

INVESTIGATION OF WOUND HEALING BY INFRARED THERMOGRAPHY IN A FULL-THICKNESS SKIN WOUND MODEL IN RATS

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ABSTRACT. Non-contact methods such as infrared thermography (IRT) stand out with their important advantages such as reducing the risk of contamination, providing real-time results, quantitatively determining a relative finding such as pain, being economical, easy, non-invasive, and safe. The study was aimed to evaluate the differences of intra-wound, wound edge, and non-wound local temperatures in untreated and treated wounds of a full-thickness excisional skin wound model by IRT in rats. Twelve Wistar albino rats (200–300 g, 8 weeks old, male) were used in the study. A full-thickness excisional skin wound (2.25 cm²) was created in the dorsal interscapular region of animals under general anesthesia. Animals were randomly divided into two groups (n=6), Group 1 (control) and Group 2 (dexpanthenol treatment), and the wound surface was treated topically once a day. Images were taken from the wound area of all animals with an infrared thermography camera 0, 1, 7, and 14 days after the wound creation. Minimum and maximum temperatures were measured with the Trotec IC-IR Report software for IRT in three different areas as intra-wound, wound margin, and non-wound. Local temperature changes were examined and analyzed statistically. In addition, the time/local temperature correlation was analyzed for the wound treatment. In the results, it was determined that the local temperature increase was less as the distance from the wound center and the wound treatment time progressed ($p<0.05$). Compared to Group 1, it was determined that there was less local temperature increase and less inflammation in Group 2 as the treatment progressed ($p<0.05$). In conclusion, this study determined the reference values for the use of IRT in wound healing studies in the full-thickness excisional skin wound model in rats. Further research is needed to standardize the methodology for using IRT in other wound models, such as infected wounds and diabetic wounds.

Keywords: Heat, local, measurement, temperature, wound model.

INTRODUCTION

Infrared thermography (IRT), used in the healthcare field, is an auxiliary examination method that displays and records temperature patterns on the body surface with an image called a thermogram. The principle of operation is based on the imaging of the heat reflection. It helps to identify changes in the examined area without ionizing radiation or other harmful effects [8]. From past to present, the use of IRT has been widespread in many different areas including health, military, industrial, construction and heat. The fact that the new thermal cameras are more convenient and useful in terms of both price and size increases their use even more. It has significant advantages by providing real-time images. It is expected that the areas where it is used will continue to increase over time.

Infrared thermal imaging has been used in healthcare since 1959 [27]. IRT measurements are performed with infrared cameras, with drug-free and non-invasive procedures [9]. Surface temperatures in the body are determined with the thermographic images that provide detection up to 6 mm deep under the skin and subcutaneously [20]. The main thing in IRT is the measurement of temperature changes rather than the measurement of temperature intensity, and it is often used for this purpose in health research [8, 31]. Local determination of small temperature differences such as 0.1 °C with IRT is useful in the early diagnosis of various diseases. It is especially important to have a diagnostic method that shows the location and intensity of pain, which is a relative finding [8, 16].

One of the most important advantages of IRT in both human and veterinary medicine is that it is a non-contact method that can be done from a distance. Thus, it may be possible to prevent infectious diseases such as COVID-19 that can be transmitted from other people, as well as various harms from animals. Another advantage of thermography is that it helps to identify physiological changes caused by asymptomatic pathologies in tissues and organs with intact anatomical integrity, such as local circulatory disorders. Because imaging methods such as radiographs, ultrasonography, magnetic resonance, and computed tomography focus on anatomical examination in tissues and organs.

Non-contact methods such as infrared thermography stand out with their important advantages such as reducing the risk of contamination, providing fast and real-time results, quantitatively determining a relative finding such as pain with temperature, being economical, non-invasive, easy, and safe. This study was aimed to evaluate the differences of intra-wound, wound edge, and non-wound areas local temperatures by IRT in untreated and treated wounds of a full-thickness excisional skin wound model in rats.

MATERIALS AND METHODS

Twelve healthy adult male Wistar rats (200–300 g, eight weeks old) were purchased from Hatay Mustafa Kemal University Experimental Research and Application Center. The study approval was obtained from the Local Ethics Board of Animal Experiments of Hatay Mustafa Kemal University (Decision no: 2021/02-04). Experiments were performed in accordance with Turkish Code of the Welfare and Protection of Animals Used for Experimental and Other Scientific Purposes and also Directive 2010/63/EU on the protection of animals used for scientific purposes. One week prior to the study, the animals were taken to the study place to undergo routine health checks and time for adaptation was provided. All rats were maintained individually in polycarbonate cages with water and food provided ad libitum, on a 12:12-h light-dark cycle under constant temperature (23±2 °C), humidity (55%) in a climatically controlled room.

