 100 μg/ml (1:10,000).

Blood perfusion was measured before each series of injections of pharmacological agents to establish the baseline value. The perfusion was allowed to stabilize for at least 5 minutes after each injection, after which perfusion measurements were made. The results were analyzed to determine the dose–response relation and define
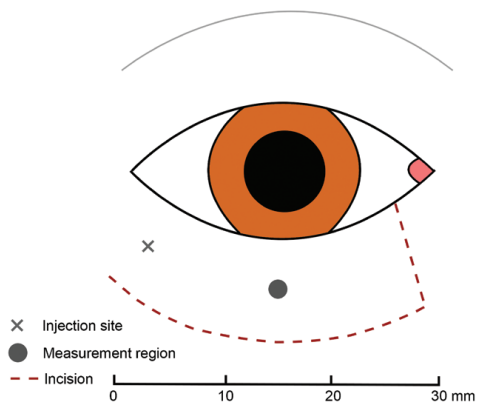

FIG. 1. Schematic illustration of the location of the injection site and measurement point along the length of a full-thickness lower eyelid flap.

the lowest concentration of epinephrine leading to maximal effect (measured using LDV, LSCI, and DRS after injection of increasing concentrations of epinephrine). The time to maximal vasoconstriction was measured continuously using LSCI after injection of 10 μg/ml epinephrine. The current DRS setup takes 30 seconds for each measurement and was therefore not suitable for recording time response in enough resolution. After completion of the series of injections in the flaps, the blood supply to the pedicle of the flap was occluded using a ligature. Another measurement was made and the perfusion at this time was assumed to be zero.

Laser Doppler Velocimetry. Laser Doppler velocimetry is based on the emission of a beam of laser light. The light is scattered and partly absorbed in the tissue being studied. Light that is reflected by moving blood cells undergoes a change in wavelength (Doppler shift), while light reflected from static tissue is unchanged. The magnitude and frequency distribution of these changes in wavelength are related to the number and velocity of blood cells. A filament probe (MT A500-0, straight microtip with slanted tip; Perimed, Stockholm, Sweden) was inserted 15 mm from the flap base (Fig. 1), using a 22 G Venflon infusion cannula. This technique allows measurement of the perfusion in a 1 mm^3 volume surrounding the tip of the probe.[11] The filament probe was attached to a master probe (Probe 418), which was then connected to the main LDV unit (PF5010; Perimed).

Laser Speckle Contrast Imaging. A LSCI system (PeriCam PSI NR System; Perimed) was used to obtain images of perfusion over the surface of the tissue. The system uses a 785 nm invisible laser beam that is spread over the skin surface by a diffuser, creating a speckle pattern that consists of dark and bright areas formed by random interference of the light backscattered from the area illuminated by the laser. The blood perfusion is determined by analyzing the variations in the speckle pattern. The speckle pattern is recorded in real time at a rate of up to 100 images per second, with a resolution of 100 μm/pixel. Perfusion was measured over an area of 5 mm^2, 15 mm from the flap base.

Diffuse Reflectance Spectroscopy. Diffuse reflectance spectral signatures were collected using a portable spectroscopic system comprising a contact fiber optical probe, a tungsten halogen light source (HL-2000-HP; Ocean Optics, Dunedin, FL), and 2 miniature spectrometers. The 2 spectrometers resolve light in the visible wavelength range from 350 nm to 1100 nm (QE65000-VIS-NIR; Ocean Optics), and in the near-infrared
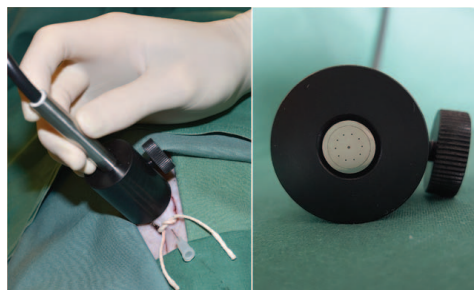
FIG. 2. **Left**, The DRS probe with the 10-mm-diameter fiber bundle attached to a custom-made probe holder with a diameter of 25 mm. The probe is gently held against the eyelid flap. **Right**, A close-up of the probe holder and the probe in which 1 central fiber serves as the light source and the surrounding fibers as collection fibers. DRS, diffuse reflectance spectroscopy.

region from 900 nm up to 1700 nm (NIRQuest512; Ocean Optics). The probe is a 10-mm-diameter trifurcated fiber bundle and consists of a 10-fiber, 200-μm-diameter signal collection ring around a single illuminating 400-μm-diameter fiber with a source–detector separation of 2.5 mm. The distal end of the probe is fixed in a custom-made black cylindrical probe holder with a diameter of 25 mm, which is held against the tissue (Fig. 2). Reference spectra were acquired before and after each set of tissue measurements using a reflectance standard (Spectralon SRS-99-010; Labsphere, North Sutton, NH). Data acquisition from the spectrometers is controlled by Ocean-View software (Ocean Optics) on a standard laptop computer. Measurements were made directly on the skin of the flaps and the overlapping wavelength range of the 2 spectrometers was used to combine the 2 spectra to form 1 continuous spectrum from 450 nm to 1550 nm (Fig. 3).
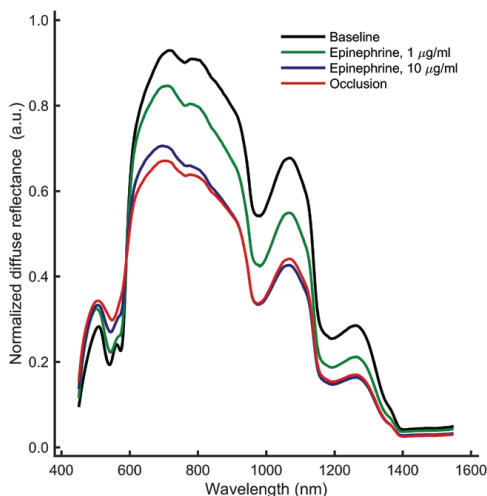
Calculations and Statistics. Eight pigs were used in this study. The number of repeated experiments is denoted *n*. For practical reasons, not all experiments were performed on all pigs, and multiple experiments were performed on some pigs. Both upper and lower eyelids were used in the study and the results were combined. It could be argued that the results for the 2 eyelids should be considered separately. However, no differences were found between the upper and lower eyelids, and they were thus considered together. Statistical analysis was performed using the Kruskal–Wallis test with Dunn's post hoc test for multiple comparisons. Significance was defined as $p < 0.05$ (*) and $p > 0.05$ (not significant). Calculations and statistical analysis were performed using GraphPad Prism 7.0a (GraphPad software Inc., San Diego, CA).

Laser-Based Methods. Perfusion measured with the 2 laser-based techniques was expressed in the arbitrary units, perfusion units. The baseline values, recorded before injection of the pharmacological agents, were set to 100%, and the value recorded when the flow had been occluded by a ligature in the base of the flap was set to 0%. In experiments where both LDV and LSCI were used, all values were used in the calculations. The time from the injection of epinephrine until stabilization of hypoperfusion was determined by nonlinear regression analysis (1-phase decay). The results are presented either as median values and ranges or mean values and 95% confidence intervals.

Diffuse Reflectance Spectroscopy. Partial least squares regression was used to quantify the relationship between the diffuse reflectance measurements and tissue perfusion. The standard normal variate pretreatment was calculated as follows. Each diffuse reflectance spectrum was normalized by subtracting the mean diffuse reflectance from all the spectra at each data point and then dividing each point by the standard deviation of all the diffuse reflectance spectra. The partial least square regression model was calibrated using reference values as independent data and the preprocessed diffuse reflectance spectra as dependent data. A total of 265 optical measurements performed on 53 measurement occasions were used for calibration. Due to the limited sample size, the
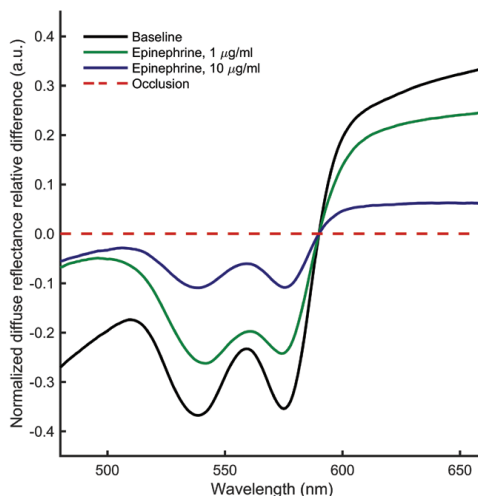

FIG. 3. Graphs show the normalized diffuse reflectance as a function of wavelength in an eyelid flap before (baseline) and after injecting 1.0 epinephrine, 10 μg/ml epinephrine and occlusion of the blood flow. The shapes differ, especially in the region of 550 nm, where the absorption of deoxygenated and oxygenated hemoglobin is greatest. Studying this wavelength more closely (graph to the right), it can be seen that the double dip is visible after the injection of saline, is less pronounced after the injection of 0.1 μg/ml epinephrine and even less pronounced after the injection of 10 μg/ml epinephrine, and disappears completely when the blood flow is occluded. This coincides with the decrease in oxygenated hemoglobin that occurs as a result of vasoconstriction.

calibration was validated using leave-one-out cross-validation. The optimum number of partial least square components was selected based on the lowest cross-validated calibration error. The model was evaluated based on its explained variance and prediction error. Finally, the partial least square model was used to predict the perfusion after each injection. Spectrum processing and modeling were performed using Matlab R2015b (The Mathworks Inc., South Natick, MA).

RESULTS

According to LDV and LSCI measurements, neither saline nor lidocaine affected the perfusion in eyelid flaps (107 perfusion units (range 81–161) at baseline, 104 perfusion units (78–156) after saline, and 100 perfusion units (76–149) after lidocaine, *p* = not significant, n = 8).

Concentration of Epinephrine. Increasing the concentration of epinephrine resulted in a gradual decrease in eyelid perfusion according to the laser-based measurements (Figs. 4 and 5A). Maximum effect was approached following the injection of 10 µg/ml epinephrine. Perfusion was 26% (95% confidence interval = 10–42) after 10 µg/ml epinephrine and 15% (95% confidence interval = −1 to 30) after 100 µg/ml epinephrine (*p* = not significant).

