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ORIGINAL PAPER

Intraoperative use of dynamic infrared thermography and indocyanine green fluorescence video angiography to predict partial skin flap loss

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Abstract Inadequate perfusion is the most common cause of partial flap loss in reconstructive surgery. Intraoperative monitoring of flap perfusion may prevent such loss. This study compared indocyanine green fluorescence angiography (ICG-FA) and dynamic infrared thermography (DIRT) in their ability to predict intraoperatively the percentage of flap survival in a caudally based McFarlane flap in 10 male Wistar rats. The intraoperative images of both techniques were subjectively and objectively analysed. The percentage of flap survival, as judged from the digital colour photographs 7 days post-operatively, was 69±3%. Objective analysis of flap survival based on intraoperative DIRT and ICG-FA (74 vs 63%, respectively) correlated quite well with the subjective measurements (75±2 vs 59±4%, respectively). However, intraoperative ICG-FA images underestimated flap survival by 6–10%, while intraoperative DIRT images overestimated the flap survival by 5–6%. The authors conclude that intraoperative use of ICG-FA and DIRT can provide valuable information on areas with inadequate perfusion as long as their limitations are respected.

Keywords Pedicled skin flap · Indocyanine green fluorescence angiography · Infrared thermography · Flap necrosis

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Introduction

The increased understanding of the vascular anatomy of the skin has contributed to a profound development in reconstructive surgery. Nowadays, the reconstructive surgeon can choose from a large variety of flaps, pedicled as well as free flaps. Although high success rates for tissue transfer are reported, partial flap loss still occurs. Inadequate perfusion is the most common cause of partial flap loss. Ideally, areas with compromised perfusion that will eventually become necrotic should be removed during the operation. Intraoperative assessment of these areas using clinical signs such as capillary refill, skin colour, dermal bleeding and skin turgor requires considerable experience. Prevention of partial flap loss is important as it causes significant morbidity to the patient and often requires surgical intervention.

Several monitoring techniques have been devised to assist the surgeon during the operation for the detection of inadequately perfused areas within a flap. The second-generation dye, indocyanine green (ICG), used in the relatively new and invasive technique ICG fluorescence angiography (ICG-FA), has replaced the previously used fluorescein as a fluorescent marker of cutaneous blood flow [10, 26]. Clinical and experimental studies have reported good correlation between the distribution of ICG and flap viability [4, 9, 10, 20], although there are conflicting reports on its ability to predict the level of necrosis [15]. The short half life (3–4 min) of ICG makes repeated measurements possible. ICG is also reported to cause significantly fewer side effects than fluorescein [2, 12, 21].

Unlike ICG-FA, dynamic infrared (IR) thermography (DIRT) is a non-invasive monitoring technique and is used in clinical medicine as a method to measure skin temperatures [1]. Dynamic IR thermography is based on the relationship between dermal perfusion and the rate of change in skin surface temperature following transient thermal challenges [8, 29]. The rate and pattern of rewarming provide indirect information on skin perfusion. Modern IR cameras are capable of producing high-resolution radiometric digital IR images with a temperature sensitivity of 0.1°C or better. The DIRT technique has been successfully used to indirectly study perfusion dynamics in flaps and to assist in flap design [6, 13, 24, 28, 31] but has, to our knowledge, not been used to estimate the area of inadequate perfusion that may become necrotic.

The aim of this study was to investigate whether the two techniques, ICG-FA and DIRT, can be used intraoperatively to predict the area of skin necrosis in a caudally based McFarlane rat skin flap model [17]. To our knowledge, a similar comparative study has not been published.

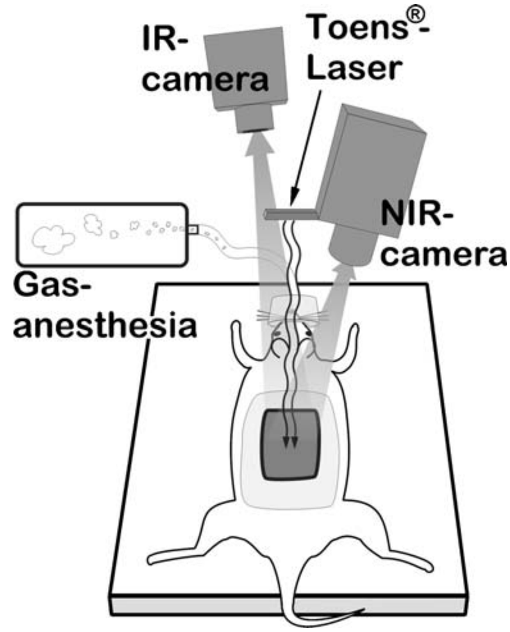

Fig. 1 A schematic diagram of the experimental set-up showing the placement of the IR digital camera and the near IR-filtered digital camcorder with Toens® laser light source on the top

