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**C. Holm · M. Mayr · J. Tegeler · A. Becker
U. Pfeiffer · W. Mühlbauer**

Laser-induced fluorescence of indocyanine green: plastic surgical applications

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Abstract The objective assessment of tissue perfusion is of utmost importance to plastic surgeons. Nevertheless, clinical observation remains the accepted gold standard for assessment of microcirculation. Dynamic laser fluorescence videography is a new technique for objective assessment of blood flow. We describe our clinical experience using this technique in plastic surgical patients. Possible implementations include evaluation of pedicle (random and axial pattern) flaps, monitoring of free tissue transfer and replants, and the objective determination of burn depth. Compared with standard clinical assessment techniques indocyanine green imaging provides significant additional information which allows a rational and evidence-based planning of surgery.

Keywords Laser · Fluorescence · Indocyanine green · Perfusion · Plastic surgery

Introduction

Being able to assess the microcirculatory state of a given tissue is of utmost importance to plastic surgeons. Early differentiation between superficial and deep burns [8], perioperative recognition of compromised microcirculation of axial and random pattern skin flaps [30], and regular detection of arterial or venous occlusion of microsurgical flaps [19] can definitively lower morbidity and enhance the rationale and quality of treatment provided to patients. Other applications include the postoperative monitoring of replanted digits in replantation surgery [37], the prediction of extremity viability, and the selection of amputation levels in patients with high voltage injury or with peripheral vascular disease [35]. Still, clinical observation remains the almost universally accepted gold standard for assessment of microcirculation. With this method the inspection of skin color, dermal bleeding, and capillary blanching on pressure are regarded as sufficient to reveal the vascular status of the skin. Obviously such parameters are open to subjective interpretation depending on the experience and judgment of the examining physicians or nurses. Furthermore, interpretation of that examination requires a considerable amount of clinical experience. This makes clinical assessment insufficiently predictable, and in addition it does not meet the growing demand for evidence-based medical practice. As a consequence there is a continuous search for more objective and reliable tests for assessment of the vascular status of the skin.

Laser induced fluorescence of indocyanine green (ICG) currently provides the most accurate information on dermal and subdermal microcirculation. It is based on the same objectives as the fluorescence technique using fluorescein, which was introduced as early as 1943 by Lange and Boyd [20]· Unfortunately, that technique was limited by the physiological properties of fluorescein and never found its way into universal clinical practice. These shortcomings of fluorescein include a long half-life, diffusion into the interstitium, and an excitation maximum in the ultraviolet spectrum, allowing penetration into the superficial dermis only. However, the pharmacokinetic, physiological, and spectral properties of ICG allow totally different clinical applications and justify the clinical reevaluation of the fluorescein technique.

Experimental studies with dynamic laser fluorescence videography have shown promising results [1, 2, 27]; clinical studies are few, however, and are limited to the assessment of circulation in axial and random pattern skin flaps [32]. Recently the use of ICG perfusography for the determination of burn depth was reported in a limited number of patients [29, 33]. The use of laser-induced fluorescence of ICG for monitoring of free microvascular flaps has not been previously reported.

C. Holm (✉) · M. Mayr · J. Tegeler · A. Becker · U. Pfeiffer
W. Mühlbauer
Department of Plastic and Hand Surgery, Burn Unit,
Klinikum Bogenhausen,
Technical University Munich,
Englschalkingerstrasse 77, 81925 Munich, Germany
e-mail: charlotte_holmjakobsen@hotmail.com
Tel.: +49-89-92702030, Fax: +49-89-92702036

We describe the ICG fluorescence technique and its possible indications in plastic surgery. The technique is compared with other techniques, and costs, convenience, advantages, and limitations are evaluated.

Methods and materials

Indocyanine green

ICG (ICG-Pulsion, Pulsion Medical Systems, Munich, Germany) is a water-soluble tricarbocyanine dye which is efficiently removed by the liver and excreted in the bile. It has been used for more than 40 years for measuring cardiac output [5], as a liver function test [21], and for fluorescence angiography of the choroidea [4]. Recently we reported the use of ICG for invasive monitoring and guidance of fluid therapy of severely burned patients during the resuscitation period [13, 14].