In line with laser-based measurements, there was a similar gradual change in tissue response according to the DRS measurements, as shown in Figure 5B, after multivariate analysis to take full advantage of the spectra obtained from the tissue. Representative examples of the spectra obtained from DRS measurements are shown in Figure 3 and illustrates that a broader wavelength range (450–1550 nm) provides
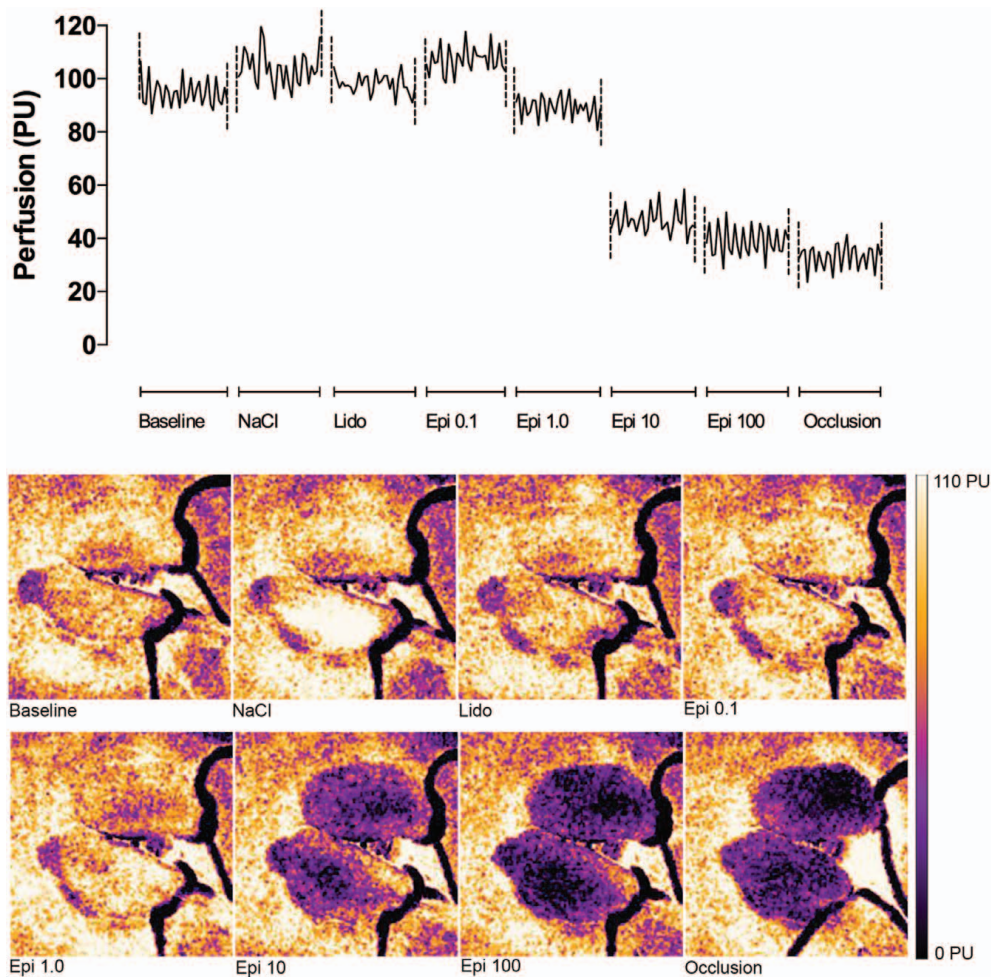
FIG. 4. LSCI raw data in which the top graph are 30-second period recordings and the bottom images are the eyelid flaps, at baseline and after the injection of saline, lidocaine, and increasing concentrations of epinephrine, and after occlusion of the eyelid flap pedicle (the ligature is visible in black). In both the graphs and the images, it can be seen that the perfusion decreases substantially after the injection of 10 µg epinephrine/ml. LSCI, laser speckle contrast imaging.

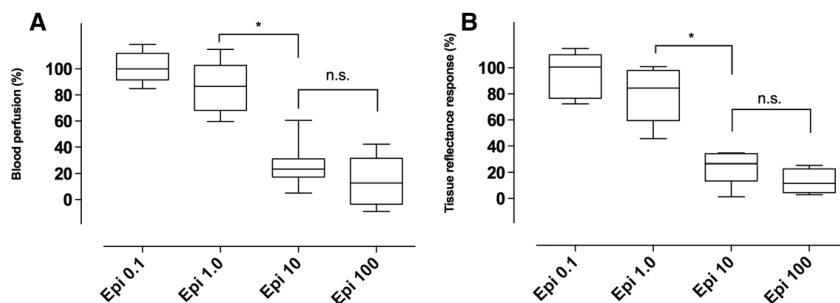**FIG. 5.** Graphs showing the results obtained after the injection of increasing concentrations of epinephrine (0.1, 1.0, 10, or 100 μg/ml) into eyelid flaps. **A**, Data from measurements with the laser-based techniques (LDV and LSCI, combined, n = 8), and (**B**) data from DRS measurements (n = 6) after multivariate analysis of the full spectra (450–1550 nm). The results are expressed as percent of the baseline value (defined as 100%), with occlusion using a ligature set to 0%. It can be seen, using both laser-based techniques and DRS, that maximum hypoperfusion was approached after the injection of 10 μg/ml epinephrine. DRS, diffuse reflectance spectroscopy; LDV, laser Doppler velocimetry; LSCI, laser speckle contrast imaging.
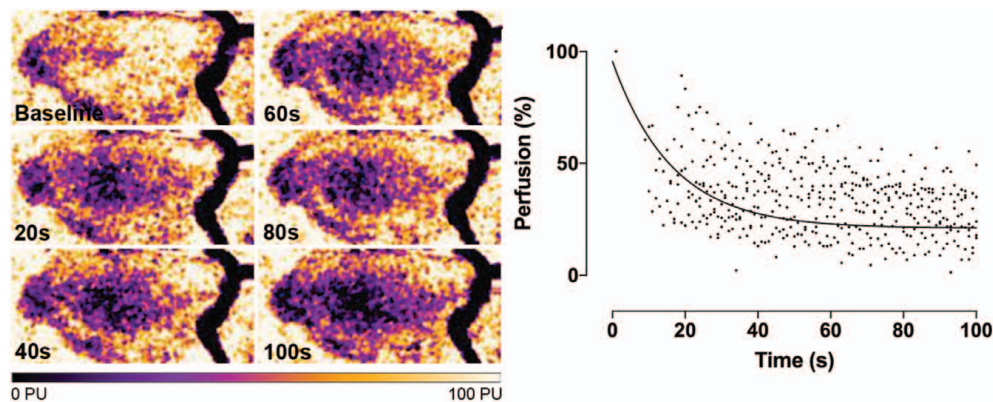

FIG. 6. Decrease in perfusion over time after the injection of 10 μg/ml epinephrine in an eyelid flap, measured using LSCI. The images on the left are representative examples of LSCI images, and the right-hand image is a scatterplot showing all the data from 6 experiments. The results are expressed as percent of the baseline value (defined as 100%) and were obtained using nonlinear regression analysis (1-phase decay). Maximum hypoperfusion was reached after 75 seconds (95% confidence interval: 57–92 seconds). LSCI, laser speckle contrast imaging.

detailed information of the tissue response. The diffuse reflectance changed gradually after injecting epinephrine and ligation of the flap pedicle for occlusion of blood flow. The shapes of the reflected spectrum differ, especially in the region of 550 nm, where the absorption of deoxygenated and oxygenated hemoglobin is greatest. Studying this wavelength more closely (Fig. 3), it can be seen that the double dip is visible after the injection of saline, is less pronounced after the injection of 0.1 μg/ml epinephrine and even less pronounced after the injection of 10 μg/ml epinephrine, and disappears completely when the blood flow is occluded. This coincides with the decrease in oxygenated hemoglobin that occurs as a result of vasoconstriction.

Time to Maximum Hypoperfusion. The time from the injection of 10 μg epinephrine/ml until the stabilization of hypoperfusion was measured in the eyelid flaps using LSCI. Blood perfusion decreased over time (Fig. 6), and reached a plateau at a lower level after 75 seconds (95% confidence interval: 57–92 seconds), according to nonlinear regression analysis (1-phase decay).

DISCUSSION

This study was performed to investigate hypoperfusion in response to epinephrine when used for local anesthesia in oculoplastic surgery. The tissue response was measured by DRS employing an extended-wavelength range, and the results were compared with those obtained with conventional laser-based techniques. The broader wavelength range (450–1550 nm) was used to provide more detailed information on the response of the tissue. The wider spectral window makes it easier to measure components such as lipids, cholesterol, and collagen,[12] as well as water content, which is likely to change after vasoconstriction. Multivariate analysis was used to take full advantage of the spectra obtained from tissues with different levels of perfusion. The results show a clear change in reflected spectra after the drop in perfusion following the injection of epinephrine, indicating that the technique can be used to monitor the tissue response upon changes in blood perfusion.

Different parts of the diffuse reflectance spectrum represent different penetration depths and depends on the absorption of the tissue and the source–detector distance of the fiber optical probe.[13] The separation between the central light source fiber and the ring of detection fibers in the probe used for the DRS measurements was 2.5 mm which will give a recording approximately 1 mm to 2 mm deep in the tissue. This is the depth of the outer tarsal lamella, in which the tarsal arcades and their circulation are included. If this technique can be implemented in clinical practice for other plastic surgery reconstructions, the distance between the light source and the detectors can be adjusted to provide deeper or more superficial measurements.

The concentration of epinephrine that has maximal effect on the perfusion and tissue response was measured using both laser-based techniques (LDV and LSCI) and DRS. Maximal effect was approached at a concentration of 10 μg/ml, which is similar to the value found in one of the previous studies in porcine full-thickness skin flaps on the flank,[14] and other studies on local cutaneous hypoperfusion in patients.[15,16] It is also similar to the concentration of epinephrine in the commonly used Xylocaine Dental adrenaline (Dentsply Ltd, Weybridge, Surrey, UK), which contains 12.5 μg epinephrine/ml. However, other local anesthetics with lower concentrations of epinephrine are used clinically, for example, Xylocain adrenalin (Astra Zeneca, Södertälje, Sweden) containing 5 μg epinephrine/ml. In the clinical situation, achieving maximal vasoconstriction of the peripheral circulation must be weighed against the possibility of systemic effects. Nonetheless, an appropriate concentration of epinephrine should be given with the local anesthetic to guarantee sufficient depth and duration of local anesthesia to avoid patient pain and stress.

