



# The use of laser speckle contrast imaging to predict flap necrosis: An experimental study in a porcine flap model



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## KEYWORDS

Flap monitoring;  
Reconstructive surgery;  
Laser speckle contrast imaging;  
Partial flap necrosis

**Summary Background:** We evaluated the use of laser speckle contrast imaging (LSCI) in the perioperative planning in reconstructive flap surgery. The aim of the study was to investigate whether LSCI can predict regions with a high risk of developing postoperative necrosis. Our hypothesis was that, perioperatively, such regions have perfusion values below a threshold value and show a negative perfusion trend.

**Methods:** A porcine flap model based on the cranial gluteal artery perforator was used. Images were acquired before surgery, immediately after surgery ( $t = 0$ ), after 30 min ( $t = 30$  min), and after 72 h ( $t = 72$  h). Regions of interest (ROIs) were chosen along the central axis of the flap. Clinical evaluation of the flap was made during each time point.

**Results:** At  $t = 72$  h, a demarcation line could be seen at a distance of  $15.8 \pm 0.4$  cm away from the proximal border of the flaps. At  $t = 0$ , perfusion decreased gradually from the proximal to the distal ROI. At  $t = 30$  min, perfusion was significantly lower in the ROI distal to the final demarcation line than that at  $t = 0$ , and in all flaps, these ROIs had a perfusion  $< 25$  PU. At  $t = 72$  h, perfusion in the ROI proximal to this line returned to baseline levels, whereas perfusion in the distal ROI remained low.

**Conclusions:** In our model, a decrease in perfusion during the first 30 min after surgery and a perfusion  $< 25$  PU at  $t = 30$  min was a predictor for tissue morbidity 72 h after surgery, which indicates that LSCI is a promising technique for perioperative monitoring in reconstructive flap surgery.

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## Introduction

When tissue reconstruction is done using microvascular free flaps, random flaps, pedicled flaps or other types of flaps, there is always a risk of tissue morbidity that is much dependent on the tissue perfusion related to the feeding vessels. Postoperative loss of tissue viability due to compromised circulation may result in complete flap loss or additional revision procedures. This leads to increased risk for infections, poor wound healing, and fat necrosis. This affects both the functional and esthetic outcome of the surgery.

New noninvasive and minimally invasive technical advancements have been adopted in reconstructive flap surgery with the aim to improve pre-, peri-, and postoperative tissue monitoring.<sup>1,2</sup> Despite this, clinical judgment by the surgeon remains the golden standard for perioperative evaluation of the tissue on the operating table. However, clinical judgment is subjective, dependent on clinical experience and not always reliable for the assessment of flap perfusion and long-term viability.

In this experimental study, we evaluated laser speckle contrast imaging (LSCI) as an alternative method for perioperative assessment in reconstructive flap surgery. LSCI is a laser-based technology that, unlike the much slower laser Doppler imager (LDI), allows for a very fast (fraction of a second) assessment of an area of tissue, with a high spatial resolution.<sup>3,4</sup> LSCI has been used in a number of studies to assess superficial perfusion in surgical flaps. For example, Ngyen et al and Sheikh et al studied blood perfusion in porcine eyelid flaps using LSCI, laser Doppler velocimetry, and thermography, and Du et al used LSCI to assess microcirculation in rat skin flaps.<sup>5-8</sup>

In a previous study, we showed that LSCI is able to detect postoperative partial and full venous and arterial obstruction.<sup>7</sup> One of our conclusions was that even if LSCI has a lower variability in detecting changes in venous blood flow than laser Doppler flowmetry, the current LSCI systems might be better suited for perioperative assessment than for continuous long-term postoperative measurements.<sup>9</sup>

The aim of this study was to investigate whether LSCI can be used perioperatively to identify flap areas with compromised circulation and thereby predict areas with a high risk of postoperative necrosis. Our hypothesis was that a decrease in perfusion could be seen in areas with compromised circulation during the first 30 min after the flap was raised. We also hypothesized that threshold values of the perfusion could be identified, which may potentially be used perioperatively to assist in planning the flap and for safe removal of tissue parts that would have a poor viability postoperatively.

