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Infrared thermography use for postoperative monitoring of rat skin flaps compering effects of vasoactive drugs

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Abstract - The technologies used in flap design and monitoring have progressed significantly in recent years. Extremely variable vascular anatomy, associated with the complexity of modern flaps, require dynamic, real-time intraoperative information about flap perfusion and hemodynamic changes. Unfortunately, most surgeons still evaluate flap perfusion and viability based solely on clinical experience. Incorrect preoperative planning and intraoperative or postoperative assessment of perfusion leads to major complications for the patient. Nowadays, surgeons can use several systems capable of evaluating the flaps. The evaluation of the viability of a flap, especially in the case of clinical studies in which various substances are applied, that may or may not improve microcirculation is of certain importance. The purpose of this experimental study was to show that infrared thermography can be used as a non-invasive method to assess the flap survival and perfusion and to compare the use of various vasoactive drugs in improving the survival of skin flaps, on rats.

Index Terms - medical thermography, flap surgery, cutaneous flap survival, image analysis, infrared thermography

I. INTRODUCTION

In an era of treating chronic or acute wounds with rapidly evolving stem cells and organ printing devices, and the dressings technologies provide doctors with a huge variety of choices, there is still a big interest in the research regarding skin microcirculation, ischemia-reperfusion phenomenon or endothelial response mechanisms. Skin flaps cannot be totally replaced, for the moment, when needed to cover vital structures such as arteries, nerves, bones or joints [1].

Although preventive medicine tries to lower the incidence of diseases [2], a lot of patients get to need reconstructive surgery. Flap necrosis is a major complication in all types of flap surgery [3], affecting the quality of life through medical, psychological and social issues of the patients and their families, during devastating pathologies such as cancer and the indicated surgical treatment after it [4–6]. Many studies showed the influence of different drugs on the survival of the cutaneous flaps [7–10]. The results of these studies suggest that administration of certain drugs may have significant effects in the reduction of ischemic necrosis in the distal parts of skin flaps.

Over time, there has been a thorough study of a plethora of substances that can influence the vascularity of the modified McFarlane skin flap in rats [10]. This type of flap provides a very well described arterial anatomy, being a so called rando-axial flap, with a consistent vascular pedicle when caudally based and a predictable area of skin necrosis cranially. Usually, the flap is designed to measure 9 x 3 cm, raised at the level of the deep fascia [1,11,12].

The use of thermography for flaps can make a difference between arterial or venous flow problems. Changes in tissue perfusion often result in a change in tissue temperature. This is the principle of thermography for microvascular imaging: It uses a video camera with infrared thermal imaging that determines the tissue temperature in the human body. The relationship between skin temperature and microvascular dermal perfusion depends on location. Thermography provides a real-time imaging solution for vessels around 1 mm or smaller. Infrared thermography is a non-invasive technique increasingly used as a monitoring and diagnostic technique today. Monitoring flaps using infrared thermography involves the use of an infrared camera that generates a color map based on the heat emitted by the tissues [13–15].

So far the applicability of various thermographic techniques has been evaluated in diagnosis of radiation and burn injury, early diagnosis of cancer or evaluation of cutaneous flap survival [1,13–16]. Temperature distribution of the skin can be closely related to the arterial blood supply. Superficial temperature as an indicator of cutaneous blood circulation can be visualized easily by thermography (see Figure 1).