The authors also investigated the time from the injection of epinephrine (10 μg/ml) to maximum hypoperfusion in eyelid flaps, and found this to be 75 seconds. This is much shorter than that found in cutaneous flank flaps, where a plateau was reached after approximately 120 seconds.[14] The eyelids have a rich vascular supply that originates from both the lateral and medial canthus, where branches of the palpebral arteries supply the tarsal arcade.[17] This rich perfusion may ensure that the epinephrine spreads quickly throughout the eyelid flap. Also, the fact that the epinephrine was injected in the flap pedicle could result in immediate constriction of the tarsal arcades, rapidly reducing blood flow to the eyelid flap. These results are in contrast to those reported by McKee et al.,[4] who found that the mean time at which the lowest cutaneous hemoglobin level was obtained in the arm skin of healthy volunteers after the administration of 1% lidocaine with epinephrine was 26 minutes. In a later study McKee et al.[18] measured the quantity of blood loss after 7 or 30 minutes from the time of injection of lidocaine + epinephrine to the time of incision and found less blood loss after 30 minutes. These results are also different from the results from the present study. Future human studies in different anatomical locations, combining different techniques, may be helpful in describing the true effect of epinephrine in local anesthetics.

However, these results are in accordance with clinical experience that perfusion in the periorbital region drops rapidly following local anesthetics with epinephrine, and also in line with the short times often given in textbooks, that is, 7 to 10 minutes, and previous scientific studies.[19] The results confirm that oculoplastic surgery could be started soon after infiltration anesthesia has been administered.

Even though vasoconstriction occurred quickly in this study, a plateau was reached at a level of about 20% perfusion, supporting the view that epinephrine can be used safely in periorbital skin procedures. Indeed, in the clinical setting, periorbital surgery is very permissive, allowing advanced flaps to be made without the risk of necrosis.

In conclusion, an epinephrine concentration of about 10 μg/ml appears to be adequate for vasoconstriction in tissue before eyelid surgery, and incisions need only be delayed for about 1 minute following lidocaine + epinephrine injection to allow for the maximal effect to be reached. The remaining 20% blood perfusion observed after the administration of 10 μg/ml epinephrine in the present study supports the safe use of epinephrine in flaps with a narrow pedicle. However, in the experimental setup of this study, only a small volume was injected into the base of the eyelid flap. In eyelid surgery, usually a more infiltrative injection technique is used, which might cause a more pronounced hypoperfusion. Diffuse reflectance spectroscopy using a broad spectrum (450–1550 nm) showed similar results to those obtained with the laser-based techniques, LDV and LSCI. Diffuse reflectance spectroscopy thus appears to be a promising technique for measuring tissue response following changes in blood perfusion in plastic surgery reconstructions. This was a pilot study with a limited number of DRS recordings, and more rigorous validation of the technique is required before it can be implemented in clinical practice.

REFERENCES

1. Brown RS, Rhodus NL. Epinephrine and local anesthesia revisited. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005;100:401–8.
2. Bhatia N, Ghai B, Mangal K, et al. Effect of intramucosal infiltration of different concentrations of adrenaline on hemodynamics during transsphenoidal surgery. *J Anaesthesiol Clin Pharmacol* 2014;30:520–5.
3. Bader JD, Bonito AJ, Shugars DA. A systematic review of cardiovascular effects of epinephrine on hypertensive dental patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;93:647–53.
4. McKee DE, Lalonde DH, Thoma A, et al. Optimal time delay between epinephrine injection and incision to minimize bleeding. *Plast Reconstr Surg* 2013;131:811–4.
5. Zötterman J, Bergkvist M, Iredahl F, et al. Monitoring of partial and full venous outflow obstruction in a porcine flap model using laser speckle contrast imaging. *J Plast Reconstr Aesthet Surg* 2016;69:936–43.
6. Myers DE, Anderson LD, Seifert RP, et al. Noninvasive method for measuring local hemoglobin oxygen saturation in tissue using wide gap second derivative near-infrared spectroscopy. *J Biomed Opt* 2005;10:034017.
7. Glennie DL, Hayward JE, Farrell TJ. Modeling changes in the hemoglobin concentration of skin with total diffuse reflectance spectroscopy. *J Biomed Opt* 2015;20:035002.
8. Egawa M, Arimoto H, Hirao T, et al. Regional difference of water content in human skin studied by diffuse-reflectance near-infrared spectroscopy: consideration of measurement depth. *Appl Spectrosc* 2006;60:24–8.
9. Reistad N, Nilsson J, Timmermand OV, Sturesson C, Andersson-Engels S. Diffuse reflectance spectroscopy of liver tissue. Proceedings of SPIE. 2015;9531.
10. Phelps JE, Vishwanath K, Chang VT, et al. Rapid ratiometric determination of hemoglobin concentration using UV-VIS diffuse reflectance at isosbestic wavelengths. *Opt Express* 2010;18:18779–92.
11. Zografos GC, Martis K, Morris DL. Laser Doppler flowmetry in evaluation of cutaneous wound blood flow using various suturing techniques. *Ann Surg* 1992;215:266–8.
12. Wilson RH, Nadeau KP, Jaworski FB, et al. Review of short-wave infrared spectroscopy and imaging methods for biological tissue characterization. *J Biomed Opt* 2015;20:030901.
13. Hennessy R, Goth W, Sharma M, et al. Effect of probe geometry and optical properties on the sampling depth for diffuse reflectance spectroscopy. *J Biomed Opt* 2014;19:107002.
14. Sheikh R, Memarzadeh K, Torbrand C, Blohme J, Malmsjo M. Hypoperfusion in response to epinephrine in local anesthetics: Investigation of dependence on epinephrine concentration, spread of hypoperfusion, and time to maximal cutaneous vasoconstriction

[published online ahead of print November 11, 2016]. *J Plast Reconstr Aesthet Surg*. 2016. doi: 10.1016/j.bjps.2016.10.017.

15. Liu S, Carpenter RL, Chiu AA, et al. Epinephrine prolongs duration of subcutaneous infiltration of local anesthesia in a dose-related manner. Correlation with magnitude of vasoconstriction. *Reg Anesth* 1995;20:378–84.

16. Larrabee WF, Jr, Lanier BJ, Miekle D. Effect of epinephrine on local cutaneous blood flow. *Head Neck Surg* 1987;9:287–9.

17. Codner MA, McCord CD, Mejia JD, et al. Upper and lower eyelid reconstruction. *Plast Reconstr Surg* 2010;126:231e–45e.

18. Mckee DE, Lalonde DH, Thoma A, et al. Achieving the optimal epinephrine effect in wide awake hand surgery using local anesthesia without a tourniquet. *Hand (N Y)* 2015;10:613–5.

19. Ghali S, Knox KR, Verbesey J, et al. Effects of lidocaine and epinephrine on cutaneous blood flow. *J Plast Reconstr Aesthet Surg* 2008;61:1226–31.

Paper IV

A waiting time of 7 min is sufficient to reduce bleeding in oculoplastic surgery following the administration of epinephrine together with local anaesthesia

Jenny Hult,[#] Rafi Sheikh,[#] [iD] Cu Dinh Nguyen, Kajsa Tenland, Ulf Dahlstrand and Malin Malmsjö [iD]

Department of Clinical Sciences, Lund, Ophthalmology, Skane University Hospital, Lund University, Lund, Sweden

ABSTRACT.

Objective: The time taken to reach maximal haemostatic effect following local anaesthesia with epinephrine is generally believed to be <10 min. This is based on clinical experience and indirect measurements of perfusion using methods such as laser Doppler flowmetry and oxygen spectroscopy. However, the only study in which bleeding has been measured quantitatively in an intra-operative setting in humans showed that the full haemostatic effect was not achieved until 30 min after anaesthesia. The aim of this study was to determine the time taken to reach maximum haemostatic effect when using epinephrine for local anaesthesia in oculoplastic surgery.

Methods: Intra-operative bleeding following infiltration anaesthesia with either lidocaine 20 mg/ml (2%) or lidocaine + epinephrine 12.5 μg/ml (1:80 000) was measured after 7, 15 and 30 min in the eyelids of 16 patients undergoing upper eyelid blepharoplasty.

Results: Bleeding was decreased by 74.6% (with 95% CI, 6.16–87.6%) 7 min after the injection of lidocaine + epinephrine (p = 0.0048) compared with lidocaine without epinephrine. There was no further decrease in bleeding after 15 or 30 min (p = n.s.).

Conclusion: The optimal time for skin incision in eyelid surgery is within 7 min of injection of lidocaine with epinephrine. Waiting longer does not lead to a further decrease in bleeding.

Key words: epinephrine – eyelid – lidocaine – local anaesthesia – timing – vasoconstriction

[#]Equally contributed.

Introduction

The combination of epinephrine and lidocaine is frequently used for local anaesthesia in plastic surgery procedures. The use of epinephrine has been shown to decrease bleeding by vasoconstriction, prolong the analgesic effect and reduce the systemic effects of local anaesthetic agents (Fink et al. 1978; Larrabee et al. 1987). Surgeons usually wait several minutes for epinephrine to act before commencing surgery in order to minimize bleeding. The time taken to reach maximal haemostasis following the administration of epinephrine has recently become the subject of debate. The time commonly given in textbooks is 10 min (Collin & Rose 2001). However, in a study by McKee et al. (2013), who measured the relative haemoglobin concentration over time in the arm skin of healthy volunteers with oxygen spectroscopy, the lowest cutaneous haemoglobin level was observed after a mean time of 26 min. In another study, McKee et al. (2015) measured the amount of blood loss from the skin of patients undergoing carpal tunnel surgery and found that there was a significant reduction after 30 min, compared to 7 min, following the administration of lidocaine + epinephrine. If this is representative of skin in other regions of the body, there is a need to significantly increase the preparation time for surgery. To the best of our knowledge, the study by McKee et al. (2015) is the only one performed to date in which the effect of local anaesthetic with epinephrine has been evaluated quantitatively by measuring the amount of blood resulting from bleeding in an intra-operative setting on humans, and no such study has yet been performed in the ocular region.