After intravenous injection ICG binds strongly to plasma proteins and is exclusively distributed in the intravascular space [26]. This makes it a suitable tracer for vessel perfusion. ICG absorbs light in the near-infrared spectral range with a maximum at 805 nm and emits fluorescence at 835 nm. These absorption and emission qualities lie in the "optic window" of the skin and are responsible for the relatively high penetration depth of approximately 3 mm. Penetrating deeper into the skin, the excitation light induces fluorescence from blood vessels within the deep dermal plexus and subcutaneous fat instead of only the superficial dermis (as with fluorescein). Because the skin is relatively transparent to the ICG fluorescence wavelength, the induced fluorescence is not trapped in the skin and can be recorded by a suitable camera. The pharmacokinetic properties of ICG present a further advantage of this tracer. The very short half life of 3–4 min allow sequential monitoring of skin perfusion with short intervals between injections. Previous examinations therefore do not prevent a clear detection of microvascular compromise.

Laser-array, video camera, and image processing software

Flap perfusion was measured using the technique of dynamic laser-fluorescence video-angiography (IC-VIEW, Pulsion Medical Systems). Under illumination with a laser (energy P_i=0.16 W, wavelength λ=780 nm) an intravenous bolus injection (0.5 mg/kg) of ICG dissolved in 5% dextrose (2 mg/ml) was given. The resulting fluorescence was recorded with a digital video camera using an infrared filter. This form of laser exposure does not cause local tissue damage since the energy absorbed lies far below the damage threshold of the skin.

A special software (IC-Calc, Pulsion Medical Systems) was used for quantitative analysis of the recorded video sequences. The fluorescence intensity served as a function of tissue perfusion. In regions of interest defined by the investigator the increment of fluorescence (the slope of the intensity curve during inflow of the ICG) was calculated for proportional comparison of different tissue areas. The slope of the intensity curve of normal, well perfused tissue was used as a reference and was set to 100%.

In addition, a false color representation of the recorded fluorescence images was used for improved demonstration of tissue areas with high and low fluorescence intensities compared to the reference. In the false color representation the mean signal intensity of the reference region (green circular region in the respective false color images) was represented by the color green, while higher fluorescence intensities were represented by yellow, orange, and red (red representing highest intensity) and lower fluorescence intensities were represented by blue-green, blue, and pink (pink representing the lowest intensity).

Patients

The Medical Ethics Committee of our hospital approved the study. After informed consent was obtained, a single dose of 0.5 mg/kg ICG was injected intravenously, using either a central venous or a peripheral venous line. White light, ICG fluorescence, and false color images were obtained as described above. Pregnant women, patients with severe hepatic insufficiency, and patients with a history of allergic reactions to ICG and iodide were excluded from this study.

Clinical cases

Burn depth assessment

While several supplemental diagnostic methods have been described, the continued clinical gold standard for assessment of burn depth remains clinical judgement by an experienced burn surgeon [10]. Clinical diagnosis of superficial and deep dermal burns may be accurate during the first few days after injury, but even experienced clinicians have difficulty differentiating between intermediate partial-thickness burns, capable of healing with minimal scarring, and deeper partial-thickness burns that require excision and grafting [11]. The early accurate evaluation of these kinds of burns has remained an imprecise clinical problem. Figure 1a shows a full-thickness electric burn of the medial malleolus region in a 28-year-old man. The false color image obtained from the laser-fluorescence videography 72 h following the burn indicates that the whole area is blue in color (perfusion index <1) and therefore a deep burn (Fig. 1b). Note the surrounding area of increased perfusion indicated by red, which corresponds to an inflammatory reaction in the surroundings. Figure 2a shows a flame burn of the right hand in a 35-year-old man. The false color image obtained from the laser fluorescence videography 48 h after injury reveals normal to increased perfusion of the whole burn, suggesting a superficial partial thickness burn (Fig. 2b). The burn healed on day 14.

