Visualising vascular perfusion in isolated human abdominal skin flaps using dynamic infrared thermography (DIRT) and indocyanine green fluorescence video angiography (ICG-FA)

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Short title: Visualising vascular perfusion using DIRT and ICG-FA

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Summary

**Background** In this experimental study skin perfusion in an isolated human transverse abdominal flap model was visualised using indocyanine green fluorescence angiography (ICG-FA) and dynamic infrared thermography (DIRT). The aim of the study was to compare the results obtained with the direct technique ICG-FA and the indirect technique DIRT.

**Method** Eight isolated human transverse abdominal skin flaps, obtained from female patients undergoing abdominoplasty, were used in the study. A total of 19 selected vessels were individually perfused. Indocyanine green was used for visualising skin perfusion with ICG-FA. Warm and cold perfusate was used for visualising skin perfusion with DIRT. Both techniques were tested for repeatability, making up a total of 34 perfusions.

**Results** Qualitative analysis of the rate and pattern of perfusion visualised by both techniques was carried out. The perfusion pattern shown in the ICG-FA images was characterised by a uniform distribution of fluorescence. The extent of the perfusion pattern seen in the DIRT images during warm perfusion was comparable to the perfused area seen with the ICG-FA technique. The appearance of distinct hot spots in the DIRT images provided additional information on the distribution of perforating vessels.

**Conclusion** The extent of the perfused area indicated by the indirect DIRT technique corresponds well with the perfused area indicated by the direct ICG-FA technique. It is concluded that in clinical or experimental situations the non-invasive DIRT technique is a good alternative to the invasive ICG-FA technique for visualising skin perfusion.

**Keywords**

dermal perfusion; fluorescence intensity; hot spots; infrared thermal imaging; skin temperature; vascular imaging
Introduction

Evaluation of skin perfusion is essential in clinical situations such as flap surgery, burns and vascular disease. Clinical evaluation of skin perfusion based on subjective signs such as skin colour, capillary reperfusion and dermal bleeding requires considerable experience. To assist in the clinically evaluation of skin perfusion, objective methods such as capillaroscopy, laser Doppler flowmetry, ultrasound Doppler and photoplethysmography are used.

Two other monitoring techniques used to visualise skin perfusion are ICG fluorescence video angiography (ICG-FA) and dynamic infrared thermography (DIRT). The second-generation dye, indocyanine green (ICG) has replaced the previously used fluorescein as a fluorescent marker of cutaneous blood flow (Still et al., 1999; Holm et al., 2002). ICG binds strongly to plasma globulins, limiting the washout time for the dye. Clinical and experimental studies have reported good correlation between the distribution of ICG and viability of skin tissue (Green et al., 1992; Holm et al., 2002; Mothes et al., 2004; Giunta et al., 2005; Braue, Jr. et al., 2007). ICG is also reported to cause significantly less side effects than fluorescein (Hope-Ross et al., 1994).

Unlike ICG-FA, infrared thermography is entirely non-invasive and is used as a monitoring technique to measure skin temperatures in humans (Jiang et al., 2005). Infrared thermography provides an image of the temperature distribution on the surface of the body. Dynamic infrared thermography (DIRT) is based on the relationship between dermal perfusion and the rate of change in skin surface temperature following transient thermal challenges (Wilson & Spence, 1989; Mercer & de Weerd, 2005). The rate and pattern of rewarming provide indirect information on skin perfusion. The DIRT technique has been successfully used to study indirectly perfusion dynamics in flaps (Salmi et al., 1995; Zetterman et al., 1999; de Weerd et al., 2006). Its usefulness in
estimating depth of burn injury has also been reported (Hackett, 1974; Liddington & Shakespeare, 1996; Renkielska et al., 2006).

In this experimental study ICG-FA and DIRT were compared as techniques to visualise skin perfusion in an isolated human transverse abdominal flap model. A modification of the model described by Kreidstein and co-workers (Kreidstein et al., 1991) was used. This model allows for repeated perfusions of selected vessels under controlled conditions. By eliminating blood born factors and central nervous stimulation, only local factors such as temperature and perfusion pressure affect the tonus of the vessels during perfusion.