The aim of this study was, thus, to determine the optimal time for skin incision following the administration of local anaesthesia with epinephrine in oculoplastic surgery. Bleeding in eyelids undergoing blepharoplasty was measured after the infiltration of lidocaine (20 mg/ml, 2%) or lidocaine + epinephrine (12.5 μg/ml, 1:80 000) at 7, 15 and 30 min.

Patients and Methods

Ethics

The experimental protocol for this study was approved by the Ethics Committee at Lund University, Sweden. The research adhered to the tenets of the Declaration of Helsinki as amended in 2008. All the patients participating in the study were thoroughly informed about the study and the voluntary nature of participation. The information was given by a person, not the surgeon, who did not participate in the treatment of the patient. All patients gave their fully informed consent.

Subjects

Patients that were admitted at the Department of Ophthalmology, Skåne University Hospital, for upper eyelid blepharoplasty, for correction of dermatochalasis, during March 2017 were consecutively recruited for the study. The patients' previous medical records were unknown to us before the day of surgery. Exclusion criteria were inability to provide informed consent, and physical or mental inability to co-operate during the local anaesthetic procedure. Patients with pronounced fat prolapse in need for separate fat excision were planned to be excluded from the study. No patients were excluded. Surgery was performed under local anaesthesia. Sixteen patients were included in the study.

Surgical procedure

The patients underwent conventional upper eyelid blepharoplasty, for correction of dermatochalasis with skin excision only, or skin and muscle excision with some liposculpting using diathermy. A traction suture between the eyelid and the sterile drape covering the face was used to keep the eyelid still throughout the procedure. The local anaesthetic and epinephrine were preheated to 37 °C in a water bath.

Local anaesthesia was applied using 1.0 ml lidocaine (Lidokain®; Mylan Hospital AS, Oslo, Norway; 20 mg/ml) or 1.0 ml lidocaine with epinephrine (Xylocain Dental® Adrenalin, consisting of 20 mg/ml lidocaine + 12.5 μg/ml epinephrine, from Dentsply Ltd., York, PA, USA), as depicted in Fig. 1. The local anaesthetics was injected subcutaneously and not in the orbicularis muscle. It was infiltrated as uniformly as possible in an area of 2 cm × 4 cm to achieve a standardized anaesthesia. Furthermore, all patients were operated by the same surgeon, who made the best efforts to make the infiltration as similar as possible in all patients.

In Region 1, lidocaine only was injected, and the first incision was made immediately after the onset of analgesia (approximately 1 min). These measurements were used to establish a control value. Lidocaine + epinephrine was injected into Region 4 in this eyelid, and the first incision was made after 30 min. Lidocaine + epinephrine was injected into the two regions of the other eyelid (one dose of 1 ml in each region). The first incision was made in Region 2 after 7 min, and in Region 3 after 15 min. This sequence of measurements, that is performing the 'lidocaine' measurement and the 'lidocaine + epinephrine (30 min)' measurement on the same eyelid, was adopted to make the surgical procedure as short as possible. A doctor not involved in the study randomly assigned the patients to one of two groups. Regions 1 and 4 were assigned to the right eye in one group, and to the left eye in the other.

Blood was collected from the site of excision using viscose non-woven surgical swabs (ocular sticks, Pro-Ophta®; Lohmann & Rauscher GmbH & Co. KG, Rengsdorf, Germany) for a total of 5 min at each site (see Fig. 2 for representative example of the blood collection from one patient). This was divided into two fractions: (i) the first 2.5 min after the first incision and (ii) the second 2.5 min after the first incision (2.5–5 min). The ocular sticks containing the blood were placed in pill cups and weighed on a calibrated scale (Ohaus Scout, model STX223; OHAUS Corporation, Parsippany, NJ, USA). The weight of the clean ocular sticks and pill cups was subtracted to obtain the weight of the blood. The blepharoplasty procedure was then completed according to clinical praxis. There were no adverse events, and the surgical results were good.

Calculations and statistics

Sixteen patients (31 eyelids) were included in the study (one patient did not have surgery on the right eyelid). Bleeding following lidocaine + epinephrine administration was calculated as the percentage of control (lidocaine without epinephrine). Calculations and statistical analysis were performed using GRAPHPAD PRISM 7.2 (GraphPad Software Inc., San Diego, CA, USA). One outlier was identified in the 0–2.5 min interval, two outliers were identified in the 2.5–5 min interval, and one outlier was identified in the total interval 0–5 min using the robust regression and outlier removal (ROUT) (Q = 1%) test, and were not included in the analysis. Results are presented as median values with 95% confidence intervals. Statistical analysis was performed using the Kruskal–Wallis test with Dunn's test for multiple comparisons. Significance was defined as $p < 0.05$ (*), $p < 0.01$ (**) and $p > 0.05$ (not significant, n.s.).

Results

Patient characteristics

Patient characteristics are given in Table 1. One of the patients had not ceased the use of blood thinners before surgery. This patient exhibited bleeding that was higher than the median for the control measurements and the 7-min measurements, but lower than the median for the 15 and 30.

Bleeding

Profuse bleeding was observed upon skin excision in the control (lidocaine) region: 2639 mg (1356–7355 mg) during the 5 min after skin excision. Bleeding was markedly reduced, 73% lower, after injecting lidocaine + epinephrine and waiting 7 min: 711 mg (265–2659 mg) during the 5 min after incision. No further decrease in bleeding was seen upon extending the waiting time to 15 or 30 min (p = n.s.). Figure 3 shows the detailed results regarding the blood loss during the first and second 2.5 min of the whole 5-min measurement period.

Discussion

The results of the present study show a 75% reduction in bleeding when the surgical procedure was started 7 min after the injection of lidocaine + epinephrine compared to after the injection of only lidocaine. Waiting for 15 or 30 min had no further effect on bleeding. These findings are in
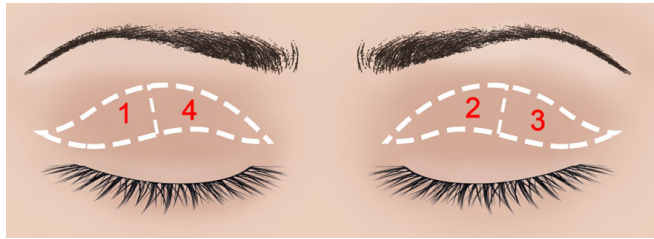
Fig. 1. Schematic illustration showing the regions subjected to local anaesthesia, and the order of skin excision. 1: Lidocaine. 2: Lidocaine + epinephrine, 7 min. 3: Lidocaine + epinephrine, 15 min. 4: Lidocaine + epinephrine, 30 min.
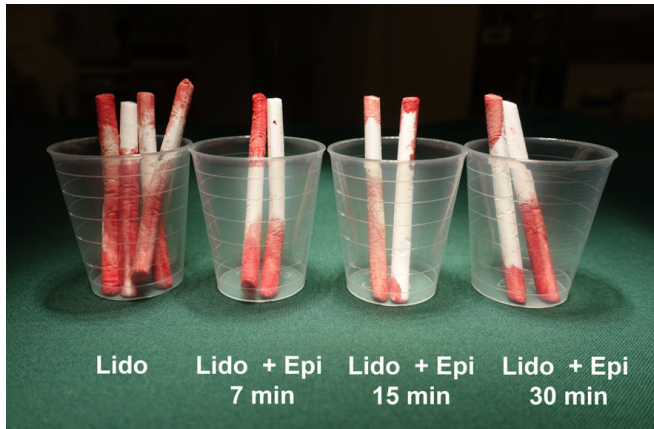

Fig. 2. Representative examples of blood collection, using ocular sticks, during the first 2.5 min after the injection of lidocaine only (Lido) or lidocaine + epinephrine (Lido + Epi), and incision at different times, as described in the text.

Table 1. Patient characteristics.

Total number of patients/eyelids	16/31
Gender: Women/men	9/7
Median age (year; range)	72 (51–78)
Median systolic blood pressure before surgery, range (mmHg)	143 (120–160)
Median diastolic blood pressure before surgery, range (mmHg)	85 (75–100)
Median pulse before surgery, range (BPM)	74 (60–90)
Median pulse during surgery, range (BPM)	73 (60–105)
No. of patients using antihypertensives	10
No. of patients with diabetes	2
No. of patients using blood thinners <1 week prior to surgery	1
No. of patients using blood thinners, but paused for >1 week prior to surgery	5
No. of patients/eyelids that had undergone previous eyelid surgery	3/5

agreement with those from previous studies. Larrabee et al. (1987) injected epinephrine with a carrier solution of lidocaine or saline into the skin of piglet trunks and measured vasoconstriction using laser Doppler flowmetry. They found that maximal vasoconstriction occurred at about 5–7 min in most cases, and in all cases by 10 min. O'Malley et al. (1995) found maximal vasoconstriction to occur after 3–4 min in the neck of patients undergoing head and neck surgery, also using laser Doppler flowmetry. Similarly, Ghali et al. (2008) used laser Doppler flowmetry to measure blood flow in the forearm and face of healthy volunteers, and found the maximal decrease in blood flow to occur after 8 min in the face and 10 min in the forearm. In a recent study by our group on porcine eyelid flaps, using laser speckle contrast imaging to measure blood perfusion, it was found that the time from the injection of epinephrine together with lidocaine to maximum hypoperfusion was 75 seconds (Sheikh et al. 2017a). In a similar study on porcine flank flaps, using laser Doppler flowmetry and laser speckle contrast imaging, a low level of perfusion was observed after approximately 120 seconds (Sheikh et al. 2017b).