Materials and methods

The experiments were carried out on 10 male Wistar rats weighing 300–350 g. The animals were caged individually and were fed a standard diet and water ad libitum. The procedures involving animal treatment and their care were conducted in conformity with guidelines on accommodation and care of laboratory animals by the European Convention for the protection of vertebrate animals. The study was approved by the Norwegian Committee on Ethics in Animal Experiments.

All surgical procedures were performed under standard aseptic conditions. Anaesthesia was induced and maintained by spontaneous inhalation of isoflurane (Forene "Abbott") and oxygen using a U-400 Anaesthesia Unit (Univentor Limited, Zejtun, Malta). A schematic drawing of the experimental set-up is shown in Fig. 1.

Under anaesthesia and with the rat in a prone position on a heating blanket, an area of ca 4×9 cm was shaved with electric clippers. A caudally based standard McFarlane flap of 2×7 cm was dissected free from the underlying fascia and sutured back in position after haemostasis was obtained with diathermy.

Indocyanine green fluorescence angiography (ICG-FA)

Perfusion of the flap was measured using the technique of dynamic laser-induced fluorescence video angiography (IC-View, Pulsion Medical Systems, AG, Munich, Germany). Under illumination with a laser (energy: Pi=0.16 W, wavelength: λ=780 nm), an intravenous bolus injection (0.3 mg/kg) of ICG (ICG-PULSION, Pulsion Medical Systems, AG) dissolved in injection water was given through a tail vein. The resulting fluorescence pattern was recorded with a digital video camera equipped with a near-IR filter.

Dynamic infrared thermography (DIRT)

A FLIR IR camera (FLIR ThermaCAM S65 HS, FLIR Systems, Boston, MA, USA) was used for monitoring skin surface temperature of the flap, and the rate and pattern of rewarming after transient cooling was used as an indirect indicator of flap perfusion. Through a fire wire interface connected to a laptop computer, it was possible to record video sequences of high-definition digital IR images. All IR images were electronically stored and afterwards processed using image analysing software ThermaCAM Researcher Pro 2.8 SR-1 (FLIR Systems AB).

Experimental protocol

After the caudally based McFarlane flap was harvested and before it was re-sutured into position, the flap was bent backwards at its base to obtain haemostasis. The flap was then returned to its original position and a plastic sheet (0.5 mm) was temporarily placed on the underside of the flap. The IR-camera revealed that the flap had cooled down most likely because of a reduced blood supply when it was turned backwards. The rate and pattern of rewarming of the flap was recorded with IR-thermography. The plastic sheet prevented warming of the flap by conductive heat transfer from the underlying tissue (donor area), so the flap could therefore only be rapidly rewarmed by perfusion with blood. After approximately 5 min, the plastic sheet was removed and the flap was sutured back into its original position. The perfusion of the flap was then examined with ICG-FA. Flap perfusion was also examined with ICG-FA 24 h after the operation.

Seven days after the surgical procedure, survival of the flaps was assessed. Digital colour images were taken, which included both the surviving and the necrotic area of the flaps. After assessment, the animals were euthanised with an overdose of Mebumal 10% (pentobarbital 100 mg/kg i.p).

Image analysis

Two methods were used to analyse the intraoperative IR- and ICG-FA-images. (1) A subjective method involving visually dividing the flap images into two separate areas; respectively, an area of low and warm temperatures and an area of low and high fluorescence intensities. (2) An objective method based on absolute temperature and fluorescence intensity measurements along a line in the midline of the flap extending from the proximal base (pedicle) to its distal end (see Fig. 3).