Thus, the aim of the study was to compare the two imaging techniques DIRT and ICG-FA in their ability to visualise skin perfusion in isolated human transverse abdominal skin flaps.
Method

Procurement of experimental tissue

The protocol for the isolated perfusion experiments in the human skin flap model was approved by the Norwegian Regional Committee for Research Ethics (Region V). The experiments were carried out on excised skin panni from patients undergoing abdominoplasty. These panni serve no purpose to the patients and are normally disposed of by incineration. Written informed consent was obtained before surgery. The procurement and disposal of the human skin specimens were in accordance with the policy of the Department of Plastic Surgery at the University Hospital of North Norway.

Skin flap model

Eight human transverse abdominal skin flaps were included in the study, all of them from female patients. The experimental design using an isolated perfused transverse abdominal skin flap model was initially described by Kreidstein (Kreidstein et al., 1991). The design was modified to permit heating and cooling of the perfusate (Figure 1). The flaps used had a mean weight of 977 g (range, 430-1360 g). In each flap one to three vessels were selected for perfusion. Each selected vascular pedicle was cannulated with an 18-gauge angiocatheter, which was secured with 3-0 silk ties. The vascular pedicle originated from either the superficial inferior epigastric artery (SIEA) or vein (SIEV), the superficial circumflex iliac artery (SCIA), or from perforators of the deep inferior epigastric artery (DIEP) (Figure 2). Venous outflow was possible through SIEV on both sides. Following cannulation heparinised saline (5 U/ml) was gently injected into the angiocatheter until venous outflow was observed. The skin flap was then connected to a perfusion apparatus. All perfusion experiments were initiated within 90 minutes after excision of skin panni. It has been demonstrated by others that the skin
The skin flap was placed on a metal-grid, which was positioned on top of a sink (Fig. 1). At the beginning of the perfusion, any identifiable leaking vessels were ligated with pressure clips. The perfusate consisted of modified Krebs-Hensleit bicarbonate buffer with the following composition (in mM): 118.5 NaCl, 25 NaHCO₃, 4.4 KCl, 1.2 MgSO₄, 1.2 KH₂PO₄ and 2.5 CaCl. Human serum albumin (30 mg/ml) (Octapharma AG, Lachen, Switzerland) was added to the buffer, resulting in a final concentration of 6.5%. The buffer was then gassed with 95% O₂/5% CO₂ (pH 7.4) using a rotating surface oxygenator and filtered through a 5.0 µm filter.

The perfusion rate was controlled using a variable-speed peristaltic pump (Watson-Marlow Alitea, Stockholm, Sweden). The flow rate was monitored using a drop counter (Bratkovsky et al., 2004) that was placed in the perfusion line (Fig. 1). A bubble trap was included in the perfusion line to prevent air embolisation. Perfusion pressure was monitored with an inline pressure transducer (Transpac IV; Abbott Laboratories, North Chicago, IL, USA). To achieve a baseline perfusion pressure of ca. 50-60 mmHg, the perfusion flow rate was kept between 6-8 ml/min. Previous studies with similar human skin flap models have shown that a baseline of ca. 50 mmHg provides adequate tissue perfusion with minimal leakage and oedema formation (< 10%) (Kreidstein et al., 1991; Kreidstein et al., 1992).

Two thermostatically controlled water baths (Heto Lab Equipment A/S, Denmark and Pharmacia LKB MultiTemp II, Pharmacia, Uppsala, Sweden), one with a temperature of 38°C and the other with a temperature of 22°C, were connected to a heat-exchanger in order to control the temperature of the perfusate. A thermocouple was
inserted in the flow line at the perfusion inlet of the catheter to monitor the temperature of the perfusate. Flow, pressure and temperature data were monitored on-line using locally designed software (LabVIEW based).

**Experimental protocol**

The experiments were performed at room temperature (ca. 22°C). After a stabilisation-period of 20 min with a perfusion temperature of 22°C, the heat-exchanger temperature was switched to 38°C, which resulted in the temperature of the perfusate entering the angiocatheter to increase to 34-35°C. The period of warm perfusion varied from 10-30 min. The perfusion with warm perfusate was normally repeated 2-3 times. Intervening cooling periods (22°C) were used in order to obtain a uniform temperature on the skin surface before the subsequent warming sequence. The skin areas perfused in the flap were documented with DIRT and ICG-FA. During all phases of the experiment, skin surface temperature was continually recorded by the IR camera. After changing the temperature of the heat-exchanger, a period of at least 5 min was allowed to elapse before an ICG-FA measurement was made. Following completion of the ICG-FA and DIRT measurements, one of the cannulated vessels was selected for final perfusion with a contrast medium (Ultravist 300 mgI/ml, Shering AG, Berlin, Germany) for x-ray imaging. Due to its high viscosity, the contrast medium had to be injected by hand and thus, the perfusion pressure was not controlled. The perfusion experiments lasted for maximum 4 hours.