The results of the present study, showing maximal haemostasis within 7 min, are in contrast to the controversial findings of McKee et al. (2013, 2015) that maximal haemostasis was not achieved until 30 min after anaesthesia with epinephrine. The reason for the difference between the results of the present study and those of McKee studies et al. (2013, 2015) cannot be determined from the present study, but
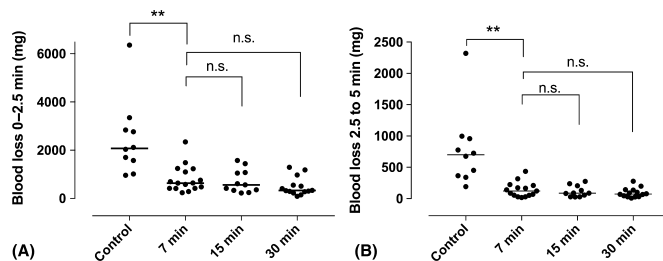
Fig. 3. The amount of blood loss after the injection of lidocaine only (control), and after the injection of lidocaine + epinephrine, and waiting 7, 15 and 30 min. (A) Bleeding during the first 2.5 min after incision (one point for control at 6257 mg is included in the calculations but not shown in graph) and (B) during the second 2.5 min after incision (one point for control at 2320 mg is included in the calculations not shown in graph). Significance was defined as $p < 0.01$ (**) and $p > 0.05$ (not significant, n.s.).

may be due to the difference in anatomical location. McKee et al. studied bleeding in the hand and arm, while we investigated the upper eyelid. The methods of blood collection also differed (micropipettes versus ocular sticks), the time during which blood was collected (1 min versus 5 min), the number of cases (15 versus 31) and patient demographics (higher mean age in the present study). The epinephrine concentrations used were similar in both studies (1:100 000 and 1:80 000). In the present study, 20 mg/ml (2%) lidocaine was used for control measurements of bleeding. It has been reported in the literature that lidocaine has an initial vasodilatory effect in skin (Gessler et al. 2001; Ghali et al. 2008). However, we observed no change in perfusion following lidocaine infiltration in our recent studies on porcine eyelid flaps and flanks (Sheikh et al. 2017a,b).

In conclusion, the maximal haemostatic effect was achieved within 7 min after the injection of lidocaine with epinephrine, confirming the 10-min waiting time for surgical incision recommended in textbooks (Collin & Rose 2001) and commonly adopted in clinical practice, and calling into question the findings of McKee et al. (2013, 2015) that a delay of 30 min is necessary. Our first measurements were made after 7 min, and further studies are needed to pinpoint the exact time at which maximal haemostasis is achieved.

References

Collin R & Rose GA (2001): Plastic and orbital surgery. London: BMJ Books.

Fink BR, Aasheim GM & Levy BA (1978): Neural pharmacokinetics of epinephrine. Anesthesiology **48**: 263–266.

Gessler EM, Hart AK, Dunlevy TM & Greinwald JH Jr (2001): Optimal concentration of epinephrine for vasoconstriction in ear surgery. Laryngoscope **111**: 1687–1690.

Ghali S, Knox KR, Verbesey J, Scarpidis U, Izadi K & Ganchi PA (2008): Effects of lidocaine and epinephrine on cutaneous blood flow. J Plast Reconstr Aesthet Surg **61**: 1226–1231.

Larrabee WF Jr, Lanier BJ & Miekle D (1987): Effect of epinephrine on local cutaneous blood flow. Head Neck Surg **9**: 287–289.

McKee DE, Lalonde DH, Thoma A, Glennie DL & Hayward JE (2013): Optimal time delay between epinephrine injection and incision to minimize bleeding. Plast Reconstr Surg **131**: 811–814.

McKee DE, Lalonde DH, Thoma A & Dickson L (2015): Achieving the optimal epinephrine effect in wide awake hand surgery using local anesthesia without a tourniquet. Hand (N Y) **10**: 613–615.

O'Malley TP, Postma GN, Holtel M & Girod DA (1995): Effect of local epinephrine on cutaneous bloodflow in the human neck. Laryngoscope **105**: 140–143.

Sheikh R, Dahlstrand U, Memarzadeh K, Blohme J, Reistad N & Malmsjo M (2017a): Optimal epinephrine concentration and time delay to minimize perfusion in eyelid surgery: measured by laser-based methods and a novel form of extended-wavelength diffuse reflectance spectroscopy. Ophthal Plast Reconstr Surg [E-Pub ahead of Print].

Sheikh R, Memarzadeh K, Torbrand C, Blohme J & Malmsjo M (2017b): Hypoperfusion in response to epinephrine in local anaesthetics: investigation of dependence on epinephrine concentration, spread of hypoperfusion and time to maximal cutaneous vasoconstriction. J Plast Reconstr Aesthet Surg **70**: 322–329.

Correspondence:
Malin Malmsjö, MD, PhD
Clinical Sciences, Lund
Ögonklinik A
Lund University and Skåne University Hospital
Kioskgatan 1
SE-221 85 Lund
Sweden
Tel: +46 722 335060
Fax: +46 462115074
Email: malin.malmsjo@med.lu.se

Paper V

Hypoperfusion following the injection of epinephrine in human forearm skin can be measured by RGB analysis but not with laser speckle contrast imaging

Rafi Sheikh[a], Josefine Bunke[a], Rannveig Linda Thorisdottir[a], Jenny Hult[a], Kajsa Tenland[a], Bodil Gesslein[a], Nina Reistad[b], Malin Malmsjö[a],*

[a] Lund University, Skane University Hospital, Department of Clinical Sciences Lund, Ophthalmology, Lund, Sweden
[b] Department of Physics, Lund University, PO Box 118, SE-22100 Lund, Sweden

A R T I C L E   I N F O

A B S T R A C T

Background: The time taken for epinephrine to achieve its optimal effect during local anesthesia has recently become the subject of debate. The time from injection to commencement of surgery is traditionally quoted to be 7 to 10 min, while recent reports claim that it may take 30 min to achieve maximum hypoperfusion, which would prolong the time required for surgical procedures. The discrepancy may be related to difficulties associated with the techniques used to measure blood perfusion. The aim of this study was to test two methods of determining the time to maximum hypoperfusion.
Methods: Laser speckle contrast imaging (LSCI) and red, green, blue (RGB) analysis of images obtained with a commercial digital camera, were used to monitor the effect of infiltration with commonly used local anesthetic preparations: lidocaine (20 mg/ml) + epinephrine (12.5 μg/ml), lidocaine (10 mg/ml) + epinephrine (5 μg/ml), and lidocaine (20 mg/ml) alone, in healthy subjects.
Results: LSCI showed a paradoxical increase in signal after the injection of local anesthetics containing epinephrine, probably due to a change in the laser penetration depth resulting from blanching of the skin. However, RGB analysis of digital photographs gave more reliable results, showing skin blanching that corresponded to the expected effect of epinephrine in local anesthetics. The time to maximum effect was found to be 7 (range 5–10) minutes for 12.5 μg/ml epinephrine, and 9 (range 7–13) minutes for 5 μg/ml epinephrine in lidocaine.
Conclusions: RGB analysis of digital images proved to be a valid technique for monitoring the effect of local anesthetics with epinephrine in human skin. The technique requires only a commercial digital camera and constitutes a cheap, simple method. The optimal delay between epinephrine injection and incision, to minimize bleeding, was found to be 7 to 9 min, which is in good agreement with common surgical practice.

1. Introduction

The optimal delay between the injection of local anesthetics containing epinephrine and incision, to ensure vasoconstriction and minimize bleeding, has recently become the subject of debate. The time commonly given in textbooks is around 7 to 10 min (Collin and Rose, 2001). However, in a study by McKee et al. (2013), who measured the relative hemoglobin concentration over time in the arm skin of healthy volunteers using oxygen spectroscopy, the lowest cutaneous hemoglobin level was not observed until 26 min after injection (McKee et al., 2013). In another study, McKee et al. measured the blood loss from the skin of patients undergoing carpal tunnel release surgery, and found a significant reduction after 30 min, compared to 7 min (McKee et al., 2015). However, when bleeding was measured during oculoplastic surgery procedures, we observed minimum bleeding after only 7 min (Hult et al., 2017). Furthermore, laser Doppler velocimetry (LDV) studies have shown maximal vasoconstriction to occur after about 8 to 10 min in the forearm and face of healthy volunteers (Ghali et al., 2008), and after 3 to 4 min in the neck of patients undergoing head and neck surgery (O'Malley et al., 1995). Similarly, short times have also been found in previous LDV and laser speckle contrast imaging (LSCI) studies in pigs (Larrabee Jr et al., 1987; Sheikh et al., 2017b; Sheikh et al., 2017a). We believe that these apparently conflicting results could be explained by difficulties associated with the techniques used for

* Corresponding author: Kioskgatan 1B, Skåne University Hospital, SE-221 85 Lund, Sweden.
E-mail address: malin.malmsjo@med.lu.se (M. Malmsjö).

blood flow measurements. The use of LDV to measure blood perfusion in humans has been reported not to be as reliable as invasive techniques such as microdialysis (Farnebo et al., 2011).

The most common tools available today for monitoring perfusion and oxygenation include spectrometry, LDV and LSCI. Spectrometry determines the degree of oxygenation by irradiating the tissue with light in the wavelength range of 500 nm to 650 nm, in which the absorption changes upon oxygenation of hemoglobin (Kasler et al., 1990). The imaging depth in spectrometry is dependent on the wavelength, from a few micrometers in the ultraviolet part of the spectrum to several millimeters in the near infrared. In the red part of the spectrum the penetration depth in tissue is typical 3–5 mm (Randeberg, 2005). LDV is based on the change in frequency, i.e., the Doppler shift, that occurs when laser light is scattered by moving objects (in this case, erythrocytes) (Fredriksson et al., 2009). LDV has been applied extensively to measure blood flow in flaps during plastic surgery procedures (Pietila et al., 1987), and the measurement depth in forearm skin is about 1 mm (Fredriksson et al., 2009). The high spatial variability in blood flow in the skin limits the accuracy of the single point measurements that are possible with LDV (Allen and Howell, 2014). LSCI measures the interference pattern created when laser light is scattered from an illuminated surface (Yamamoto et al., 1993). LSCI is a fast, full-field technique for the imaging of microvascular perfusion (Allen and Howell, 2014), and the imaging depth is approximately half that for LDV (0.5 mm). Laser-based techniques measure motion in the tissue and are affected by movement artifacts. There is therefore considerable interest in developing new techniques that do not suffer from these problems for non-invasive monitoring of perfusion. In a previous report, Leahy described a method of visualizing red blood cell content in the microcirculation with a RGB camera and reported a measurement depth of approximately 350 μm to 490 μm (Leahy et al., 2006). Jakovels et al. has showed that RGB-analysis with a simple camera can be used to quantify hemoglobin distribution and perfusion dynamics (Jakovels et al., 2011). Radioisotope clearance was previously considered the gold standard for measuring skin perfusion However, the method is no longer widely used due to the need to inject radionuclides, and the complexity of the procedure (Pan et al., 2018). To the best of our knowledge, no other method has replaced radioisotope clearance as the gold standard for skin perfusion measurements.