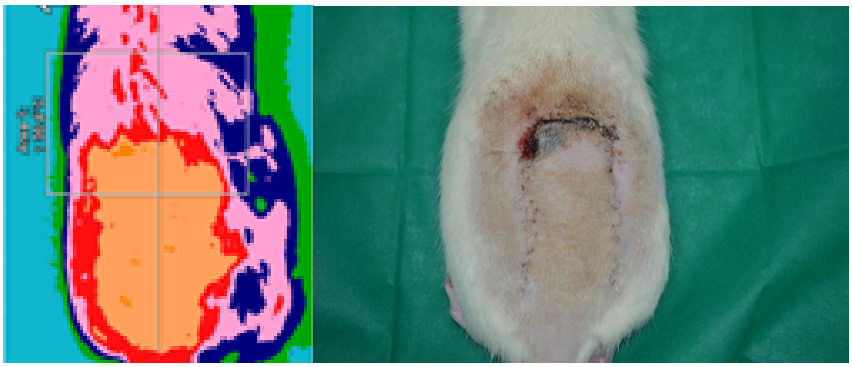


Figure 1. Image with skin flap on rat subject IR versus photography.

Infrared thermography is non-invasive diagnostic method which offers two-dimensional representation of the surface temperature of the skin and it is a sensitive method to detect altered temperature distribution expressed through the histograms, evaluating the vitality of the skin flap by computerized image analysis. Enough blood supply of the flap is a significant factor for proper wound healing and cutaneous flap survival. Thermography of the skin is an easy method for estimating the blood circulation of the skin flap, but it is limited to hair-free or shaved skin areas [13,17–19].

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 use intraoperatively for identifying areas of risk. Such technique would be of great benefit to the surgeon because it requires considerable clinical experience to identify area of inadequate perfusion by assessment of skin color capillary refill and turgor. ICG-FA (indocyanine green-fluorescein angiography) and DIRT (dynamic infrared thermography) are reported to be capable of monitoring the dynamics of flap perfusion [17–19].

II. MATERIALS AND METHODS

Subjects

The experimental study was performed at the Advanced Research and Development Centre for Experimental Medicine (CEMEX) of the Grigore T. Popa University for Medicine and Pharmacy from Iasi. We used 40 male adults Wistar rats, which were hosted in special cages, with constant temperature and a day/night cycle from 7 a.m. to 7 p.m. The animals received water and food 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.

All surgical procedures were performed under standard aseptic conditions. The animals were divided in six groups of six animals each: control group, PRP (platelet-rich plasma) group, L-NAME (N(ω)-nitro-L-Arginine Methyl Ester) group, PRP and L-NAME group and 2 groups with CAP (Cold Atmospheric Plasma activated solution) treatment. Before any surgical procedure, the animals were shaved with a hair clipper and the design of the caudally based dorsal flap (8 x 3 cm) was drawn after the induction of the anesthesia with Isoflurane.

Technical details of the thermal camera

In our study, infrared thermograms were collected using an OPTRIS PI160 IR medical camera with a measurable temperature range of -20 ° C to 900 ° C, Spectrum 7.5-13 μm, Recording Rate of the 120Hz image and an optical resolution: 160 x 120 pixels. This device allows the transfer of images using an O6 lens with the 35.5 focus. This compact model is suitable for postoperative use and the high thermal resolution enables the detection of minimal temperature differences [18].

Object parameters	
Reflected temperature [°C]	20.5
Emissivity	0.65
Humidity	50%
Distance [m]	0.54
Atmosphere temperature	20.5
Acquisition parameters	
Acquisition time [min]	1
Frame rate [Hz]	120
Window size [pixel x pixel]	160x120

Table 1. Parameters of the thermographic camera OPTRIS PI160.

In the working protocol, the thermographic camera was positioned at a fixed distance of 54 cm from the subject, and the thermographic images were individually recorded on the 5th day after the flap was performed (see Figure 2). After each recording, the thermal camera was recalibrated to eliminate any errors. In real-time, images were sent to a laptop and further processing included filtering and color conversion.

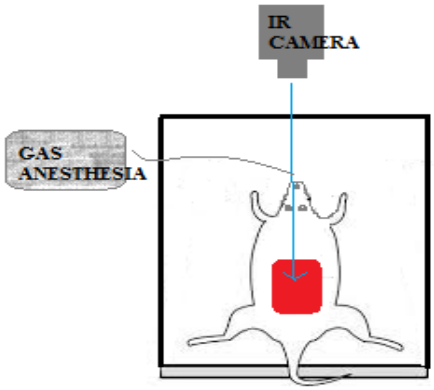


Figure 2. The in situm position of the regarding protocol.