A porcine pedicled flap model based on the cranial gluteal artery perforator (CGAP) was used in this study. A similar model was used by our group in the previously mentioned study to show the usefulness of LSCI for postoperative flap monitoring compared to laser Doppler flowmetry.<sup>9</sup> In this former study, wherein flaps were designed as 12 cm by 15 cm rectangles, we could see signs of stasis in the most distal parts, which indicates threatening ischemia. Therefore, in the current study, the flap was elongated to 20 cm and narrowed to 10 cm to promote that the most distal part of the flap would be nonviable after three days. The model

simulates partial necrosis, which is a known complication in reconstructive flap surgery.<sup>10</sup>

## Materials and methods

### Animals

Six mixed breed pigs (mean age 4 months, 45 kg, Swedish Landrace pigs) were used in the study.

### Surgery

The pigs were preanesthetized with Dexdomitor 0.1 mg/kg, Zoletil 5 mg/kg, and atropine 0.05 mg/kg. Anesthesia was maintained with pentobarbital sodium 8 mg/kg/h and fentanyl 0.5 µg/kg/h dissolved in Ringer's acetate given continuously intravenously using a motorized infuser along with crystalloid fluids (Ringer acetate). Body temperature, blood pressure, heart rate, and oxygen saturation were monitored during the whole procedure.

A 10 × 20 cm fasciocutaneous island flap based on the CGAP was raised from the right buttock of each pig. The flap was dissected along the surface of the muscle including the skin, subcutaneous tissue, and muscle fascia. The pedicle containing the perforator artery along with comitant veins was isolated (Figure 1). The flap was then reinserted in its original position using surgical staples. No barrier was inserted between the flap and the underlying muscle. After the initial measurements, the flaps were dressed in dry swabs and Tegaderm (3 M, St. Paul, Minneapolis, MN, USA), and the animal was returned to its box and awakened from anesthesia.

After 72 h, the animal was anesthetized in the same manner as that previously described. After the final measurements were made, the animal was euthanized with an overdose of pentobarbital sodium and 70% ethanol without regaining consciousness.

### Clinical evaluation

The visual appearance and capillary refill of the flaps were evaluated by two clinically experienced surgeons (the authors SF and JZ) at  $t = 0$  min,  $t = 30$  min, and  $t = 72$  h. This approach was adopted to mimic the clinical setting, where the surgeon constantly evaluates the color and capillary refill of the flap.

### Equipment

A laser speckle contrast imager (PeriCam PSI System, Perimed AB, Järfälla, Sweden) was used for all measurements. In short, LSCI measures perfusion illuminating the tissue with a divergent laser beam at a wavelength of 785 nm. This creates a speckle pattern over the illuminated area. A monochrome CMOS camera captures the speckle image, and the perfusion is calculated in every image pixel by measuring the local speckle contrast. A separate camera captures a



**Figure 1** The cranial gluteal artery perforator flap (CGAP). The flap was dissected along the surface of the muscle, including the skin, subcutaneous tissue, and muscle fascia. The pedicle containing the artery along with comitant veins was isolated.

color image of the measured area. The principle of the measurement technique of LSCI has been previously described in detail.<sup>3,11</sup>

### Measurements

After the pig was anesthetized, the area of the flap was marked on the buttock of the pig. Room temperature and vital parameters including blood pressure and heart frequency were continuously monitored. The distance between the LSCI camera and the skin was kept between 20 and 25 cm. The settings were the same for all measurements, with an acquisition rate of 21 images/s and averaging of the perfusion data from 42 consecutive images, which resulted in a total measurement time of 2 s for each image. The image size was set to correspond to the size of the flap, and the spatial resolution of the perfusion image is 0.2 mm/pixel at a measurement distance of 20–25 cm. Baseline perfusion of the flap was measured during anesthesia, before surgery. Immediately ( $t=0$ ), as well as 30 minutes ( $t=30$  min) after the flap was raised and reinserted, perfusion was measured with the same settings. After 72 h ( $t=72$  h), a final perfusion measurement was made with the same settings as those before. Clinical evaluation of the flaps was made at  $t=0$ ,  $t=30$  min, and  $t=72$  h. Digital photographs of the flaps were taken at the respective time points, and

demarcation lines were overlaid on each image to indicate where the surgeons believed that viability was questionable. In each flap, the distance from the pedicle base to the demarcation line was measured using ImageJ software.<sup>12</sup>