**Dynamic infrared thermography (DIRT)**

An IR camera was used for monitoring skin surface temperature of the flap, and the rate and pattern of skin warming was used as an indirect indicator of areas where the flap was perfused by the perfusate. Two different IR cameras were used. In four skin flaps a
Nikon Laird S270 (Tokyo, Japan) IR camera and in the other four skin flaps a FLIR IR camera (FLIR ThermaCAM S65 HS, FLIR Systems) were used. For both cameras it was possible to record digital IR images of high definition (0.1°C). The IR images taken with the both cameras were stored electronically. The FLIR camera, connected to a laptop computer through a FireWire interface, had the advantage of recording video sequences. All IR images were processed using image analysing software PicWin-IRIS (EBS system technik GmbH, München, Germany) and ThermaCAM Researcher Pro 2.8 SR-1 (FLIR Systems AB, Boston, MA, USA), respectively. Silver tape or silver paint, visible in the IR images, were used to mark the midline and left and right side of the flap.

**Indocyanine green fluorescence angiography (ICG-FA)**

The skin area of the flap perfused was also imaged using dynamic laser-induced fluorescence video angiography (IC-View, Pulsion Medical Systems, AG, Munich, Germany). Under illumination with a laser (energy: $P_0 = 0.16$ W, wavelength: $\lambda = 780$ nm), a bolus injection of indocyanine green dissolved in injection water and buffer (0.3 ml/20 ml buffer) (ICG-PULSION, Pulsion Medical Systems, AG, Munich, Germany) was given through the angiocatheter until venous outflow through one or both SIEVs were observed. The resulting fluorescence pattern in the skin was recorded with a digital video camera equipped with a near-infrared filter. In order to obtain optimal images, it was important that the camera and laser source were perpendicular to the flap surface. During recording, the room was darkened to achieve maximum image resolution.
Results

In 8 skin flaps, 19 different vessels were perfused. Repeatability was tested by perfusing 12 of the vessels up to 3 times, making a total of 34 perfusions.

The resulting perfusion patterns from 7 different vessels in 4 flaps are presented in Figure 3, 4, 5 and 6. IR images, ICG-FA images and x-ray images are shown. The images show perfusion with time and repeated perfusions with warm/cold perfusate and ICG-solution. Images comparing perfusion of vessels of lateral and central origin, and a single vein are also presented. In all images, the navel is orientated to the top and the skin side is facing up.

Prior to warm perfusion, the inlet temperature of the perfusate was maintained at 22°C, which resulted in a nearly uniform surface temperature. During warm perfusion, distinct hot spots became visible in the thermographic images. The first hot spots appeared within 30 seconds of warming, becoming gradually more evident (i.e. warmer and larger) with time. Both the number and the size of the hot spots varied for the different vessels tested. In addition to the appearance of hot spots, a distinct background area, both surrounding and in between the hot spots, also increased in temperature.

Following ICG injection, a well-defined area with nearly uniform distribution of fluorescence became immediately visible. The fluorescence intensity reached its maximum after 30-60 seconds, thereafter decreasing in intensity. Wash out of ICG was observed through one or both of the SIEVs. The total skin area heated during warm perfusion was comparable to that illuminated in the fluorescent images.

Figure 3 presents images from two periods of warm perfusion in a single flap (A1-A4 and B1-B4), which clearly demonstrate the repeatability of both methods. The arrows mark the inlet of the perforating artery being perfused (DIEP), verified from x-
ray images. Images A1 and B1 show ICG-FA recordings and the IR images A2-A4 and B2-B4 show the subsequent warming of the flap at time 30 sec, 6 min and 10 min.