Surgical procedure

After the induction of the anesthesia, the flaps were raised at the level of the deep fascia, including skin, subcutaneous tissue, panniculus carnosus, and superficial fascia. The flaps were sutured back to their places after the interposition of a sterile synthetic material, to prevent neovascularization from the subjacent tissue. The control group (BO1-BO6) had no substance administrated.

The viability of the flaps was assessed in the 5th postoperative day, by clinical inspection (color, suture state, suppuration, turgor, temperature), photographic documentation with Nikon D3200 device and by thermographic imaging. We compared the flaps by the percentage of necrosis related to the normal area of the flaps, using a Fiji software.

Substances administration

The PRP solution was obtained from 4 donor rats, by cardiac puncture. The donor rats were sacrificed. From each rat a volume of 6 to 8 mL of blood was collected. The blood was transferred in special heparinized tubes. The tubes were then centrifugated at a speed of 3100 rotations/min, for 10 minutes. After this, the superior layer was separated from the sediment and the content was transferred in small tubes which were again centrifugated for 15 minutes, at a rate of 3600 rotations/min. The PRP solution was then transferred in 1 mL recipients. In the PRP group (NO21-NO26), 1 mL of PRP solution was injected subcutaneously in the flap at 5 cm from the pedicles, before the suture. In the third group (NO14-NO19), a 30 mg/kg dose of L-NAME was administrated in each rat, 30 minutes before the elevation of the flap. The same procedure was applied to the fourth group (NO27-NO32) but, after the flap elevation, 1 mL of PRP solution was injected into the flap, in the same manner as for the PRP group.

The CAP solution was obtained through an air dielectric barrier discharge (DBD) plasma source which was designed to produce the pulsed plasma directly into 24 well plates [20]. The electrodes were connected using high voltage cables to a power supply that excited the plasma. During this procedure, optical and electrical parameters of the discharge are checked using current probes and fixed grating monochromator. The two groups of animals that had CAP administrated followed the same protocol as for the PRP administration, 1 mL being injected 5 times at variable distances (0.5 cm) from each other, keeping 5 cm from the pedicles.

Analysis of the IR data

The flap's surface temperature and the values of the temperatures measured by the IR camera depend on multiple factors. In particular, flap size and localization as well as body temperature, fever, respiratory flow and vascular circulation depending on the applied anesthesia, angle between the tissue surface and the camera lens, room temperature and air humidity. To eliminate these confounding factors the temperature difference between flap surface and regular surrounding tissue was measured instead of just measuring the absolute temperature of the flap's tissue surface.

III. RESULTS AND DISCUSSIONS

Improved image enhancement techniques have been used to help visualize and analyze images easily. It has been chosen to use the imaging techniques in the thermography to process the image so that the resulting image contains more useful information than the original image. In our case, the improvement technique was performed as a post-processing step. Smooth Spatial Filters (LPF) was also used to reduce noise. With their help, we succeeded in selecting low-frequency components and reduced or in some cases eliminating high frequencies [18].

The first batch (control group), analyzed in July 2018, covered subjects BO1-BO3, BO5, BO15 and BO16. After the thermographic analysis it was noticeable that in this case the average of maximum temperature was 35.12 degrees, the temperature of the flap did not increase, it had maximum differentiation points and there were no visible hot spots. According to the thermographic images and the performed interpretations we can conclude that there was no problem with the arterial flow, which was also confirmed by the low rate of necrosis. Diffusion of the flap temperature was uniform and there were no visible signs of ischemia, both at the center and at the edges of the flap (see Figure 3).