Replantation monitoring

Figure 3a shows the result of microsurgical replantation of the left index finger at the level of the proximal interphalangeal joint in a 38-year-old man. On the fourth postoperative day the replanted digit appeared pale with significantly delayed capillary refill, and operative revision was considered. The false color image obtained from the laser fluorescence videography revealed sufficient perfusion of the distal phalanx (Fig. 3b). Note the hyperperfusion of the thumb and the radial part of the dorsum of the hand. The replanted finger healed without further operative intervention.

Assessment of pedicle flaps

Axial pattern flap The axial pattern flap with a preexisting, anatomically recognized arteriovenous system leaves it with a great increase in vascular reserve and makes it safer, more robust, and better able to cope with adverse circumstances than the random pattern flap. The length of this kind of flap is governed by the territory of the feeding axial vessel. In practice it has been found that this kind of flap can be safely extended beyond the territory of the arteriovenous system. Thus the distal segment can be viewed as random on the end of the axial segment. How far this random part of the flap can be extended is unclear and is left very much to the subjective judgement of the surgeon [23]. The oldest and classic axial pattern flap is the groin flap [25]. The patient shown in Fig. 4a was a 76-year-old woman with an ulcerating squamous cell carcinoma of the abdominal wall. She had distant metastases to the vertebral spine and to the liver and was in a very poor condition. A limited surgical operation was planned with palliative re-

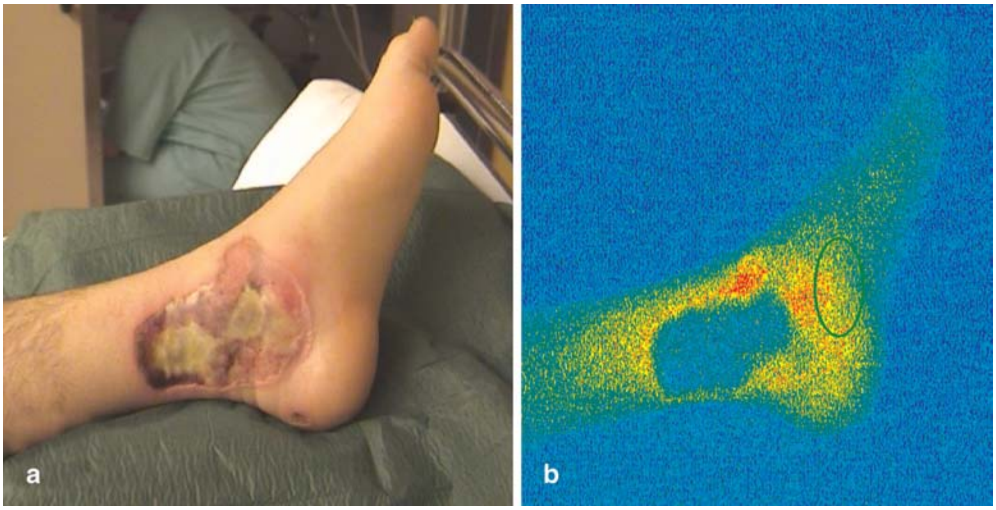


Fig. 1 **a** Clinical photograph of a full thickness electric burn of the medial malleolus region in a 28-year-old man. **b** False color image 72 h postinjury, indicating deep burn