Figure 4 presents images from three different perfusions of the same vessel (DIEP) in a single flap. These images demonstrate the repeatability of ICG-FA and DIRT. Images A1-A3 are ICG-FA recordings and B1-B3 are IR images following 6 min of perfusions with warm, cold and warm perfusate, respectively. The warm shadows shown by the black arrows in images B1 and B3, coincide with the position of the SIEV marked with the white arrow in the x-ray image (B4), indicating venous drainage of warm perfusate. The arrow in image B2 marks cold spots, resulting from changing the perfusion inlet temperature back to 22°C. These correspond exactly with the hot spots in images B1 and B3, resulting from perfusing the same artery at 34°C. Image A4 is a colour photograph of the flap, where the silver ink stripe marks the midline and the silver tape marks an appendectomy scar.

Figure 5 shows images from perfusions of two vessels in a single flap, a DIEP in panel A and a SIEA in panel B. The image A1 is an x-ray image of the flap, where a circle marks the perfused vessel. Images A2-A3 in the figure show the skin area warmed by hot perfusate 3 and 7 min after start of the perfusion. Image A4 shows the corresponding ICG-FA recording. The IR images B2-B4 show what happens after prolonged perfusion (up to 27 min). Note how the size of the heated area changes very little with time. Image B1 shows the corresponding ICG-FA recording. There is a striking similarity in the perfusion pattern seen with ICG-FA and DIRT.

The images in Figure 6 show examples of perfusions of three different kinds of source vessels, namely a SCIA in one flap (A1-A4), and a SIEV (B1-B2) and a DIEP (B3-B4) in a second flap. The results demonstrate that the distribution patterns seen with the ICG-FA show great similarity with those seen in the DIRT images, irrespective
of the origin of the vessels. Furthermore, panel A in the figure also demonstrate repeatability of both techniques when perfusing a SCIA.
Discussion

The aim of this study was to compare two monitoring techniques for blood perfusion, dynamic infrared thermography (DIRT) and indocyanine green fluorescence angiography (ICG-FA), in an isolated perfused human transverse abdominal skin flap. DIRT is a non-invasive indirect technique, while ICG-FA is an invasive direct technique. Both techniques have been reported to be capable of monitoring the dynamics of flap perfusion (Rübben et al., 1994; Salmi et al., 1995; Zetterman et al., 1999; Still et al., 1999; Yamaguchi et al., 2004; Mayr et al., 2004; Holm et al., 2006; de Weerd et al., 2006). The overall results show a good qualitative correlation between these two imaging methods with respect to the extent of perfusion, also during repeated perfusions. These observations were independent of the origin of the vascular pedicle used for perfusion (DIEP, SIEA, SIEV, or SCIA).

The advantage of the in vitro experimental model used in this study, is that one is able to compare the two imaging techniques under identically controlled conditions. An in vivo situation differs from the in vitro situation in that there is, for example, pulsatile flow, blood as perfusate and innervation. Despite these differences we believe that the results obtained in this study are relevant. For example, although the imaging techniques have different response rates, both visualise the extent of distribution already within a few minutes. In free flap surgery Holm et al. used ICG-FA to determine intraoperatively the reliability of flap perfusion of the SIEA or DIEP flap (Holm et al., 2007). The results of our study indicate that the indirect and non-invasive DIRT technique provides the surgeon with the same information. The extent of distribution was comparable for both techniques, however, the pattern of distribution showed a clear difference. IR thermography shows the presence of hot spots that correlate with the location of perforating blood vessels to the skin (Itoh & Arai, 1995; Binzoni et al.,...
2004; de Weerd et al., 2006), which normally are registered with ultrasound Doppler. In a clinical setting, the appearance of hot spots occurs earlier than the Doppler sound, confirming perfusion (de Weerd et al., 2006). In our experiments, the ICG-FA images did not show clear fluorescent focal points, as reported by Krishnan et al (Krishnan et al., 2005). In their study, bright points seen in some of the flaps were assumed to be skin perforators.

ICG-FA has been reported to be a quick, reliable and cost-effective method for assessing tissue perfusion (Raabe et al., 2003). However, one of its drawbacks is that the assessment of skin area visualised by fluorescence is short lived and has to be ascertained subjectively during the recording period. While the IC-calc software available with the IC-view equipment permits one to objectively elaborate the intensity of fluorescence, this could only be done on processed images and was not possible to perform in real time. Furthermore, since the quantitative analysis also requires having an area of tissue with normal blood flow for reference, this was, for obvious reasons, not possible in our experiments. In addition, some subjective variables can influence the objective findings, including the shape of the flap surface that should be as flat as possible to allow the camera to be perpendicular to the flap surface in order to accurately record the fluorescence intensity.