The aim of the present study was to compare the ability of red, green, blue (RGB) image analysis to LSCI in monitoring skin perfusion. The techniques were used to examine the change in skin perfusion upon administration of epinephrine in local anesthetics, which is known to cause vasoconstriction in the subcutaneous vascular plexus. The time to maximum response was evaluated.

2. Materials and methods

2.1. Ethics

The protocol for this experimental study was approved by the Ethics Committee at Lund University, Sweden. The research adhered to the tenets of the Declaration of Helsinki as amended in 2008. All the patients participating in the study were given information about the study and informed of the voluntary nature of participation. All patients gave their informed written consent.

2.2. Study subjects

The study subjects were adult volunteers. The exclusion criterion was the presence of any medical condition that would contraindicate the administration of local anesthesia with epinephrine, such as heart conditions or previous allergic reaction to local anesthetics, about which the subjects were particularly asked. Two participants were excluded from the study before analysis: the first due to large movement artifacts making RGB analysis difficult, and the second due to too deep

Table 1
Subject characteristics.

Total number of subjects	14
Total number of subjects included in the analysis	12
Gender women/men	8/6
Median age (y) (range)	53 (31–72)
Median resting pulse before surgery, range (BPM)	60 (56–86)
Median maximum pulse during surgery, range (BPM)	65 (63–91)
Median pulse 30 min after infiltration, range (BPM)	61 (60–70)
No. of patients with diabetes	0
No. of patients using antihypertensives	1 (excluded)
No. of patients Fitzpatrick type I	1
No. of patients Fitzpatrick type II	11
No. of patients Fitzpatrick type III	1
No. of patients Fitzpatrick type IV	1 (excluded)

administration of the anesthetic agent, resulting in insufficient anesthesia and the suspicion of unreliable results. The subject excluded because of movement artifacts was taking medication for hypertension, while the others had no known medical conditions, and were considered healthy. The subjects were classified according to the Fitzpatrick scale for skin pigmentation (Fitzpatrick, 1988). One subject was type I, eleven subjects type II, one type III and one type IV. The subject with skin type IV was the one excluded due to too deep administration of the anesthetic agent. The characteristics of the subjects are given in Table 1.

2.3. Local anesthetics

The following three anesthetics were chosen as they are often used for local anesthesia in plastic surgery.

1. Lidocaine (20 mg/ml) + epinephrine (12.5 μg/ml) (Xylocaine Dental® Adrenaline, Dentsply Ltd., York, PA, USA), denoted LIDO (20.0) + EPI (12.5)
2. Lidocaine (10 mg/ml) + epinephrine (5 μg/ml) (Xylocaine Adrenaline®, AstraZeneca, Södertälje, Sweden), denoted LIDO (10.0) + EPI (5.0), buffered to pH ≈ 7.4 with a 5:1 solution of sodium bicarbonate (50 mg/ml) (Natriumbikarbonat Fresenius Kabi®, Fresenius AG Bad Homburg, Germany)
3. Lidocaine (20 mg/ml) (Xylocaine®, AstraZeneca, Södertälje, Sweden), denoted LIDO (20.0)

2.4. Experimental procedure

The light sources in the room were turned off and the windows were covered to achieve a controlled light environment before the perfusion measurements were started. The studies were performed in a draft controlled room, at a temperature of 20 °C, in which the equipment had been placed at least 24 h before starting the experiments. All subjects rested in the supine position for 10 min, after which their heart and lungs were auscultated. Heart rate was then monitored continuously 10 min before and during the perfusion experiments. The subject's arm was stabilized using a vacuum pillow (Germa Protec, Germa AB, Kristianstad, Sweden). The anesthetic solutions were preheated to 37 °C before injection, and 0.5 ml was injected into the subcutaneous tissue on the volar side of the forearm, at least 8 cm apart. The volume used was 0.5 ml as this represents a clinically significant amount, without the risk of "interference" between the injection locations. The anesthetics were infiltrated as uniformly as possible by the same surgeon within a 1.5 cm^2 area in order to achieve standardized anesthesia. The response of perfusion to local anesthetics was imaged with LSCI or a digital camera for RGB analysis.

2.5. Laser speckle contrast imaging

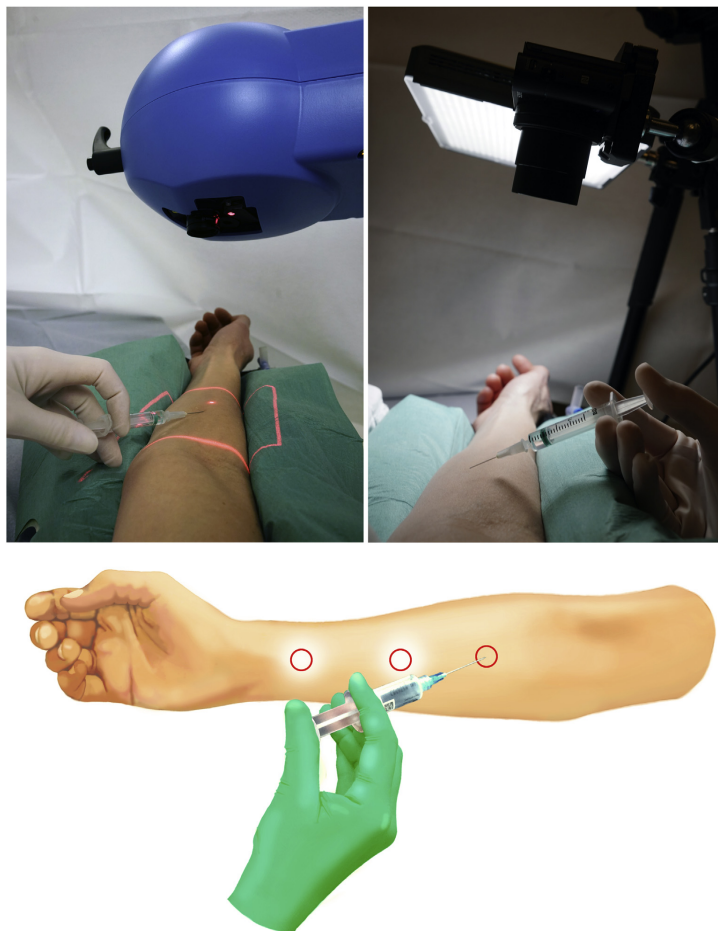Perfusion was measured with LSCI using the PeriCam PSI NR System

Fig. 1. Photographs of the laser speckle contrast imaging (LSCI) setup (upper left) and digital camera for red, green, blue (RGB) analysis (upper right), together with a schematic illustration of the injection sites on the arm (lower image). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Perimed AB, Stockholm, Sweden), set to visualize a scanning area of 10 cm^2, located over the injection site. The LSCI detector was mounted 50 cm above the subject's arm. To avoid movement artifacts, the subjects were asked not to move, and the examiner paid close attention to this issue. Furthermore, the arm was stabilized as described above. Changes in the speckle pattern were recorded at a rate of up to 100 images per second, with a spatial resolution of up to 100 μm/pixel, resulting in an instantaneous image of motion, interpreted as perfusion. Blood perfusion was expressed in arbitrary perfusion units (PU). Image frames were analyzed every 30 s during the study period of 25 min.

Our initial intention was to use LSCI to measure perfusion as a means of determining when a state of hypoperfusion had been established. However, after LSCI measurements had been performed three times in two subjects, i.e., a total of six experiments, it became evident that the LSCI data could not be interpreted correctly, since the LSCI

signal increased (indicating an increase in blood perfusion), while there was simultaneous blanching of the skin (indicating a decrease in blood perfusion). We therefore used RGB analysis of images obtained with a commercial digital camera in the remaining subjects.

2.6. RGB color analysis of photographs

The effect of injection was filmed using a commercial digital camera (Sony Cybershot DSC-RX100 V, Sony Corporation Tokyo, Japan), with a resolution of 1280 × 720 pixels at 30 frames per second, with the white balance preset to daylight mode. The camera was mounted 20 cm above the subject's arm, on a Manfrotto 244rc Variable Friction Magic Arm and a Manfrotto 290 series tripod. A daylight color temperature high CRI (> 95) LED panel with softbox diffuser (Amaran HR672C, Aputure Imaging Industries Co. Ltd., Shenzhen, P.R. China), was used as a light
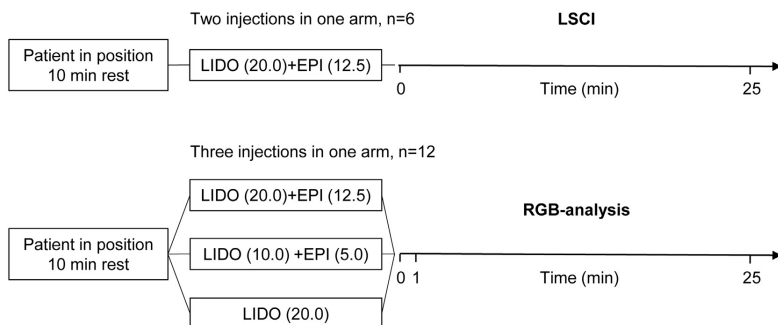
Fig. 2. Flow chart showing the two experimental procedures. The subjects rested 10 min before the procedure was started. The upper figure shows injection with 0.5 ml LIDO (20.0) + EPI (12.5) three times in two subjects ($n = 6$), followed by monitoring with laser speckle contrast imaging (LSCI) every 30 s for 25 min. The lower figure shows injection with 0.5 ml LIDO (20.0) + EPI (12.5), LIDO (10.0) + EPI (5.0) and LIDO (20.0) in one arm of 12 other subjects ($n = 12$). After injection, the sites were monitored with red, green, blue (RGB) analysis every 3 s during the first minute, and then every 30 s for the rest of the study period (25 min). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
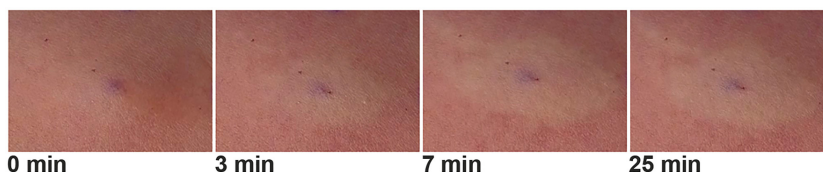

Fig. 3. Representative example showing the progression of blanching following the injection of LIDO (20.0) + EPI (12.5).

source.