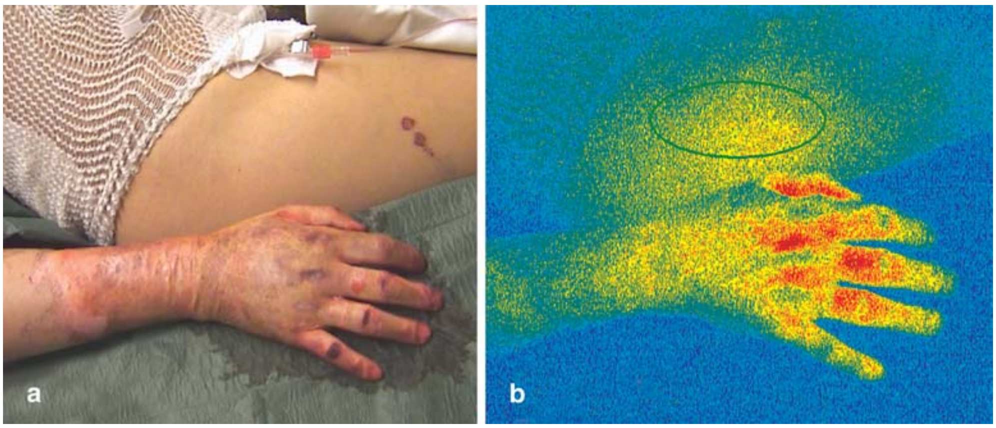


Fig. 2 **a** Clinical photograph of a flame burn of the right hand in a 35-year old man. **b** False color image obtained 48 h postinjury, revealing superficial partial thickness burn

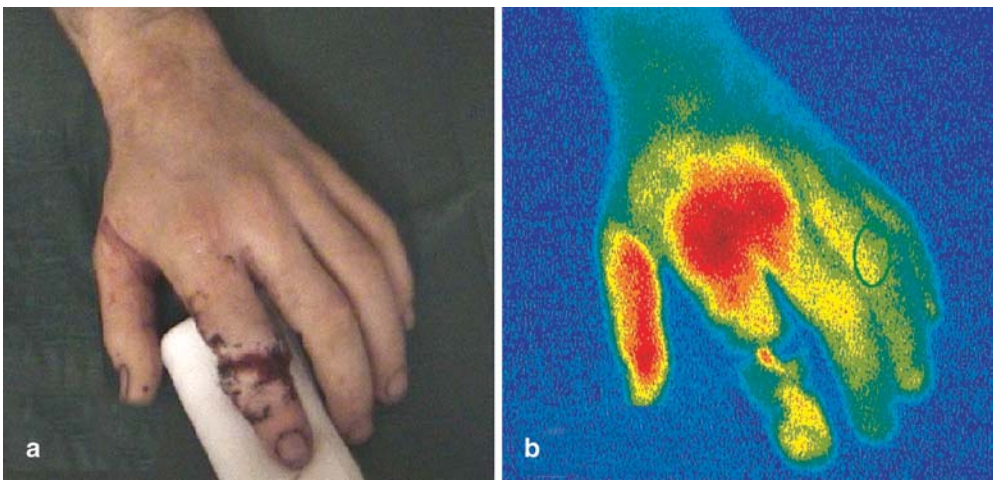


Fig. 3 **a** Clinical photograph of the result of microsurgical replantation of the left index finger in a 38-year-old man. **b** False color image obtained on the 4th postoperative day, revealing sufficient perfusion of the distal phalanx

section of the tumor and coverage with bilateral groin flaps. We had to extend this flap considerably over on to the back so as to be able to cover the defect in the upper abdomen (Fig. 4b). Thus the distal part of the flap was far beyond the limit of the superficial circumflex iliac artery and was regarded a random pattern flap. Perioperative ICG imaging provided support in retrospect for the chosen flap design since it demonstrated the axial vessels and showed a good perfusion of the distal random part of the flaps (Fig. 4c, d).

The rigid limitations of length-breadth ratio of a random pattern flap are well known [23]. These rules, however, depend on the richness of the subdermal plexus, and can be considerably relaxed, for example, in the face, as facial skin is extremely vascular. In the limbs these restrictions must be applied much more