### Data analysis

Perfusion values are presented as PU (perfusion units), which is an arbitrary unit calculated from the speckle contrast analysis and is proportional to the concentration and the average velocity of the red blood cells in the measured tissue.<sup>3,4</sup> All perfusion images were analyzed using the system's analysis software (PSIWin, Perimed, Järfälla, Sweden). Ten regions of interest (ROIs), located 2 cm apart, were chosen from the LSCI images along the central axis of the flap from the proximal to the distal border.

All data are given as text and tables and presented as absolute values or mean (SD). Two-way analyses of variance for repeated measures with Tukey's multiple comparisons test were performed to test whether changes from baseline and differences between  $t=0$  and  $t=30$  min were significant. Statistical calculations were performed using GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, CA, USA). With all analyses, probabilities of less than 0.05 were accepted as significant.

### Results

Room temperature was stable throughout the protocol. No major changes in blood pressure or heart frequency were registered.

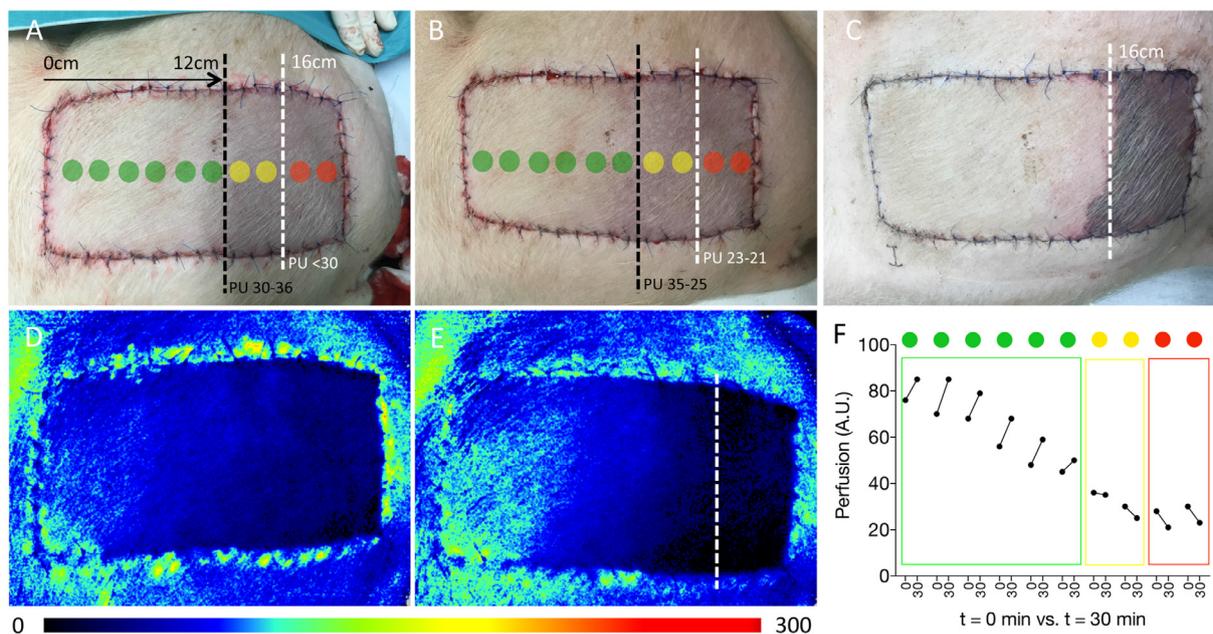
### Clinical evaluation

After the flaps were raised ( $t=0$  min), a darker area interpreted as an area with compromised circulation could be seen on all flaps at a mean (SD) distance of 10.7 (1.9) cm from the proximal border of the flaps, and beyond. At  $t=30$  min, the distance to the area with stasis was 10.1 (0.8) cm (Figure 2A and B).