Following general anesthesia induction (xylazine HCl 10 mg/kg, i.m., Alfazyne® %2 Egevet, İzmir, Türkiye and ketamine HCl 60 mg/kg Alfamine® %10 Egevet, Türkiye), surgical preparation was done with routine method and a 1.5x1.5 cm (2.25 cm²) sized square full-thickness excisional skin wound [24] was created on the dorsal neck of each animal. The animals were randomly divided into two experimental groups (n=6 each). Group 1 was untreated as control group. Group 2 was treated with dexpanthenol ointment (Dexpanthen® %5 Orion, İstanbul, Türkiye) as treatment group. The treatments were applied topically until it covered all wound area once a day. Wound areas of all animals were photographed individually by an infrared thermography camera at 0, 1, 7, and 14 days after wound creation. Trotec EC 060 V (Heinsberg, Germany) infrared thermography camera was used for IRT recordings. It was performed at an average

distance of 0.5 m and an angle of 70-90° as the most suitable distance and angle for the IRT device. In the analysis of IRT images, minimum and maximum temperatures were measured in three different areas, intra-wound, wound edge, and non-wound, using the Trotec IC-IR Report software. Sample images of the measurements are presented in Figure 1-4. In the statistical analysis, the mean and standard error of mean (SEM) values of the data of the groups were calculated. Groups were compared with the student-t test. Pearson correlation test was used to analyze the correlation between measurements made on different days and between regions. Statistical significance value was accepted as $p < 0.05$.

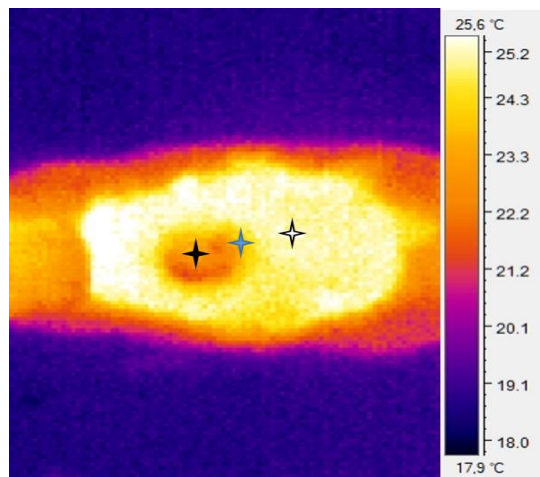


Fig. 1. Day 0 intra-wound (black star), wound edge (blue star) and non-wound (white star) areas IRT local temperature measurement thermogram from Group 1

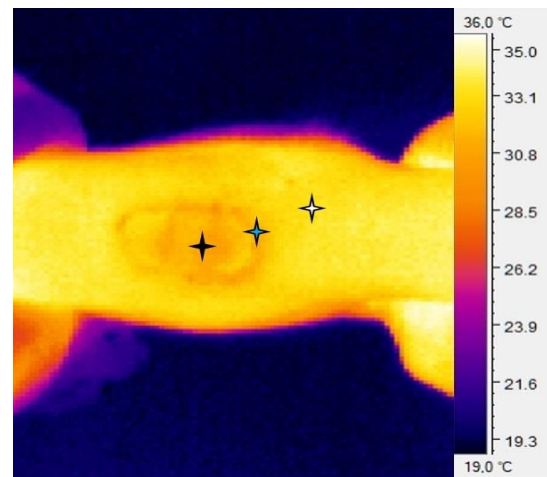


Fig. 2. Day 1 intra-wound (black star), wound edge (blue star) and non-wound (white star) areas IRT local temperature measurement thermogram from Group 1

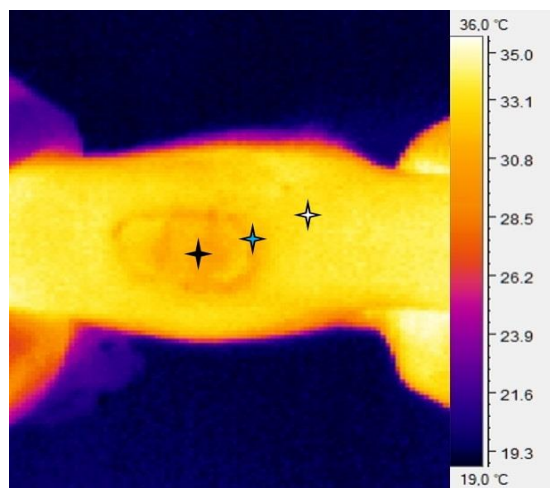


Fig. 3. Day 7 intra-wound (black star), wound edge (blue star) and non-wound (white star) areas IRT local temperature measurement thermogram from Group 1