The results of both methods were compared and evaluated with the final outcome of flap survival on the seventh post-operative day. The area of survived and necrotic skin tissue on the day 7 digital colour images of the flaps were measured using the software TRACER, version 2.1 (Medical Computing Research Group, University of Glamorgan, Wales, UK) (Fig. 2c). The same programme was used for the subjective method. In the intraoperative IR images, the flap was subjectively divided into two separate thermal areas (high and low) (Fig. 2a). In a similar fashion, the flap was subjectively divided into two different areas according to fluorescence intensities (high and low) (Fig. 2b) based on the ICG-FA images. All the subjective measurements were carried out by the same person. The argument for performing the subjective analysis is that subjective examination is commonly used intraoperatively. The rationale for doing the objective method is to support the subjective method of analysing the images.

In the objective method, the IR thermal images were analysed with the software (ThermaCAM Researcher Pro 2.8 SR-1) to obtain a temperature gradient along the centre of the flap (see Fig. 3a and 5). In a similar fashion, the ICG-FA images were analysed using ImageJ software (National Institute of Health, Bethesda, MD, USA) to obtain a fluorescence intensity gradient along the centre of the flap (Fig. 3b). Due to individual variations in the fluorescence intensities, it was necessary to normalise these data. For each individual animal, the highest pixel intensity along the centre line of the flap was set to 100 and all other values

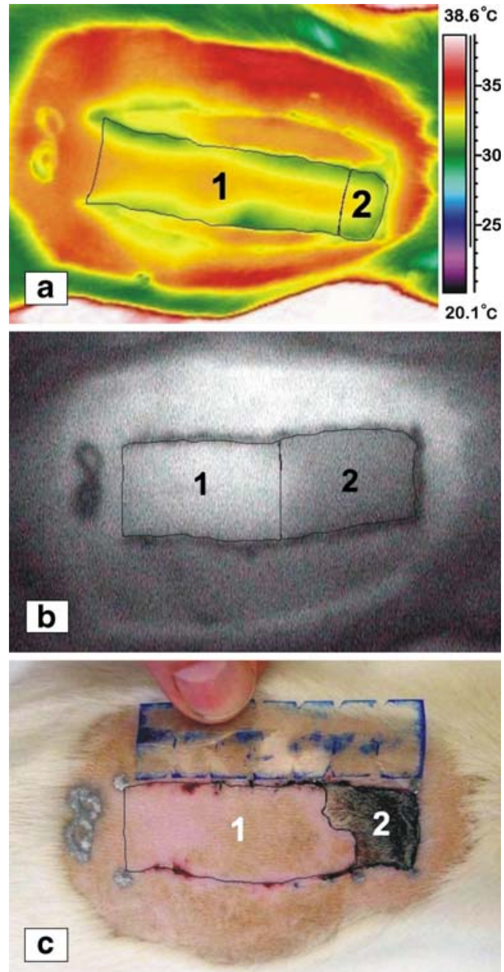

Fig. 2 **a** Intraoperative IR thermal image, **b** intraoperative ICG-FA image and **c** seventh-day post-operative digital colour image of a flap in a single animal. In each image, the flap is divided into two separate regions (*1* and *2*), the areas of which were measured using the analysing software Tracer; **a** proximal region (*1*) with high temperature and distal region (*2*) with low temperature, **b** region (*1*) with high fluorescence intensity and region (*2*) with low fluorescence intensity and **c** vital (*1*) and necrotic (*2*) region

were correspondingly adjusted. The data presented in Fig. 6 thus represent the average relative pixel intensities along the centre line for all animals.

Statistical analysis

Data are presented as mean values ± standard error of the mean (SEM). One-way ANOVA with repeated measures followed by the least significant difference test (Fischer's least significant difference test) was used in the comparison of the subjective analysis of the data (Table 1). $P<0.05$ was considered significant. Statistical calculation was performed using the statistical software SigmaStat for Windows version 3.5 (Systat Software, Erkrath, Germany). For the objective method, a receiver operating characteristic (ROC) curve was constructed (SPSS version 14.0 for Windows)