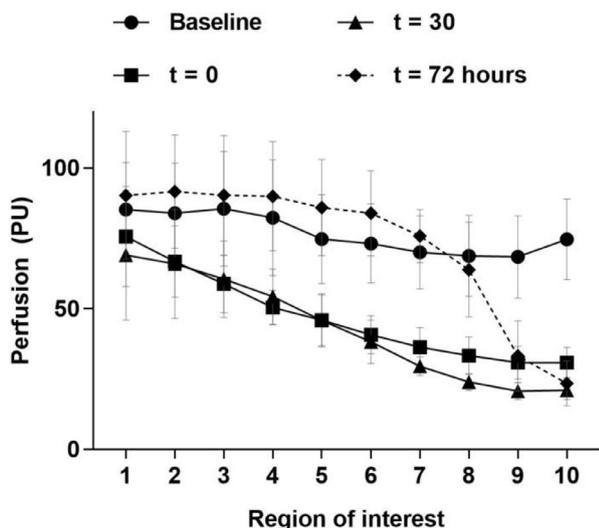
After 72 h, a clear demarcation line was seen at a distance of 15.8 (0.4) cm from the proximal border of the flap (Figure 2C). The area distal to this line had clinical signs typical for ischemia with a dark color without any notable capillary refill or arterial bleeding when punctured with a biopsy punch.

### Changes in flap perfusion

The mean perfusion of the flaps at baseline,  $t=0$ , and  $t=30$  min for 10 different ROIs is shown in Figure 3. At baseline, the highest perfusion in the flap was  $86 \pm 17$  PU (ROI 2) and the lowest perfusion was  $68 \pm 15$  PU (ROI 9). The perfusion decreased slightly from the proximal to the distal regions of the flap (at most a 20% decrease in ROI 9 compared to that in ROI 1,  $p < 0.001$ ). At  $t=0$ , a general decrease in perfusion was observed in all ROIs compared to that at baseline (ranging from  $75.6 \pm 17.8$  PU in ROI 1– $30.8 \pm 5.4$  PU in ROI 10). The decrease in perfusion from the proximal to



**Figure 2** Example of changes in visual appearance (A-C) and perfusion (D-F) in a CGAP flap, directly (A, D) and 30 min (B, E) after raising the flap. The dashed black line represents the proximal border of the area with compromised circulation as predicted by clinical assessment. The dashed white line (C) represents the proximal border of the manifested ischemic area at  $t = 72$  h. The colored dots indicate the regions of interest (ROI) wherein perfusion was measured. ROIs are numbered as 1-10, starting from the left to right (proximal to distal). (F) shows the change in perfusion from  $t = 0$  to  $t = 30$  min in different ROIs (green = viable, yellow intermediate, and red ischemic) in the same flap as that shown in A-E.



**Figure 3** The mean (SD) perfusion in ten different regions of interest (ROI) of the flaps (proximal to distal) at three different time points; baseline (before surgery), directly after raising the flap ( $t = 0$  min), and 30 min after raising the flap ( $t = 30$  min). Changes in perfusion between baseline and either  $t = 0$  or  $t = 30$  min were significant in all ROIs ( $p < 0.001$ ). Between  $t = 0$  and  $t = 30$  min, a significant decrease in perfusion could only be seen in the distal ROI (ROI 8-10,  $p = 0.03$ ).

the distal side of the flap became more pronounced with time, with ROI 10 having a 59% and 70% decrease in perfusion compared to ROI 1 at  $t = 0$  and  $t = 30$  min, respectively ( $p < 0.001$ ).