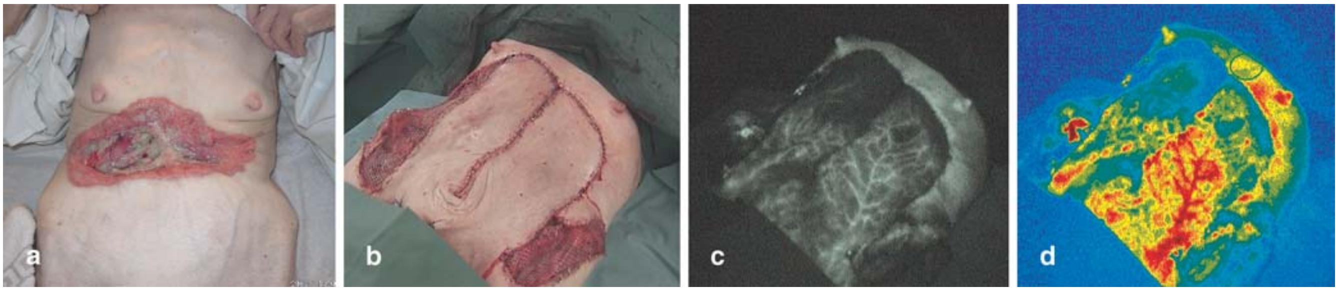


Fig. 4 **a** Clinical photograph of a 76-year-old woman with an exulcerating carcinoma of the abdominal wall. **b** Palliative resection of the tumor and coverage with bilateral groin flaps. **c** ICG fluorescence image demonstrating the axial arterial system. **d** False color image demonstrating the axial arterial system

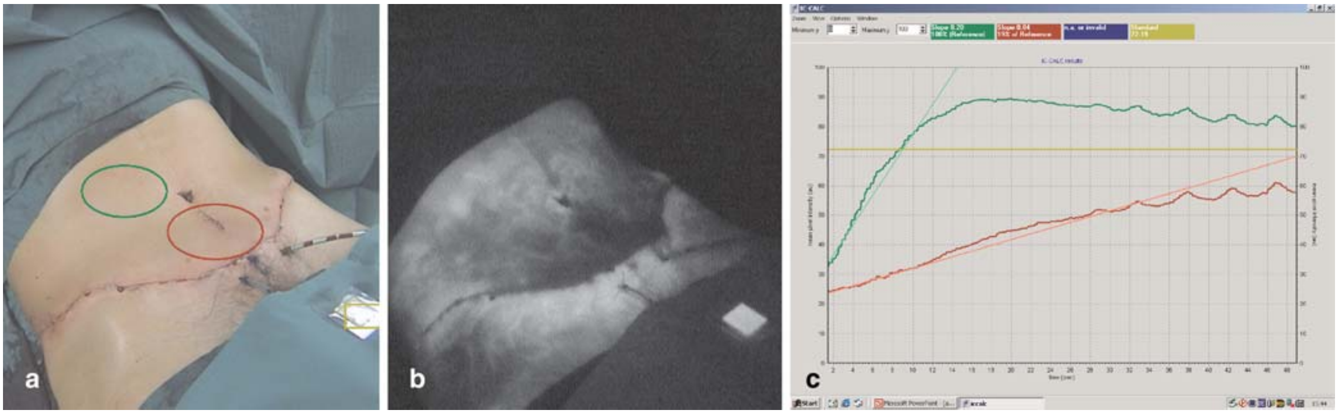
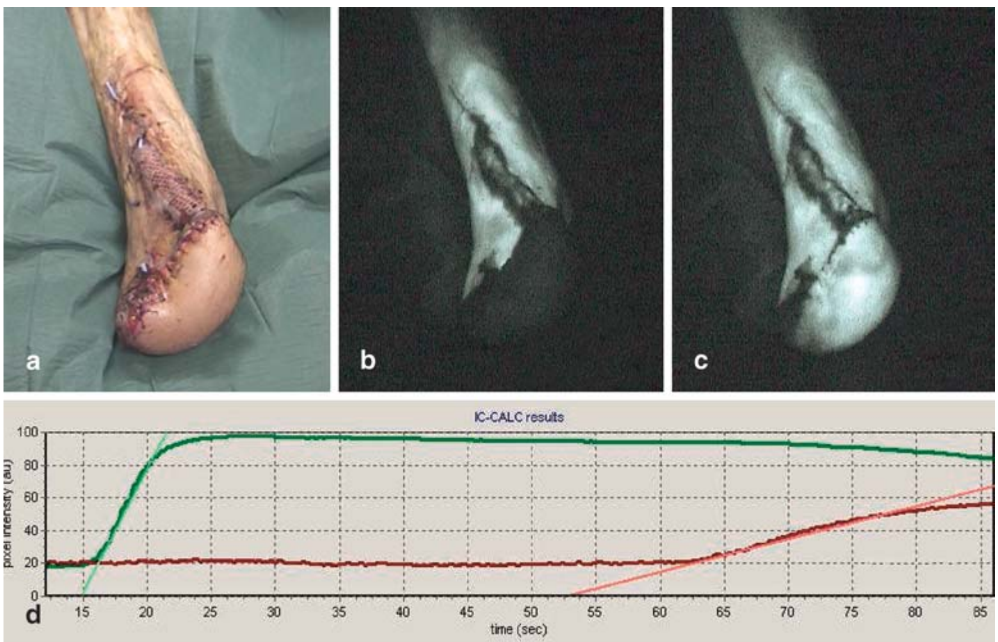