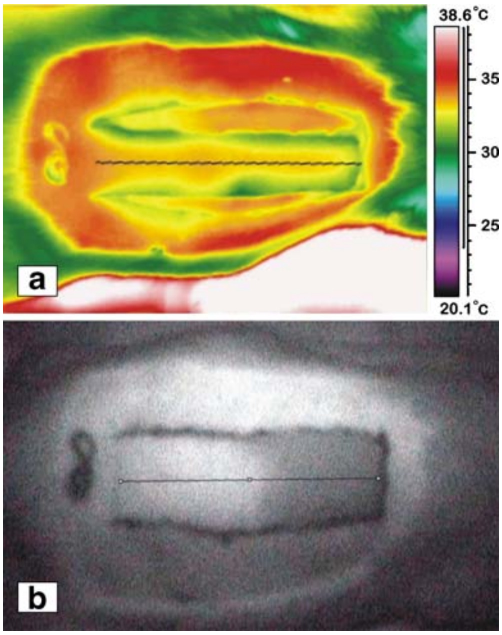

Fig. 3 **a** Intraoperative IR thermal image and **b** intraoperative ICG-FA image. The *black line* through the centre of the flap indicates the position of the temperature profile and fluorescence profile, respectively, used in the calculations presented in Figs. 5 and 6

for testing performance of the methods ICG-FA and DIRT to predict necrosis. The optimal cut-off point to predict outcome was derived from the ROC curve, including the sensitivity and specificity. Plotting sensitivity against 1-specificity allows comparison of area under the curve (AUC) as an indicator of the predictive power of the methods.

Results

In Fig. 2, images of the two different imaging techniques in a single animal are presented. The indirect method of DIRT is shown as a thermal image (Fig. 2a) and the direct method ICG-FA is shown as a fluorescence intensity image (Fig. 2b). In the same figure, a digital colour photo (Fig. 2c) of the resulting necrosis in the flap on the seventh post-operative day is shown. In all three images, two separate regions of the flap can be seen; a proximal region (1) with high temperature and a distal region (2) with low temperature (Fig. 2a), a region (1) with high fluorescence intensity and a region (2) with low fluorescence intensity (Fig. 2b) and vital (1) and necrotic (2) regions (Fig. 2c).

None of the flaps showed any signs of infection or haematoma. In most cases, necrosis became clinically apparent within 24 h after the operation. Table 1 shows the percentage of survival that was subjectively determined from the images mentioned in the section above. On day 7 post-operatively, a mean area of 10.2±0.6 cm^2 (69.3%) of the flaps was vital and a mean area of 4.4±0.4 cm^2 (30.7%) was necrotic. From the subjective area analysis of the IR-images, 74.7±2% of the flap had a high temperature and was located in the proximal area, compared to 25.4±2% which had a low temperature and was located in the distal part of the flap. The images from the intraoperative ICG-FA recordings of the flap indicated that 59.1±4% of the flap had a high fluorescence intensity, while 40.9±4% of the flap had low fluorescence intensity and may undergo necrosis. ICG-FA recordings made 24 h after the operation indicated that the area of high fluorescence intensity had increased to 72.8±3%, which was significantly different from the area measured in the intraoperative ICG-FA recordings. Intraoperatively, there was also statistically significant difference between the size of the flap area with high temperatures and high fluorescence intensity, and there was significant difference between these areas and the actual area of viable tissue at day 7 post-operatively (Table 1).

Table 1 Per cent survival measured in intraoperative DIRT and ICG-FA images, 1 day post-operative ICG-FA images and colour images from day 7 post-operatively

Rat #	DIRT (%) intraoperative	ICG-FA (%) intraoperative	ICG-FA (%) 1 day postop.	Photos (%) 7 days postop.
R1	65.7	51.2	59.4	61.8
R2	67.2	50.6	71.9	65.6
R3	74.6	66.6	77.8	79.3
R4	69.1	61.4	67.2	68.6
R5	67.8	35.0	59.0	47.6
R6	88.6	54.2	74.9	77.0
R7	83.9	72.9	80.0	72.5
R8	74.0	64.0	81.2	79.0
R9	76.6	75.8	78.3	71.8
R10	79.4	59.5	78.3	69.3
Average	74.7±2	59.1±4[a]	72.8±3[b]	69.3±3[a,b]

Values mean ± SEM

[a] Significant difference from intraoperative DIRT images, at $p<0.05$.

[b] Significant difference from intraoperative ICG-FA images, at $p<0.05$.

Using ROC analysis, we evaluated the accuracy of the methods in predicting levels of necrosis. Fig. 4a,b represents plots of all sensitivity/specificity pairs over the entire range of recordings. ROC analysis showed a good accuracy of both methods, DIRT and ICG-FA, in differentiating between viable and necrotic areas in the flap. The areas under the ROC curves were 0.86 and 0.90 for DIRT and ICG-FA, respectively.