Changes in perfusion between baseline and either  $t = 0$  or  $t = 30$  min were significant in all ROIs ( $p < 0.001$ ). Between  $t = 0$  and  $t = 30$  min, a significant decrease in perfusion could only be seen in the distal parts of the flap (ROI 8-10,  $p = 0.03$ ). In the proximal and medial ROIs, perfusion either increased or was stable during the first 30 min after raising the flap (Figure 2F).

At  $t = 30$  min, no ROI distal to the demarcation line at  $t = 72$  h had a perfusion above 25 PU (Table 1).

At  $t = 72$  h, perfusion in the area proximal to the demarcation line (ROI 1-8) had recovered to values that did not show significant difference with baseline values. In ROI 9, perfusion increased to a value that did not show significant difference with  $t = 0$  values, and in ROI 10, the perfusion remained on a value that did not show significant difference with  $t = 30$  min values.

## Discussion

Partial flap necrosis is a well-described problem in reconstructive flap surgery. In a meta-analysis conducted in 2016, Xiong et al reported 6% partial necrosis in flaps for the reconstruction of defects in lower extremities.<sup>13</sup> In 2013, Lie et al reviewed 98 articles that reported on DIEP flaps that experienced partial necrosis. The reported rate of partial flap necrosis in these articles ranged from 0% to 11%.<sup>10</sup> The authors concluded that because there is no consensus around the classification or definition of partial necrosis, it is difficult to deduce how frequent the problem is. Still, the condition undoubtedly causes great discomfort for the patient, and any means to reduce the complication rates

**Table 1** Perfusion (PU) in different ROIs in the six flaps at  $t = 30$  min.

ROI	Flap 1	Flap 2	Flap 3	Flap 4	Flap 5	Flap 6	Mean(SD)
1	85.2	55.0	57.4	104.6	40.9	71.2	69.0(23.0)
2	84.9	60.5	50.5	91.4	41.7	66.4	65.9(19.3)
3	78.9	57.8	46.5	68.7	44.2	67.1	60.5(13.5)
4	68.5	55.2	43.6	59.4	42.5	56.7	54.3(9.9)
5	59.3	49.3	34.0	48.7	37.6	46.2	45.8(9.0)
6	50.4	45.2	32.7	32.6	32.5	36.0	38.2(7.7)
7	34.5	30.3	31.8	26.4	26.5	27.4	29.5(3.3)
8	25.3	22.3*	26.4	26.8	23.7	19.2	24.0(2.9)
9	21.4*	21.2*	20.6*	24.6*	21.2*	15.5*	20.7(2.9)
10	23.2*	23.0*	20.9*	24.1*	19.4*	15.4*	21.0(3.3)

\* represents ROI from areas distal to the demarcation line at  $t = 72$  h. Note that there is no ROI with perfusion above 25 PU at  $t = 30$  min in areas distal to the demarcation line at  $t = 72$  h.

further would be beneficial, in terms of both shortened length of stay in hospital and reduced healthcare costs. A tool that could be used as a reliable indicator of flap areas with compromised circulation would be a valuable contribution in the ongoing efforts to improve surgical outcome.

One method for perioperative imaging of tissue viability that has gained ground in recent years is fluorescence angiography (FA), a vascular imaging method that combines indocyanine green (ICG) with laser angiography to visually assess superficial blood flow. The dye ICG has an excellent safety profile but has to be administered intravenously for each assessment and the cost per case is relatively high.<sup>14,15</sup>

In our study, we used the LSCI as a tool to predict partial flap necrosis in a porcine fasciocutaneous flap model. LSCI has some clear advantages compared to FA for the perioperative evaluation of tissue viability. It is a completely noninvasive method, with no need for dye injection before assessment, which means that the cost per case is negligible after the initial investment. This also implies that the measurements can be made repeatedly during the operation, and follow-up measurements can be made during the postoperative phase, in case the surgeons suspect that there may be problems with vessel patency or clot formation.

Two authors of this study have many years of experience in microsurgery, burn surgery, and replantation surgery and regularly evaluate tissue viability in a clinical setting. Nevertheless, based on visual appearance and capillary refill during the first 30 min after the flap was raised, these observers typically overestimated the areas that would present clinical signs of ischemia three days after the surgery.