Fig. 5 **a** Clinical photograph of an abdominoplasty in a 45-year-old tobacco smoking woman. **b** ICG fluorescence image demonstrating hypoperfusion of the lower medial part of the abdominal skin flap. **c** Quantitative assessment demonstrating a substantially reduced perfusion of this area when compared with the upper abdomen (19%)

Fig. 6 **a** Clinical photograph of a radial forearm flap, which was used to cover an instable scar of an amputation stump of the forefoot. **b** ICG videography 25 s after injection showing filling of the surrounding skin. **c** ICG videography 55 s after injection showing filling of the forearm flap. **d** Quantitative assessment demonstrating a 50 s delay of dye inflow to the flap (*red*) when compared with the surrounding skin (*green*)



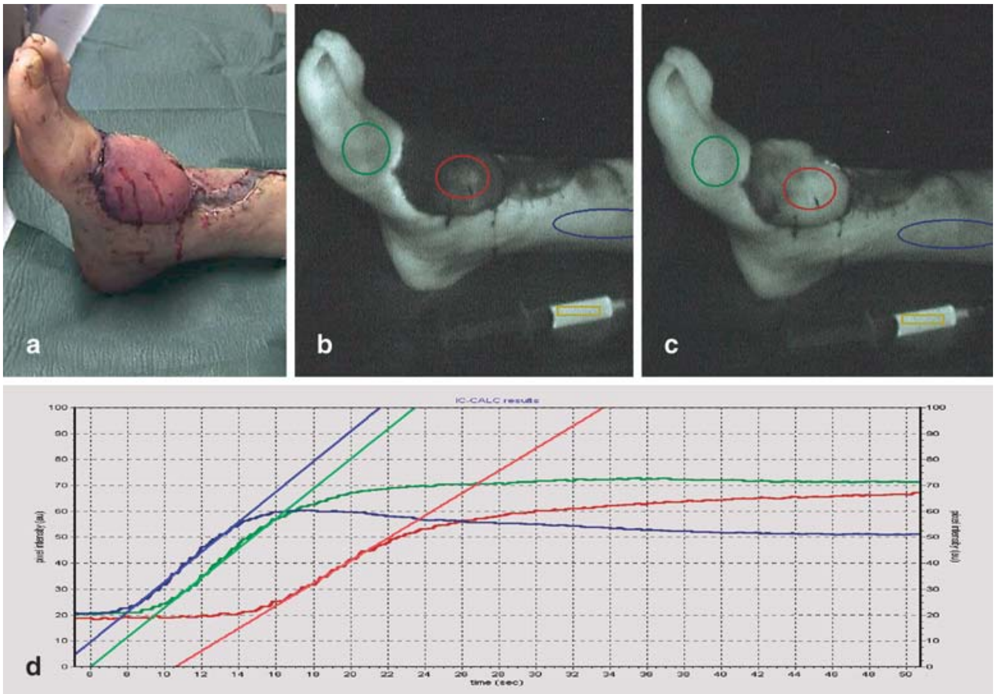


Fig. 7 **a** Clinical photograph of a radial forearm flap, which was used to cover a defect of the dorsum of the foot. The flap shows discoloration and swelling. **b**, **c** ICG videography demonstrating rapid filling of the flap but a delayed dye disappearance rate compared with the surrounding skin. **d** Quantitative assessment demonstrating a rapid dye inflow to the flap (*red*) but a delayed dye disappearance rate compared with the surrounding skin

rigidly. This leaves the length-breadth ratio concept imprecise and subject to personal interpretation.