The average temperature profile through the axial centre of the flaps measured from the intraoperative IR thermal images is presented in Fig. 5. The curve shows a high skin temperature running from its proximal base and continuing for about two-thirds of the length of the flap, with a marked fall in skin temperature towards its distal end. The curve in Fig. 6 is a profile of the average relative fluorescence intensity (in pixels) through the centre of the flap from its proximal base to the distal end of the flap. The shape of the curve is somewhat similar to the temperature profile shown in Fig. 5, with a clear fall in fluorescence intensity towards the distal end of the flap. From the ROC curves, sensitivity and specificity for the best cut-off value for both methods were calculated (Table 2). The cut-off values were then used to read off the related flap lengths in Figs. 5 and 6 (indicated by the respective vertical arrows). The objective ROC analysis showed that, with the DIRT method, 74% of the flap had skin temperature, which represented viable tissue on post-operative day 7, while the ICG-FA method had a cut-off point at 63% of relative flap length. As mentioned previously, the actual flap survival was 69.3%±3%.

Discussion

Partial flap necrosis is an undesirable outcome of surgery involving tissue transfer, which could be avoided by resecting the area at risk during the operation. However, this requires having a reliable technique that can be used intraoperatively for identifying areas of risk. Such a technique would be of great benefit to the surgeon because it requires considerable clinical experience to identify areas of inadequate perfusion by assessment of skin colour, capillary refill and turgor. ICG-FA and DIRT are reported to be capable of monitoring the dynamics of flap perfusion [6, 7, 9–11, 13, 16, 23, 24, 26, 28, 30, 31].

In this study, the percentage of flap survival, as judged from the digital colour photographs on day 7, was 69±3%. The objective measurements obtained with ROC analysis of intraoperative DIRT and ICG-FA, 74 vs 63%, respectively, correlated quite well with the subjective intraoperative measurements with DIRT and ICG-FA, 75±2 and 59±4%, respectively. This indicates that our subjective evaluation is in good accordance with the objective results, especially when it comes to the analysis of the IR-images.

However, the results from the subjective measurements of the intraoperative ICG-FA recordings underestimated flap survival by 10%, while those obtained with DIRT overestimated flap survival by 5.4%. These latter findings are clearly not ideal because overestimation of flap viability can result in partial flap loss, with a resultant open wound and delayed healing. On the other hand, underestimation of flap viability can result in resection of viable flap tissue and tight wound closure, which can also lead to impaired wound healing or even an inability to close the wound. Interestingly, the 1-day post-operative objective measurements with ICG-FA gave a better estimation of the percentage of flap survival than those obtained with intraoperative ICG-FA.

In interpreting the results, one has to be aware of the fact that the thermographic and angiographic examinations were not performed under completely identical conditions during the day of surgery. As explained in the method section, a

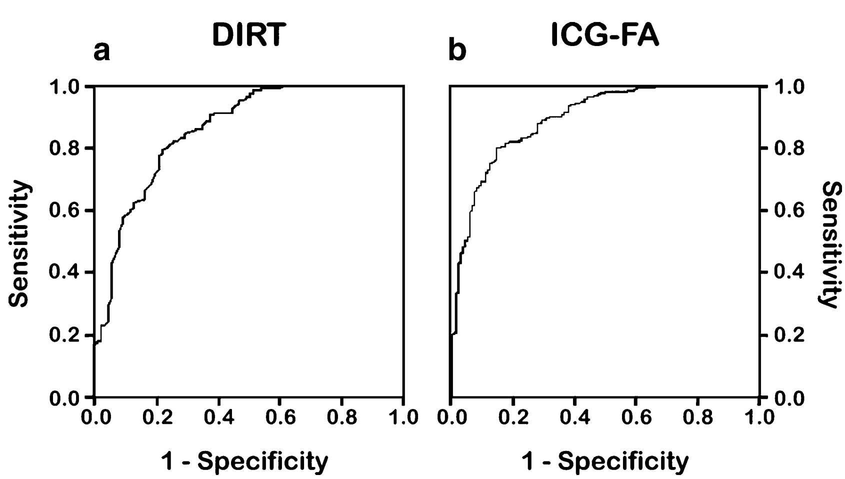

Fig. 4 ROC-curves for **a** DIRT and **b** ICG-FA. Plots of sensitivity/specificity pairs over the entire range of recordings with DIRT and ICG-FA are represented. The *x axis* is the false-positive fraction (1-specificity) of the flap without necrosis and the *y-axis* is the true-positive fraction (sensitivity) of the flap with necrosis. The areas under the curves were 0.86 (95% CI 0.81–0.91) and 0.90 (95% CI 0.87–0.93) for DIRT and ICG-FA, respectively