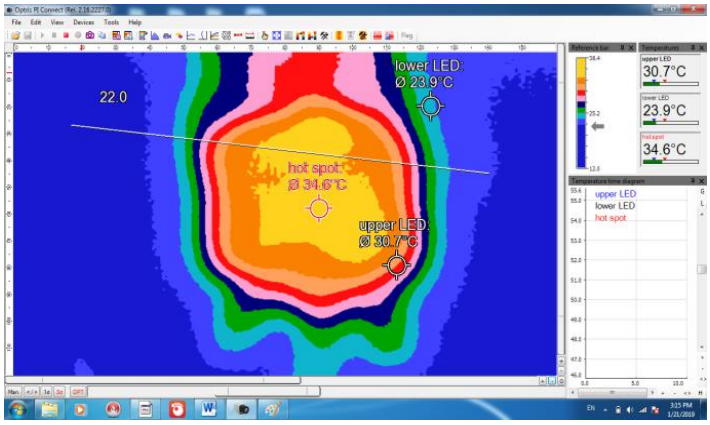
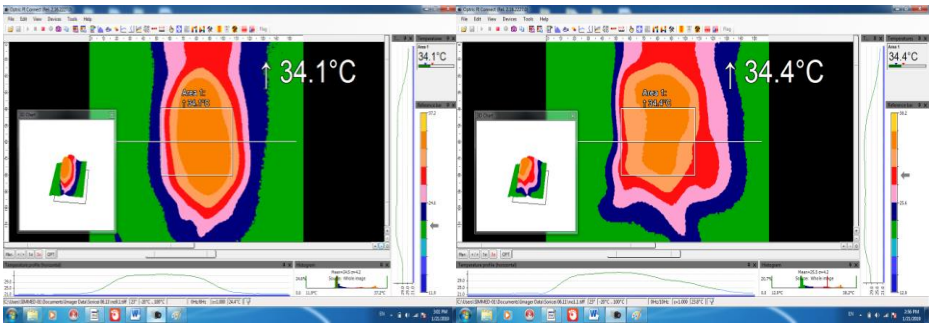


Figure 3. Thermografic image of subject NO4 from control group.

The second batch (CAP) included subjects NO7-NO13. As a result of the thermographic analysis, the mean maximum temperature of 34.1 degrees was noticeable, the temperature of the flap did not increase and there were no visible hot spots (except for NO8). Unlike the previous group, an infusion problem was observed, especially on NO10, NO12 and NO13, where we had an average maximum temperature of 33.9°C (necrosis and vascular abnormalities). In other cases where the infusion was normal, a slight vasodilation at the periphery of the flap was observed with no arterial flow problems (see Figure 4).



a) b)

Figure 4. Termographic images with subjects from CAP batch: a) subject NO7 with the mean max temperature 34.1°C with no visible hot spots; b) subject NO12 with mean max temperature 34.4°C with visible infusion problems.

The third batch in the analysis was the L-NAME (NO14-NO19) group studied in November 2018. For this group, a nitric oxide releasing vasodilator was administered. Maximum mean flap temperature was 33.6 degrees Celsius, much lower than the first batch in the analysis, but evenly distributed, with a deeper vasodilatation clearly visible thermographically, covering a larger area of the flap. The area of the flap was well perfused, with no visible ischemic signs at the entire group, except for NO17 (see Figure 5).