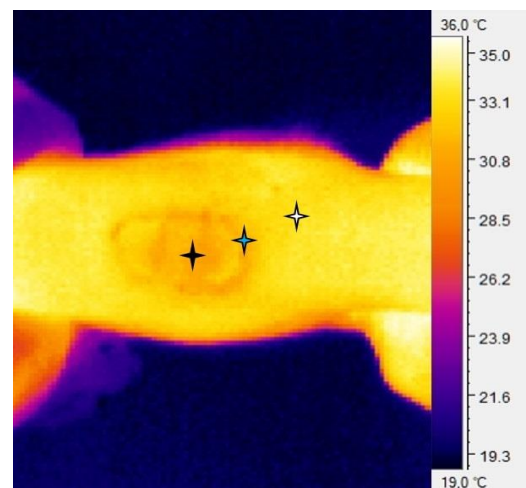


Fig. 4. Day 14 intra-wound (black star), wound edge (blue star) and non-wound (white star) areas IRT local temperature measurement thermogram from Group 1

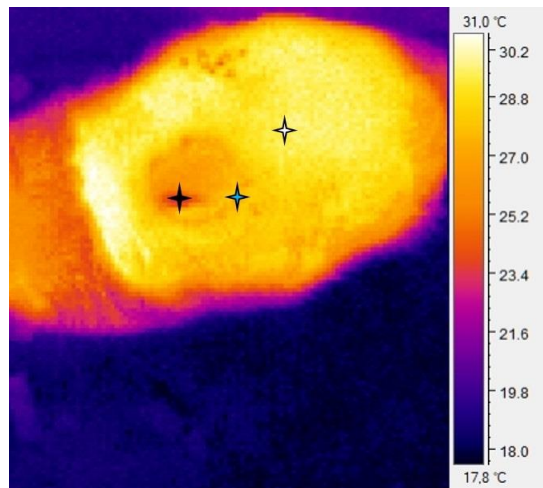


Fig. 5. Day 0 intra-wound (black star), wound edge (blue star) and non-wound (white star) areas IRT local temperature measurement thermogram from Group 2

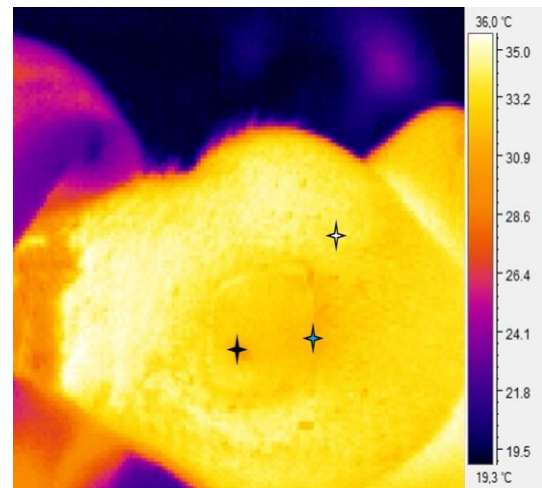


Fig. 6. Day 1 intra-wound (black star), wound edge (blue star) and non-wound (white star) areas IRT local temperature measurement thermogram from Group 2

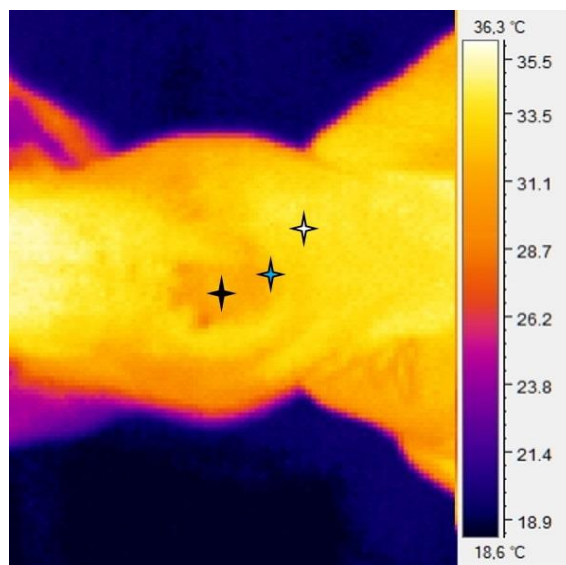


Fig. 7. Day 7 intra-wound (black star), wound edge (blue star) and non-wound (white star) areas IRT local temperature measurement thermogram from Group 2