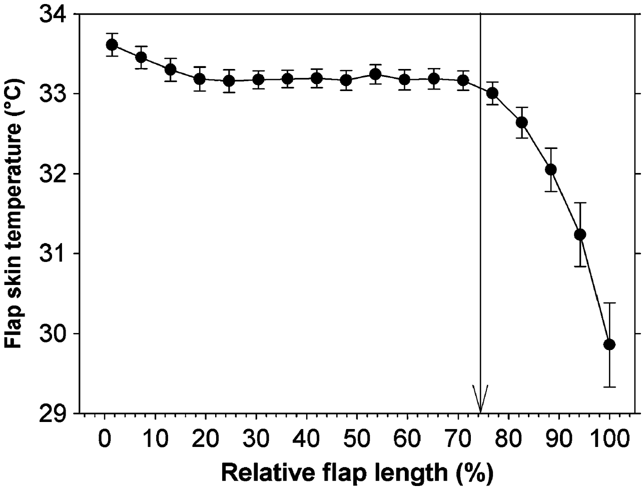

Fig. 5 The plotted curve is a temperature profile through the axial centre of the flaps from its proximal base to the distal end, measured from the intraoperative IR-images. The *vertical arrow* marks the cut-off point for the decline in temperature, as derived from the ROC analysis. Values mean ± SEM

plastic sheet was used to prevent the flap from being heated from the underlying tissue because thermographic images taken after the flap was sutured back to the donor site showed a more or less homogenous temperature distribution due to passive rewarming. The reason we chose to carry out the angiographic examination after the flap was sutured back in position was to be able to perform the two angiographic examinations (during surgery and 24 h after surgery) under similar conditions. Although it cannot be ruled out that the methodological approach we used may have affected the results, we do not feel that performing the angiographic examination during surgery with the plastic

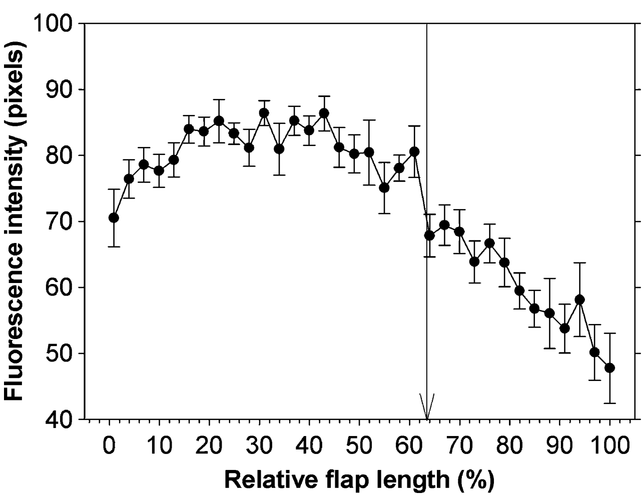

Fig. 6 The plotted curve is a profile of the average relative fluorescence intensity (in pixels) through the centre of the flap from its proximal base to the distal end of flap, measured in the intraoperative ICG-FA images. The *vertical arrow* indicates the cut-off point for the decline in fluorescence intensity, as derived from the ROC analysis. For reasons of clarity, only every 10th data point is presented in the *curve*. Values mean ± SEM

Table 2 Receiver operator curve characteristics for measures of necrosis, with cut-off points for optimal sensitivity and specificity

ROC characteristics			
Method	Cut-off point (% flap length)	Sensitivity (%)	Specificity (%)
DIRT	74	80	75
ICG-FA	63	84	78

sheet in place would affect the general outcome of the study. In fact, we feel that this approach would possibly result in a reduced blood flow due to a lower flap temperature, thereby producing a larger difference between the two techniques (a greater underestimation using angiography).