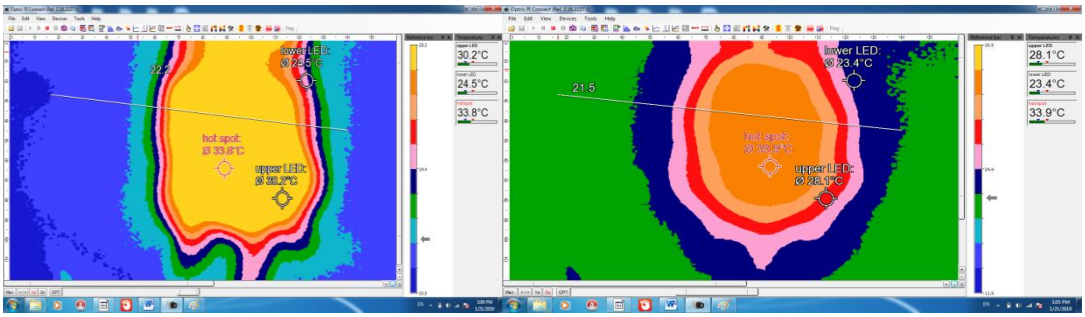


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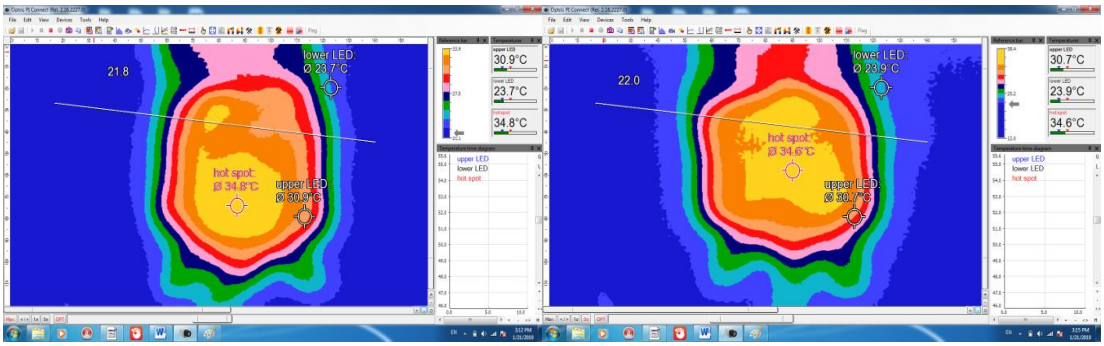
Figure 5. Termographic images with subjects from L-NAME batch: a) subject NO15 with the mean max temperature 33.6°C with a deeper vasodilatation; b) subject NO17 with mean max temperature 34.4°C with visible ischemic signs.

The fourth batch in the analysis was the PRP group (NO21-NO26). In this case a vasodilatory, angiogenic substance was administered. The average maximum flap temperature was 34.5 degrees Celsius. At 3 of the batch (NO 23, 24 and 26) it is noted that the flap temperature increased and there were visible hot spots, which means that there was a problem in the arterial flow. Hence the high necrosis rate. Due to the administered substance, there is a clearly visible thermodynamic vasodilation that covers a large area of the flap, but with signs of hypoperfusion (see Figure 6).



a) b)

Figure 6. Termographic images with subjects from PRP batch: a) subject NO23 and NO26 with mean max temperature 34.7°C with visible hot spots.

Batch number five analyzed was the PRP+L-NAME group (NO27-NO32). In this case, the maximum mean flap temperature was 35.4 degrees. The revascularization area was large (it contained the entire area of the flap), did not show signs of ischemia, the flap temperature increased in certain areas and there was no problem with arterial flow (see Figure 7).

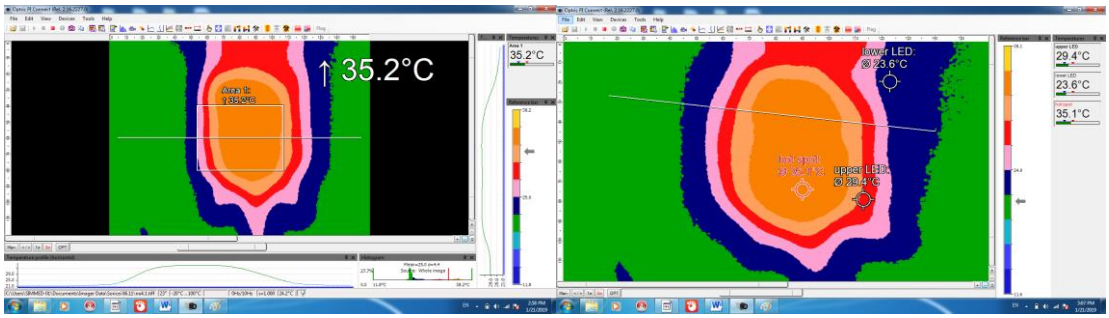


Figure 7. Termographic images with subjects from PRP+L-NAME batch: subject NO27 and NO30.