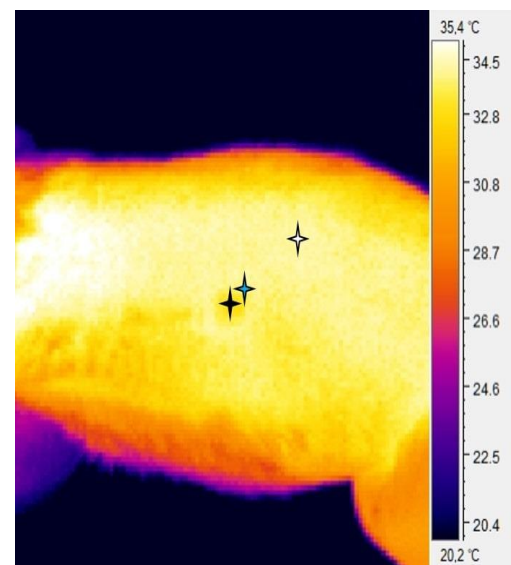


Fig. 8. Day 14 intra-wound (black star), wound edge (blue star) and non-wound (white star) areas IRT local temperature measurement thermogram from Group 2

RESULTS AND DISCUSSION

Parallel IRT measurement of the full thickness skin wound of the groups on the intra-wound, wound edge and non-wound areas revealed significant differences in minimum or maximum temperatures of days 1 and 14 after the wound creation ($p < 0.05$). Although there were limited clinical differences between the groups on days 0 and 7, no statistically significant difference could be detected. To show the precision of IRT measurements, the

means and the standard error of mean (SEM) from animals were presented (Table 1 and 2). Significant differences and positive correlations were found in in-group comparisons of local temperature values measured by IRT in the intra-wound, wound edge, and non-wound areas on the day of wound formation (day 0), 1st, 7th, and 14th days in experimental animals ($p < 0.05$). The correlation of measurements in days and regions is shown in Table 3.

Table 1. Summary of min-max values of intra-wound, wound edge, and non-wound areas at the days 0 and 1 after wound model.

		Day 0				Day 1			
		Group 1		Group 2		Group 1		Group 2	
		Min	Max	Min	Max	Min	Max	Min	Max
Intra-wound	Mean	24.48	27.07	24.88	27.18	31.85	34.10	31.62	34.53
	SEM	0.61	0.48	0.19	0.34	0.14	0.09	0.32	0.19
	P	0.29	0.43	0.29	0.43	0.28	0.04	0.28	0.04
Wound edge	Mean	26.15	27.12	26.53	27.20	32.60	33.72	33.52	34.25
	SEM	0.43	0.42	0.32	0.31	0.18	0.23	0.26	0.24
	P	0.26	0.44	0.26	0.44	0.01	0.08	0.01	0.08
Non-wound	Mean	27.27	28.45	27.16	28.72	33.38	34.88	33.62	35.22
	SEM	0.48	0.49	0.58	0.41	0.25	0.18	0.34	0.15
	P	0.45	0.36	0.45	0.36	0.31	0.11	0.31	0.11

Table 2. Summary of min-max values of intra-wound, wound edge, and non-wound areas at the days 7 and 14 after wound model.

		Day 7				Day 14			
		Group 1		Group 2		Group 1		Group 2	
		Min	Max	Min	Max	Min	Max	Min	Max
Intra-wound	Mean	31.70	34.13	31.32	33.95	33.85	34.78	33.17	35.00
	SEM	0.33	0.23	0.27	0.05	0.27	0.27	0.23	0.18
	P	0.22	0.25	0.22	0.25	0.06	0.28	0.06	0.28
Wound edge	Mean	33.08	34.28	32.75	33.93	34.37	34.72	33.55	34.62
	SEM	0.38	0.46	0.32	0.21	0.28	0.33	0.27	0.12
	P	0.28	0.27	0.28	0.27	0.04	0.40	0.04	0.40
Non-wound	Mean	33.98	35.05	33.68	35.02	33.88	34.93	32.80	34.37
	SEM	0.16	0.20	0.26	0.12	0.45	0.38	0.31	0.17
	P	0.20	0.45	0.20	0.45	0.05	0.12	0.05	0.12

Table 3. Correlation of IRT results between measurement areas and days, in-group comparisons.