Skin surface temperature measurement is one of the oldest monitoring techniques for flap perfusion. Its reliability has been discussed (Khouri & Shaw, 1992; Busic & Das-Gupta, 2004). From this study, DIRT measurements showed that in perfused flaps the temperature distribution is not uniform, due to the presence of hot spots. This may explain some of the difficulties experienced when random single point measurements of skin surface temperature are used in post-operative monitoring of free flaps (Busic & Das-Gupta, 2004). The advantage of the DIRT technique is that the
thermal images cover a large skin area clearly visualising the distribution of these hot spots.

Following the recommendations of Kreidstein and co-workers (Kreidstein et al., 1991), the perfusion pressure in our isolated preparation was kept low and as stable as possible during the experiments to prevent oedema. It has been postulated that high perfusion pressure may cause vasoconstriction due to the myogenic response of the arterial wall to high perfusion pressure (Bayliss, 1902; Cormack & Lamberty, 1994). The ICG fluid infused in connection with the ICG-FA recordings was always washed out before the subsequent recording, indicating no leakage to the extracellular space. The fact that we were able to perform repeated perfusions with the same results also confirms that conditions were stable throughout the experiments. The high viscosity of the contrast medium used for the x-ray images required that it be injected with a higher pressure than 50 mmHg. This may have resulted in x-ray images showing a greater perfusion area than the ICG-FA and DIRT images. The x-ray images presented in this study were only used to indicate the position of major veins and the position of the angiocatheters used for perfusing the selected blood vessels.

In this study we have shown that the extent of the perfused area indicated by the indirect DIRT technique corresponds well with the perfused area indicated by the direct ICG-FA technique. We conclude that the non-invasive DIRT technique is a good alternative to the invasive ICG-FA technique for visualising skin perfusion in experimental as well as in clinical situations.
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**Figure 1** Illustration of the perfusion apparatus.
Figure 2 A schematic diagram of an isolated skin flap. The vessels perfused in the experiments are illustrated: The superficial circumflex iliac artery (SCIA), the superficial inferior epigastric artery (SIEA) and vein (SIEV), and perforators of the deep inferior epigastric artery (DIEP)
Figure 3 The images presented in panels A and B are from two periods of warm perfusion in a single flap. Images A1 and B1 are ICG-FA recordings. The IR images A2-A4 and B2-B4 were recorded respectively 30 sec, 6 min and 10 min after start of warm perfusion. The arrows mark the inlet of the artery (DIEP) being perfused.
Figure 4 The images presented in panels A and B are from three different perfusions of the same vessel (DIEP) in a single flap. Image A4 is a colour photograph of the flap. Images A1-A3 are ICG-FA recordings and B1-B3 are IR images, all taken 6 min after perfusion with warm, cold, and warm perfusate, respectively. The black arrows in images B1 and B3 show warm shadows of the venous drainage, which coincide with the position of the SIEV marked by the arrow in B4. The white arrow in image B2 marks cold spots.
Figure 5 The images presented in panels A and B are from perfusions of two vessels, a DIEP and a SIEA in a single flap. Image A4 is an x-ray image, where the circle marks the perfused vessel. Images A2-A3 show the skin area warmed by hot perfusate after 3 min and 7 min of perfusion. Image A1 shows the corresponding ICG-FA recording. Images B2-B4 show the subsequent warming of the flap after 5, 10 and 27 min of perfusion. Image B1 shows the corresponding ICG-FA recording.
Figure 6 The images show examples of perfusions of three different kinds of source vessels, a SCIA in panel A and a SIEV and a DIEP in panel B. Images A1-A2 are ICG-FA recordings from two perfusions of the SCIA. Images A3-A4 are the corresponding IR images after 10 min of perfusion. B1 is an ICG-FA recording and B2 is an IR image after 19 min of perfusion of a SIEV in another flap. B3 is an ICG-FA image and B4 is an IR image after 10 min of perfusion of a DIEP in this flap.