Figure 5a shows the result of abdominoplasty in a 45-year-old tobacco smoking woman. ICG videography demonstrated a substantial reduction of skin perfusion in the infraumbilical region (Fig. 5b). This corresponds to the distal part of the abdominal skin flap, where the largest tension is applied. Quantitative assessment revealed a reduction of skin perfusion to 19% of the reference area on the upper abdomen (Fig. 5c). These findings might explain the increased wound healing complications, which have been reported in tobacco smoking individuals undergoing elective abdominoplasty.

Monitoring of microsurgical flaps

Many techniques for intra- and postoperative monitoring of free tissue transfers have been evaluated, but still there is no consensus as to which will eventually become the standard accepted method. An ideal monitoring technique should provide an immediate detection of arterial or venous occlusion. The criteria indicative of arterial or venous compromise should be easily to interpret by the nursing personnel. We present two examples of quantitative assessment of perfusion of microsurgical flaps which revealed different kinds of vascular complications.

Figure 6b and 6c show the perioperative ICG videography of a free forearm flap which was used to cover an unstable scar of an amputation stump on the forefoot. The quantitative assessment shows a 50-s delay of ICG inflow to the flap (red) compared with the surrounding tissue (green; Fig. 6d). This reveals a significant arterial spasm, which was treated with a vasodilating agent. Afterwards the flap healed uneventfully.

Figure 7b and 7c show the ICG videography of another free radial forearm flap which was used for coverage of a defect in the dorsum of the foot. This flap developed late postoperative swelling and discoloration. The quantitative assessment revealed rapid filling of the flap, but a disturbed dye outflow (red) compared with the surrounding skin (green; (Fig. 7d). This confirmed the clinical impression of venous stasis. The flap recovered completely after the application of medical leeches.

Discussion

Determination of burn depth

Inasmuch as the status of the skin perfusion is indicative of burn depth, an accurate method for assessment of the remaining viable cutaneous circulation would allow a correct estimation of the depth of burn. Previous authors have reported the use of ICG fluorescence in burn wound assessment in both experimental [8, 28] and clinical [29, 33] studies. ICG fluorescence has been consistently shown to produce an image of the perfusion of the viable dermis and the subcutis, allowing differentiation of spontaneously healing second-degree burns from burn wounds that require excision and grafting [29, 33]. A point of criticism has been the qualitative nature of the investigation results, which has required subjective interpretation of measurement results for reliable diagnosis of burn depth. This has been compared with the process of radiological interpretation of computed tomography or ultrasound examination [12]. Thus until now the desire for an objective, visual map of the burn wound has remained unfulfilled. The lack of numerically quantifiable readings certainly limits the objectivity of the technique and leaves it dependent on the judgement of the ICG image by the individual surgeon. Threshold fluorescence ratios for second- and third-degree burns and a reliable quantitative evaluation of the ICG images are needed and should be evaluated in further studies.

Pedicle flaps

Problems of blood supply dominate all aspects of flap surgery. Adequate blood supply with effective tissue perfusion is a crucial factor at every stage of the transfer [24]. Fluorescein angiography of the skin following intravenous administration of fluorescein has been previously used to confirm the viability of random pedicle skin flaps shortly following flap elevation, following flap delay and later after pedicle division procedures [22]. Experimentally Graham et al. [6] established an investigative benchmark for predictive assessment of random flap perfusion in the rat using fluorescein as injectable substance. The physiological limitations of fluorescein, however, prevented this technique from becoming generally accepted. The first human study on the use of ICG for assessing the viability of skin pedicle flaps was recently published by Still and coworkers [32]. They found that ICG imaging added significant information to the traditional clinical assessment methods because healing of the skin flaps was accurately predicted by the result of the perioperative ICG examination [32]· A recent clinical study from our group including 13 patients with 15 pedicle flaps added confirmatory evidence to these results [16]; intraoperative ICG filling defects were always associated with delayed wound healing, while all the flaps with complete ICG filling healed primarily [16]. Thus ICG imaging seems a sensitive tool for the assessment of nutritive blood flow of pedicle skin flaps.