The sixth batch in the analysis was the CAP group; a reverification batch comprised the NO19-NO24 subjects in December 2018. This group, without ischemia, from a thermographic point of view observed an average maximum temperature at the flap level 35.95 degrees with a large revascularization area that included the entire flap area. The cutaneous necrosis rate was 16%, correlated with the fact that it did not show any signs of ischemia thermographically. (see Figure 8)

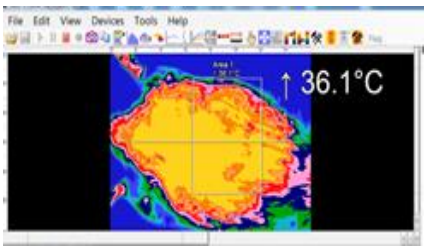


Figure 8. Termographic images with subjects NO20 from L-NAME batch with mean max temperature 34.7°C with a large revascularization area.

From a thermographic point of view, the best perfused batch without ischemic problems at the flap was the L-NAME group. With an average maximum temperature of 33.6 ° C, there were no high temperature variations at the flap level, with no visible spots boiled with a distribution uniform. It also correlated with the rate of low necrosis.

Batch	The duck of necrosis	Substance used	Temperature Max average	MEAN	Standard Deviation	Coefficient of variation
1. Control group - July	49.64%	No substance	35.1 ºC	26.12	4.54	0.173 %
2. CAP	14.47%	Cold atmospheric plasma	34.1 ºC	24.60	4.10	0.166 %
3. L-NAME	18.56%	L-NAME	33.6 ºC	24.28	4.01	0.163 %
4. PRP	23.85%	Platelet rich Plasma	34.5 ºC	24.98	4.16	0.166 %
5. PRP+L-NAME	17.76%	Platelet rich plasma and L-NAME	35.4 ºC	25.16	4.28	0.170 %
6. CAP reverification	16%	Cold atmospheric plasma	35.9 ºC	29.65	3.73	0.125 %

In addition, it had the lowest value of the coefficient of variation and of standard deviation. This translates into the fact that the thermographic layer was the most uniform batch. In this case, we had better vasodilation than the other lots, but also a good venous circulation. The group with the highest temperature variation was group 5 (PRP+L-NAME), with an average maximum temperature of 35.4 ºC, the highest value in all analyzed groups. This means hot, visible, but not very varied points, which correlated with the necrosis rate of only 18%.

From the point of view of the vasodilatory substances administered, according to the thermographic data obtained, the L-NAME lot had the best viability of the flap, also confirmed by the low rate of necrosis. From a statistical point of view, the Variation Coefficient for each lot was less than 5%, which means that the groups were homogeneous, with no temperature fluctuations of the flaps in each lot, with some exceptions in which necrosis occurred.

IV. CONCLUSION

Although advances in microvascular techniques and conditioning of vascular risk factors have contributed to the improvement of flap surgery in the recent decades, a pillar of permanent free-flap viability is its close monitoring of well-trained personnel. To our knowledge, this study is the first one to investigate IR thermography for postoperative monitoring of rat skin flaps comparing the use of vasoactive drugs. Therefore, IR thermography offers a real-time imaging solution for the control of microvasculature in surgery. The static thermography is a promising objective method for postoperative monitoring of free-flap reconstructions and for detecting perfusion issues before macroscopic changes in the tissue surface are obvio

V. ACKNOWLEDGMENT

All the authors had the same contribution to this research.

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VI. DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

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