It is well known that microcirculation at skin regions further away from the main supplying vessels, in this case the CGAP, displays lower perfusion values.<sup>16</sup> In the pig buttock, one may expect there to be multiple supplying vessels that supply the skin segment before raising the flap, but in this case, it seems likely that regions closer to CGAP will be better perfused, even before the flap is raised. Indeed, this finding is in agreement with our findings, i.e., even before raising the flap, we observed a decrease in perfusion from the proximal to distal areas of the flap.

During the first 30 min after the flap was raised, perfusion in the distal parts of the flaps (ROI 8-10) decreased significantly, while perfusion in the more proximal ROI did not decrease or even, in some flaps, increased during the same

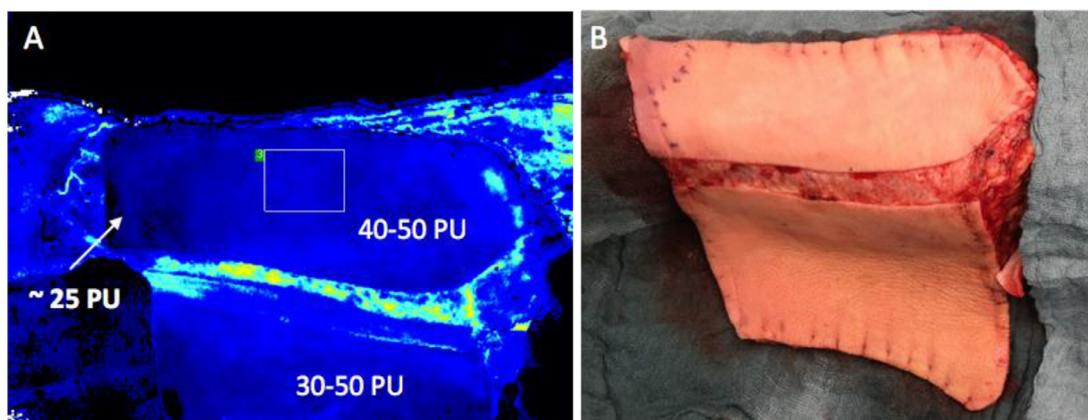
time period. Importantly, the areas in which perfusion decreased during the first 30 min showed clear clinical signs of ischemia three days after the surgery.

Directly after the flaps were raised, all ROIs distal to the final demarcation line had a perfusion below 40 PU. The variation of the perfusion in the distal parts of the flaps at this time point was large, and in five of the flaps, areas with initial perfusion between 30 and 40 PU recovered and were viable at the end of the study.

After 30 min, the perfusion in all ROIs distal to the final demarcation line was below 25 PU. At this time point, there was also a smaller variation in perfusion in the distal ROI between the different flaps. This indicates that LSCI assessment of flap viability should be done for at least 30 min after the flap is raised and that perfusion values below 25 PU after 30 min are an indicator of compromised circulation and thus could be considered a threshold value, at least in our material. However, perfusion in flaps 5 and 6 was below 25 PU 30 min after the flaps were raised in areas proximal to the final demarcation line. This means that if a threshold value of 25 PU had been used as the only indicator of threatening ischemia in a clinical setting, there would have been a risk of unnecessary wide excisions. If the threshold value had been set to 22 PU, there would not have been any unnecessary excisions in our material. Instead, later ischemic areas would have been missed in flaps 2 and 6. Thus, although a decrease in perfusion during the first 30 min after the flap was raised was found to be a strong predictor for tissue morbidity, other factors such as initial perfusion value and clinical appearance should likely be used in conjunction with the LSCI evaluation to guide surgical decisions.

Figure 4 shows a perfusion image, as acquired using an LSCI imager, of a radial forearm flap (for phalloplasty) as a clinical example. Twenty minutes after the flap was raised, an area with low perfusion (approximately 25 PU) was identified at the edge of the flap. This area was considered nonviable on the third postoperative day and was subsequently resected. It is interesting to note that the perfusion values in the nonviable areas of the flap are in the same range as those in nonviable areas in the flap model presented in the current study.