Image frames were sampled every 3 s during the first minute, and every 30 s throughout the rest of the study period of 25 min. The reason for this imaging interval is that the decrease in perfusion was expected to be steep during the first minute. Each color component, red (R), green (G), and blue (B), was analyzed separately over an area of 20×20 pixels adjacent to the injection site to provide a measure of the qualitative change in color over time. For each combination of RGB, a total Euclidean color distance was calculated. Two-phase nonlinear regression analysis was used to calculate the time to steady state hypoperfusion. The setup and experimental procedures can be seen in Figs. 1 and 2, respectively.

2.7. Calculations and statistics

The response of perfusion to local anesthetics was imaged with LSCI three times in two of the subjects, in whom LIDO (20.0) + EPI (12.5) had been injected in the volar forearm ($n = 6$). All three local anesthetics: LIDO (20.0) + EPI (12.5), LIDO (10) + EPI (5.0) and LIDO (20.0), were injected at three different locations in one volar forearm of the other 12 subjects, and the response was imaged with a commercial digital camera ($n = 12$). One of the limitations of the study is that the same subjects did not participate to the two measurement procedures. The order of the anesthetic injected was randomized using the Rand function in MS Excel. Calculations and statistical analysis of the LSCI data were performed using GraphPad Prism 7.2 (GraphPad Software Inc., San Diego, CA, USA). The RGB data were post-processed using the MATLAB software package (MathWorks Inc., Natick, MA).

3. Results

The anesthetics containing epinephrine induced blanching of the skin, which increased gradually over time and extended approximately 2 cm from the injection site (Fig. 3). However, when monitored with LSCI, no corresponding reduction in perfusion was observed. On the contrary, perfusion appeared to increase after injection with LIDO (20.0) + EPI (12.5) (Fig. 4), indicating that LSCI is not a reliable method for monitoring hypoperfusion, probably due to a change in laser penetration depth resulting from blanching of the skin.

RGB analysis of images obtained with a commercial digital camera gave more reliable results, consistent with the expected effect of epinephrine in local anesthetics. Injection caused an increase in the level of all three colors channels (Fig. 5). Since a mixture of maximum RGB color levels is white, the results indicate blanching of the skin. Color change therefore appears to be a simple but effective way of monitoring skin perfusion. Blanching reached a maximum at a median time of 7 (4 to 12) minutes following the injection of LIDO (20.0) + EPI (12.5), and 9 (4 to 16) minutes following the injection of LIDO (10.0) + EPI (5.0). No blanching was observed following the injection of LIDO (20.0) without epinephrine, indicating no effect on perfusion.

4. Discussion

Our results show that the change in skin color is a sensitive indicator of blood perfusion. Detailed analysis of the red, green and blue color channels in images obtained with a standard digital camera allowed the gradual blanching of the skin induced by the injection of epinephrine in local anesthetics in human forearm skin to be monitored. This may be due to a decrease in the concentration of red blood cells or a reduction in the velocity of the red blood cells. Similar use of RGB analysis has been reported by others. Perfusion in the human hand has been measured in 14 subjects with a RGB camera during upper limb occlusion at 50 and 250 mm Hg, indicating the possibility of visualizing the hemodynamics of subsurface skin tissue (Nishidate et al., 2011). A novel
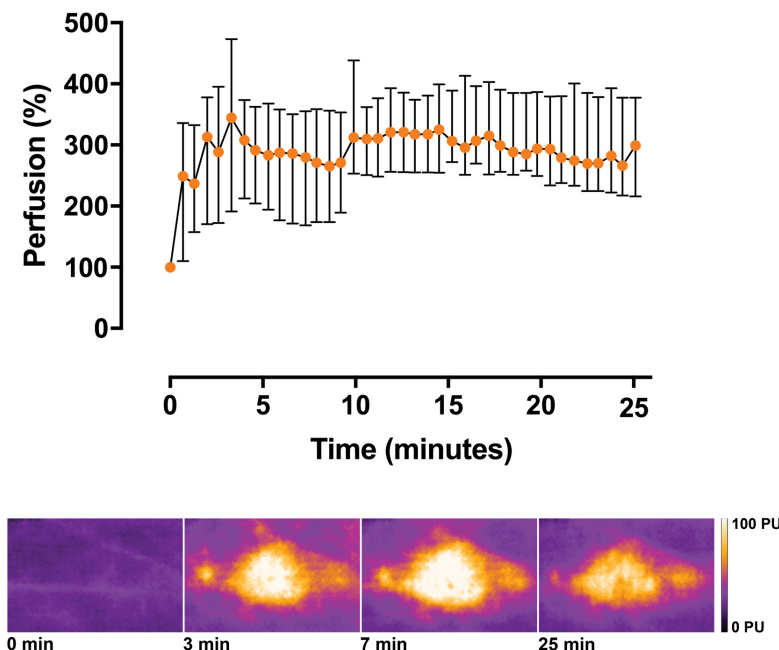
Fig. 4. Results of laser speckle contrast imaging (LSCI), showing perfusion after the injection of LIDO (20.0) + EPI (12.5). Data are presented as median values and interquartile ranges (% of baseline values). Representative examples are shown before injection and 3, 7, and 25 min after injection of LIDO (20.0) + EPI (12.5). Note the paradoxical increase in signal intensity despite the fact that epinephrine is known to induce vasoconstriction and subsequent hypoperfusion.

method of monitoring the change in oxygen saturation has also been presented using RGB analysis (Sophia). The change in oxygen saturation in five healthy volunteers was accurately quantified, compared to the values given by a conventional pulse oximeter, indicating that it is possible to use an RGB camera to determine changes in oxygen saturation (Guazzi et al., 2015). Farnebo et al., described a technique to monitor the hyperemic response in the skin upon reperfusion after occlusion of the forearm (Farnebo et al., 2010), while Zhai et al. used 'colorimetry' to measure blanching after the administration of topical steroids (Zhai et al., 2009).

We used RGB analysis to study the time required to reach the maximum effect of epinephrine in local anesthetics. Our results showed maximum hypoperfusion of the skin already after 7 to 9 min. These results are contrary to recent reports in which a 30-min delay was recommended from injection to incision, which is much longer than that used in clinical practice today. McKee et al. claimed that it took 26 min to reach maximal vasoconstriction, according to spectroscopic measurements, and recommended a delay of 25 min before commencing hand surgery (McKee et al., 2013). The 7 to 9 min determined in this study are in line with the delay traditionally applied to ensure minimal bleeding in reconstructive surgery, and is the time often prescribed in textbooks. It is also in good agreement with our previous study on the amount of bleeding after subcutaneous administration of local anesthetics containing epinephrine, which showed a 75% reduction in bleeding when the surgical procedure was started 7 min after the injection of lidocaine + epinephrine. Waiting for 15 or 30 min had no further effect on bleeding (Hult et al., 2017). We therefore see no reason to change the clinical practice of waiting 7 to 10 min between injection and the start of surgery.

It is difficult to measure hypoperfusion in human skin. Farnebo et al. showed that LDV is not a reliable technique for measuring perfusion in humans, compared to microdialysis, which is a precise but invasive technique (Farnebo et al., 2011). The initial results obtained with LSCI in the present study did not correlate at all with the visible blanching of the skin seen after the injection of local anesthetics with epinephrine, and are in direct contrast to surgical observations that epinephrine causes hypoperfusion. LSCI thus appears not to be a suitable method of measuring severe hypoperfusion. We believe that blanching affects the measurement depth of LSCI, leading to perfusion being measured deeper in the tissue, where it may be unchanged or even increased. Fredriksson et al., performed Monte Carlo simulations of light propagation in tissue and showed that the measurement depth in skin using LDV is dependent on the optical properties of the tissue (e.g. the blood concentration and blood oxygen saturation) (Fredriksson et al., 2009). Another explanation of the paradoxical LSCI results could be constriction of the blood vessels by epinephrine, reducing the diameter, and thus leading to a higher velocity of blood flow. Higher velocity of red blood cells produces a transient increase in the signal intensity and thus higher LSCI values. However, such a compensatory increase in blood flow is usually seen only as a short-term spike (Kairinos et al., 2009).