Krishnan et al. [15] used ICG-FA in the intraoperative evaluation and post-operative follow-up of nine flaps. They observed an underestimation of flap survival based on ICG-FA in both the intraoperative and post-operative phases. The underestimation of flap survival has also been reported when the predecessor of ICG, fluorescein, was used intra-operatively [5, 22, 25]. Pang et al. [22] postulated that, in the early post-operative period, arteriospasm and ischaemia due to surgical trauma induce an alteration in perfusion pressure and/or vascular blood supply within the flap that causes inadequate perfusion to the distal part of the flap. However, in the area of marginal blood flow, vascular relaxation may occur in the later stage of the post-operative period, and the capillary blood flow may be re-established. The fact that ICG-FA performed on the first post-operative day in our study was more accurate in predicting the border of skin necrosis than the intraoperative ICG-FA (Table 1) supports the postulation of Pang et al. [22].

Giunta and colleagues [9] used ICG-FA to predict flap necrosis in a rat model. In an attempt to quantify the perfusion of tissue, they used the perfusion index, which is defined as the fluorescence intensity in the area to be investigated divided by the fluorescence intensity of the normally perfused surrounding area. They found that, in all parts that became necrotic, the perfusion index ranked less than 25% of the surrounding tissue, while in the parts that survived the index, it ranked above 40%. We attempted to measure the perfusion index in the near-IR cameras' software IC-CALC (Pulsion Medical System AG) but found that the measurements were rather inaccurate and not reproducible because they were dependent on the placement of the reference area, which represents normally perfused surrounding tissue. In our view, measuring the fluorescence intensity along the flap is a more objective method in this particular case.

Skin surface temperature measurements have not generally been accepted as a reliable monitoring technique for flap perfusion [14]. DIRT, however, is based on the relationship between dermal perfusion and the rate and pattern of change in skin surface temperature following transient thermal challenges. Although the technique measures skin perfusion indirectly, the correlation between thermographic and laser Doppler velocimetric results is good [19, 27]. The rewarming pattern of a flap is related to the vascular anatomy, and knowledge on this pattern is necessary before DIRT can be used intraoperatively. Rewarming should come from blood perfusion of the flap and not by conductive and/or convective heat transfer from the underlying surface, which may be a problem if the flap is thin and without pronounced subcutaneous fat. Interestingly, the axial pattern seen in the IR-image (Fig. 3a) during rewarming of this caudally based McFarlane flap relates well to the known axial flow pattern of this flap [3]. The same observations were, on the other hand, not made when using ICG-FA (Fig. 3b).

The increased knowledge of vascular anatomy has contributed to the high success rates in flap surgery. However, the vascular territory of a given artery in a cadaver study may be different from the vascular territory of that same artery within a viable flap. In their study on axial and random pattern flaps, McGregor and Morgan [18] showed that, if one of a pair of abutting cutaneous vessels was occluded, then the other vessel would ‘extend’ its territory into the area of decreased intravascular pressure. Cormack and Lamberty [4] differentiated between anatomical territories, dynamic territories and potential territories. Flap harvest results in alteration of intravascular pressures and changes in dynamic equilibrium, leading to readjustments of flow and change in the size of the areas perfused. Dynamic territories may, therefore, be fundamentally different from anatomical ones. Both methods used in this study provide useful information on the perfusion dynamics in the flap: DIRT through the rate and pattern of rewarming and ICG-FA through the rate of dye-uptake and dye-clearance.

According to Creech and Miller [28], the ideal test for evaluation of skin flap viability should fulfil the following requirements: It should be easy to perform, provide exact results, be economical and be repeatedly applicable without danger and inconvenience to the patient. Furthermore, it should be applicable for all types of flaps and should not disturb the regular flap physiology. Although ICG-FA measures perfusion directly, this technique is invasive, has to be performed in a dark room and does not allow continuous monitoring. Another problem relates to variations in fluorescence intensity due to the inability of the projected low-power laser light to uniformly illuminate curved surfaces, indicating that this method is best suited for use on flat surfaces. In addition, adverse reactions to ICG have been reported [2, 12, 21].

Technically, we found that DIRT has the advantage of being non-invasive, it provides rapid and continuous registration of skin temperature and it does not cause any physical harm to the patient. The main drawbacks of this technique are that it is an indirect method and that it overestimates flap survival in our experiment.

In spite of these drawbacks, we conclude that intraoperative use of ICG-FA and DIRT can provide valuable information on areas with inadequate perfusion, as long as their limitations are respected.

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