In our earlier study, we could not see any significant difference in perfusion during the first 30 min after the flaps were raised in neither proximal nor distal ROI.<sup>7</sup> The distal ROIs in the previous study, wherein shorter flaps were used,



**Figure 4** A clinical example of a radial forearm flap for phalloplasty 20 min after the flap was raised. The arrow in (A) indicates an area with low perfusion ( $\sim 25$  PU). In the corresponding color image (B), the area with clinical signs of reduced perfusion is marked by the surgeon as a dotted line. The same area was considered nonviable on the third postoperative day and subsequently resected (image courtesy of Professor D'Arpa, University Hospital Gent, Belgium).

correlate to more proximal ROI in the current study (approximately ROI 1-7). Interestingly, in the current study, the perfusion in the proximal ROI (ROI 1-8) had normalized or even increased after three days, compared to preoperative measurements. This strengthens our conclusion that a decrease in perfusion during the first 30 min after the flap is raised indicates a compromised circulation and a high risk of developing necrosis.

This study has a number of limitations. As with FA, the surface of the flap has to be exposed to be able to be assessed by LSCI. This means that the technique cannot be used on buried flaps after insertion. Care should also be taken not to extrapolate these results to other flap types such as myocutaneous, osteocutaneous, or muscle flaps because these flaps may have different perfusion characteristics.

We acknowledge that LSCI assessment of skin perfusion might differ between humans and pigs. There are currently a few studies in which LSCI has been used for the assessment of microcirculation in pig skin.<sup>5,6,9,17,18</sup> Even if results from these studies might not be translated directly to clinical conditions, we assume that because porcine skin is fairly similar to the human skin, LSCI can measure similar perfusion levels in both species.<sup>19,20</sup>

We used a model based on a pedicled flap and not a free flap because the aim of this study was to evaluate the possibility of using LSCI to predict ischemic areas without actually being interested in the cause of the ischemia. A free flap model would have made the study set up considerably more complicated without any major contribution to the final results.

In this study, we looked at absolute threshold perfusion values as a measure of risk for later ischemia in different regions of the flap, as this is the most straightforward method to use LSCI in clinical practice. However, it is possible that the decrease in perfusion from baseline is more predictive of risk, and future work should be conducted to investigate the use of regional perfusion changes as a risk measure. This would, however, require a baseline measurement for each ROI, and the perfusion in the specific regions would have to be related to their respective baseline perfusion.

We choose not to use an artificial barrier between the flap and the underlying muscle, although this might have affected the viability of the flap at the final assessment because of neovascularization. However, at least one study has shown that the minimal critical period for flap survival for arterial occlusion is 6 days.<sup>21</sup> Therefore, we believe that a barrier would not have affected the tissue viability and thereby the visual appearance of the flap three days after surgery.

Another limitation is the small number of subjects. Six flaps are too few to be able to draw any certain conclusion on which threshold value should be chosen to keep both sensitivity and specificity of the method high. On the other hand, it is doubtful that any threshold value obtained from porcine studies could be translated directly to human conditions. Therefore, a large-scale animal study could not be ethically motivated because even with a large number of animals, the obtained threshold value might not be representative for patients. Instead, future studies should be directed toward pre-, peri-, and postoperative assessment of free flaps by LSCI in a larger cohort of patients.

In conclusion, we have found that a decrease in perfusion during the first 30 min after raising the flap and a perfusion below 25 PU after 30 min was a predictor for tissue morbidity 72 h after surgery in a porcine flap model. These findings suggest that LSCI is a promising technique for the perioperative monitoring of tissue and for identifying areas at risk of necrosis, and future studies will hopefully show the clinical usefulness of LSCI in reconstructive flap surgery.<sup>22</sup>

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## Conflicts of interest

None to declare.

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## Ethical approval

Ethical approval was given by the Regional Ethical Committee (Jordbruksverket), registration number 90-15.

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