One limitation of the present study is that we do not know whether the change in color is related to perfusion, the velocity of the blood cells, vasoconstriction, or an change in red blood cell concentration. However, the recorded changes in color correspond directly to the injection of epinephrine and the time taken to induce vasoconstriction. We therefore strongly believe that the change in color is dependent on vasoconstriction, i.e. a reduction in blood volume and thus the number of erythrocytes.
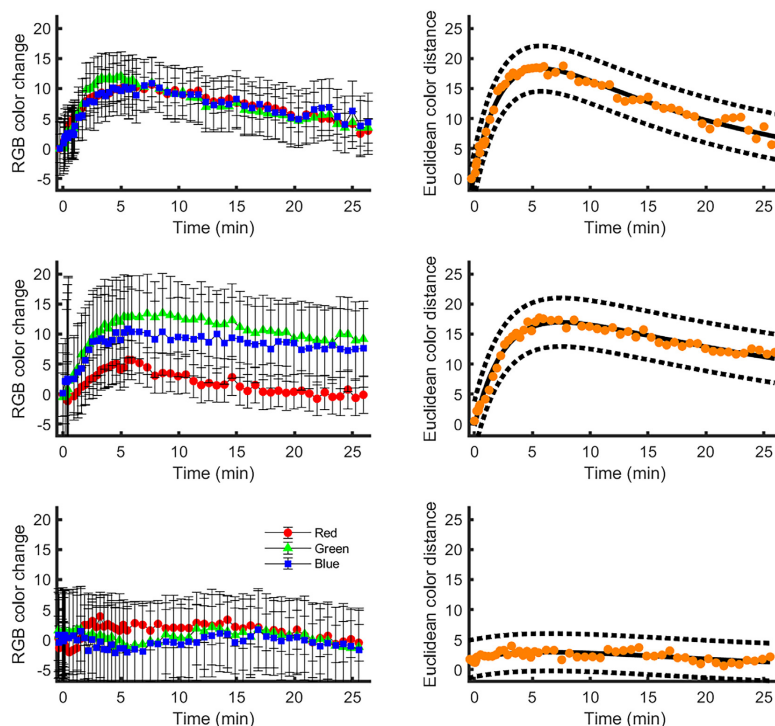
Fig. 5. Red, green, blue (RGB) analysis of digital images. Representative examples are given of the color change in the skin measured by RGB analysis and analyzed as the total Euclidean color distance, following the injection of LIDO (20.0) + EPI (12.5) (top panels), LIDO (10.0) + EPI (5.0) (middle panels) and LIDO (20.0) (bottom panels). Data are shown as mean values ± SD of a measurement area of 20 × 20 pixels adjacent to the injection site to provide a measure of the change in color over time. Note that the increase seen in all color channels indicates blanching of the skin, since a mixture of maximum RGB color levels is white. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Skin color changes were only monitored using a digital camera. One way of ensuring that hypoperfusion had been attained would have been to confirm the lack of bleeding surgically at various times. However, this is not ethically justified in healthy subjects. Another limitation of the present study is that there is no gold standard for validating the RGB technique. The only non-invasive and non-contact measurement technique available is LSCI, which is hampered by movement artifacts and a non-linear relationship between signal intensity and skin blood flow at low perfusion rates (Briers et al., 2013). LSCI is based on both blood velocity and the concentration of red blood cells. The LSCI signal is more sensitive to changes in blood velocity than red blood cell concentration (Tew et al., 2011). The other technique commonly used for validation is a pulsometer, which is a non-invasive technique, but dependent on skin contact, and is based on spectroscopy at wavelengths of 660 and 940 nm (Jubran, 2015). The drawback of this technique is that it only provides the level of oxygenation of the tissue, and thus provides only an indirect measurement of perfusion.

Monitoring the blanching of the skin with color analysis appears to be a simple and reliable technique for measuring hypoperfusion. Commercial digital cameras are compact, cheap, and easy to use, making them suitable for use in the clinical situation. They also allow non-invasive measurements, and do not need to be in direct contact with the skin. This constitutes a significant advantage over equipment in which contact probes are used.

In conclusion, RGB analysis of digital images accurately reflects the spread of hypoperfusion following the administration of local anesthetics with epinephrine. This was not possible using LSCI, probably due to skin blanching. Commercial digital cameras may thus offer an effective means of monitoring perfusion before and during plastic surgical procedures. Maximum hypoperfusion was observed within 7 to 9 min, which is in good agreement with observed blanching, and the generally accepted waiting time before commencing surgery.

Financial support

Acknowledgements

English.

References

Allen, J., Howell, K., 2014. Microvascular imaging: techniques and opportunities for clinical physiological measurements. Physiol. Meas. 35, R91–R141.

Briers, D., Duncan, D.D., Hirst, E.R., Kirkpatrick, S.J., Larsson, M., Steenbergen, W., Stromberg, T., Thompson, O.B., 2013. Laser speckle contrast imaging: theoretical and practical limitations. SPIE 10.

Collin, R., Rose, G.A., 2001. Plastic and Orbital Surgery. BMJ Books, London.

Farnebo, S., Thorfinn, J., Henricson, J., Tesselaar, E., 2010. Hyperaemic changes in forearm skin perfusion and RBC concentration after increasing occlusion times. Microvasc. Res. 80, 412–416.

Farnebo, S., Zettersten, E.K., Samuelsson, A., Tesselaar, E., Sjoberg, F., 2011. Assessment of blood flow changes in human skin by microdialysis urea clearance. Microcirculation 18, 198–204.

Fitzpatrick, T.B., 1988. The validity and practicality of sun-reactive skin types I through VI. Arch. Dermatol. 124, 869–871.

Fredriksson, I., Larsson, M., Stromberg, T., 2009. Measurement depth and volume in laser Doppler flowmetry. Microvasc. Res. 78, 4–13.

Ghali, S., Knox, K.R., Verbesey, J., Scarpidis, U., Izadi, K., Ganchi, P.A., 2008. Effects of lidocaine and epinephrine on cutaneous blood flow. J. Plast. Reconstr. Aesthet. Surg. 61, 1226–1231.

Guazzi, A.R., Villarroel, M., Jorge, J., Daly, J., Frise, M.C., Robbins, P.A., Tarassenko, L., 2015. Non-contact measurement of oxygen saturation with an RGB camera. Biomed. Opt. Express 6, 3320–3338.

Hult, J., Sheikh, R., Nguyen, C.D., Tenland, K., Dahlstrand, U., Malmsjo, M., 2017. A waiting time of 7 min is sufficient to reduce bleeding in oculoplastic surgery following the administration of epinephrine together with local anaesthesia. Acta Ophthalmol. https://doi.org/10.1111/aos.13616.

Jakovels, D., Spigulis, J., Rogule, L., 2011. In: RGB mapping of hemoglobin distribution in skin. European Conferences on Biomedical Optics. 6 SPIE.

Jubran, A., 2015. Pulse oximetry. Crit. Care 19, 272.

Kairinos, N., Voogd, A.M., Botha, P.H., Kotze, T., Kahn, D., Hudson, D.A., Solomons, M., 2009. Negative-pressure wound therapy II: negative-pressure wound therapy and increased perfusion. Just an illusion? Plast. Reconstr. Surg. 123, 601–612.

Kasler, M., Von Glass, W., Albrecht, H.P., Lang, T., 1990. Noninvasive intraoperative measurement of intracapillary hemoglobin oxygenation and relative hemoglobin concentration in surgical skin flaps. HNO 38, 375–378.

Larrabee Jr., W.F., Lanier, B.J., Miekle, D., 1987. Effect of epinephrine on local cutaneous blood flow. Head Neck Surg. 9, 287–289.

Leahy, M., Odoherty, J., Nilsson, G., Henricson, J., Sjöberg, F., 2006. A new method for visualizing red blood cell content in the microcirculation. SPIE newsroom. In: The International Society for Optical Engineering. 2006.

McKee, D.E., Lalonde, D.H., Thoma, A., Glennie, D.L., Hayward, J.E., 2013. Optimal time delay between epinephrine injection and incision to minimize bleeding. Plast. Reconstr. Surg. 131, 811–814.

McKee, D.E., Lalonde, D.H., Thoma, A., Dickson, L., 2015. Achieving the optimal epinephrine effect in wide awake hand surgery using local anesthesia without a tourniquet. Hand (N Y) 10, 613–615.

Nishidate, I., Tanaka, N., Kawase, T., Maeda, T., Yuasa, T., Aizu, Y., Yuasa, T., Niizeki, K., 2011. Noninvasive imaging of human skin hemodynamics using a digital red-green-blue camera. J. Biomed. Opt. 16, 086012.

O'Malley, T.P., Postma, G.N., Holtel, M., Girod, D.A., 1995. Effect of local epinephrine on cutaneous bloodflow in the human neck. Laryngoscope 105, 140–143.

Pan, X., Chen, G., Wu, P., Han, C., Ho, J.K., 2018. Skin perfusion pressure as a predictor of ischemic wound healing potential. Biomed. Rep. 8, 330–334.

Pietila, J.P., Von Smitten, K., Sundell, B., 1987. The rabbit ear pedicle flap. A new model for evaluating factors affecting the circulation in flaps. Scand. J. Plast. Reconstr. Surg. Hand Surg. 21, 145–147.

Randeberg, L.L., 2005. Diagnostic Applications of Diffuse Reflectance Spectroscopy (Doctoral Theses Doctoral Theses at NTNU). Norwegian University of Science and Technology.

Sheikh, R., Dahlstrand, U., Memarzadeh, K., Blohme, J., Reistad, N., Malmsjo, M., 2017a. Optimal epinephrine concentration and time delay to minimize perfusion in eyelid surgery: measured by laser-based methods and a novel form of extended-wavelength diffuse reflectance spectroscopy. Ophthal. Plast. Reconstr. Surg. 34 (2), 123–129.

Sheikh, R., Memarzadeh, K., Torbrand, C., Blohme, J., Malmsjo, M., 2017b. Hypoperfusion in response to epinephrine in local anaesthetics: investigation of dependence on epinephrine concentration, spread of hypoperfusion and time to maximal cutaneous vasoconstriction. J. Plast. Reconstr. Aesthet. Surg. 70, 322–329.

Tew, G.A., Klonizakis, M., Crank, H., Briers, J.D., Hodges, G.J., 2011. Comparison of laser speckle contrast imaging with laser Doppler for assessing microvascular function. Microvasc. Res. 82, 326–332.

Yamamoto, Y., Ohura, T., Nohira, K., Sugihara, T., Minakawa, H., Igawa, H., Shintomi, Y., Fujii, H., 1993. Laserflowgraphy: a new visual blood flow meter utilizing a dynamic laser speckle effect. Plast. Reconstr. Surg. 91, 884–894.

Zhai, H., Chan, H.P., Farahmand, S., Nilsson, G.E., Maibach, H.I., 2009. Comparison of tissue viability imaging and colorimetry: skin blanching. Skin Res. Technol. 15, 20–23.

Techniques for Measuring Perfusion during Reconstructive Surgery and Effects of Epinephrine in Local Anesthetics

Rafi Sheikh was born in Sweden, in 1980. He received his medical degree from Lund University, Lund, Sweden, in 2009. He is an Ophthalmologist and is specialized in cataract and vitreoretinal surgery. Currently his main areas of research interest are oculoplastic surgery, microvascular blood flow, neuro-ophthalmology, and photoacoustic imaging.

FACULTY OF MEDICINE

LUND UNIVERSITY