Free tissue transfer/replant monitoring

Postoperative monitoring of revascularized or replanted digits has been previously reported using serial quantitative skin fluorescein measurements [7]. However, we were the first to report on the use of laser induced fluorescence for peri- and postoperative monitoring of free flaps [15]. In a clinical, observational study we evaluated the validity of intraoperative laser fluorescence videography as monitoring tool of different kinds of free tissue transfer. We found that evaluation of circulation is possible in any type of free flaps, irrespective of the type of the transferred tissue. This includes skin, skin and muscle, skin and fascia, and pure muscle and pure fascia. Vascular complications were definitely detected by the intraoperative fluorometric examination [15]. Shortcomings of ICG videography include the inability to obtain continuous monitoring over time. Nevertheless, the dynamics of in- and outflow of the ICG, the distribution of dye within the flap, and the intensity of ICG compared with the surrounding skin provided an overall picture of the microcirculation of the flaps, which had significant impact on the course of the operation. Early repetitions of the examination were possible due to the very short half-life of ICG.

The feasibility of distinguishing between arterial and venous occlusion was not clearcut and was based on the interpretation of the dynamic video-angiography rather than on absolute values of perfusion. Thus the rate of dye uptake in the flap compared with the surrounding skin and the flap disappearance rate of the ICG was considered indicative of the patency of the arterial and the venous anastomosis. Whether these shortcomings can be overcome with alternative techniques is questionable. Doppler ultrasound scanning for surface monitoring of the arterial inflow and the venous outflow signals is an established method for monitoring free tissue transfers [31]. Although this technique can be used in the operative room and can be repeated frequently, it is noncontinuous, and when in close vicinity to larger arteries or veins, it can difficult to be sure that the Doppler is recording flow in the vascular pedicle to the free flap.

The more sophisticated implantable laser Doppler flowmeter provides a continuous, noninvasive monitoring device [17, 34]. The beat-to-beat information provided by this technique facilitates the distinction between arterial and venous obstruction [3]. Despite several favorable reports in the literature, however, this technique also has several limitations. The optical cable is extremely sensitive to the slightest degree of movement of the extremities and to respiration, and clotted blood between the probe and the muscle fibers or loss of contact between the prism and the muscle can lead to non-functioning of the probe and loss of the signal. Thus in one series the laser Doppler falsely identified an ischemic episode in 21% of cases [36].

Methods such as photoplethysmography [38] and impedance plethysmography [9] are both continuous noninvasive techniques that allow evaluation of the shape of the waveforms for distinction between arterial and venous compromise. Photophlethysmograph waveforms can even be transmitted by telephone to a remote monitoring station that allow the surgeon at home to interpret any waveform abnormalities [18]. Neither of these techniques, however, have gained widespread acceptance in clinical use.

Conclusion

Our clinical experience adds confirmatory information to the existing literature regarding the use of laser-induced fluorescence of ICG for the determination of burn depth and for the perioperative assessment of skin flap viability. The use of this technique for monitoring of free tissue transfer is new, and our initial promising results should be further evaluated in clinical studies. The advantages of the dynamic laser fluorescence videography include low costs, convenience, perfusion quantification, and short assessment time. Limitations include noncontinuity, the need to switch off room light during measurements, and the requirement for intravenous injection with the risk of possible adverse reactions to the tracer substance. When combined with clinical observation the laser fluorescence of ICG can provide the surgeon with scientific evidence, which allows a rational design and planning of surgery.

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