Group	Intra-Wound Min	Intra-Wound Max	Wound Edge Min	Wound Edge Max	Non-Wound Min	Non-Wound Max
Group 1	0.696	0.614	0.697	0.631	0.587	0.549
P	0.000	0.001	0.000	0.001	0.003	0.005
Group 2	0.687	0.602	0.555	0.594	0.449	0.46
P	0.000	0.002	0.005	0.002	0.028	0.024

IRT provides a non-contact evaluation of surface temperature changes in tissues. Its real-time, non-invasive, and side-effect-free method are its important advantages. Perhaps the most important advantage of IRT is its high sensitivity to temperature changes in asymptomatic pathological conditions [9, 33]. In measurements, body surface temperature may vary depending on age, gender, weight, metabolism, topography of the measured tissue area and blood circulation [16, 20]. IRT has been used in many species in humans and animals for the examination of local lesions or systemic diseases. In recent years, thermographic imaging has been used to determine the effects of pain and anesthesia in surgical procedures and anesthesia, especially with vasomotor changes and the response of C-sympathetic fibers [2, 12, 14]. To our knowledge, there is no study examining the local temperature measurements of the wound area with IRT in a full-thickness skin wound model. Present study was aimed to evaluate the differences of intra-wound, wound edge, and non-wound local temperatures in untreated and treated wounds of a full-thickness excisional skin wound model by IRT in rats.

In infrared thermography (IRT), since the device measures heat energy, the amount or intensity of light in the environment does not affect the results [9, 18]. However, environmental conditions such as sun rays, weather conditions, humidity, which may affect the individual during thermographic shots, may cause general or local temperature changes in the body [1, 13]. In case of thick hair covering that will affect the accuracy of the measurements, it should be shaved [11, 13]. Luzi et al. [17] reported that all areas in rabbits except the eyes are not suitable for IRT due to excess hair. There should be no activities and environmental factors that will change the physiology while IRT measurements are being made. The limitations of IRT are that thermographic measurements do not give accurate results in direct sunlight, bad weather, stress, or during feeding. This is not a disadvantage in research with experimental animals carried out under animal laboratory conditions at certain standards. In the present study, these factors that may adversely affect the IRT measurement were ruled out, thanks to the adaptation of the experimental animals and subsequent thermographic measurements in the same environment under standard conditions. The fact that it was hairless or less hairy in the following days due to shaving in creating the wound model in the areas where the measurements were made also ensured that there was no hair cover disadvantage. The effect of feather cover can be evaluated in different studies to be carried out without shaving the feathers.

Performing the thermographic image acquisition without contact provides a significant advantage in both examinations and experimental studies. In IRT, it is also possible that the images are not affected much by the movement of the region and that real-time

comparisons can be made with different individuals. In thermal camera measurements made by focusing on a part of the body, the emergence of other abnormal data in the environment will decrease [23]. In our study, IRT measurements were made without touching the wound, in an easy, practical way and away from the factors that would affect the measurements. Individual and in-group comparisons were obtained successfully.

In IRT, the distance between the thermal camera and the area to be measured should not be too much. This distance should be around 0.5-1 m on average and the measuring angle should be close to 90°, depending on the area where the measurement is made and the characteristics of the device [9, 19]. Although 90° is ideal as a measurement angle, it has been reported that angle differences up to 20° (measurements with angles such as 70-110°) are appropriate [35]. Some researchers say that the distance should be reduced to 0.5 m depending on the region [3, 7]. Due to the small size of the rats compared to many species and the characteristics of the IRT device used in our study, thermographic measurements were made at a distance of 0.5 m and an angle of 70-90°, which was found to be the most appropriate during the applications, in order to obtain accurate results. In our study, it was thought that the most accurate results were obtained with this distance and angle.

There may be differences between the IRT values measured in the studies, depending on many factors [11, 22]. Factors affecting blood pressure can indirectly change the body (general) or tissue (local) temperature by affecting blood flow in capillaries [4]. In a study that evaluated pain in laboratory animals with IRT, it was stated that the changes in images and measurements were mostly affected by vasodilation and vasoconstriction. IRT has been found to be a convenient method in pain assessment and has been recommended for use in both research and animal health practices [22]. In a pilot study, a thermography assessment of stress in dogs was performed. IRT was found to be a useful tool in determining stress and emotional changes in dogs, but it was emphasized that more studies should be done [30]. According to a study, obese individuals may have lower local temperatures that can be noticed in measurements due to the fact that subcutaneous adipose tissue is more and this increases thermal insulation [5]. These disadvantages did not arise due to the fact that the animals in our study had certain standards and similar characteristics, were not obese, and were not regions with dense fat tissue.

A study carried out on mice after a surgery event recording skin temperature variation on selected body areas reported that the temperatures increase until day 7 and then decrease reaching values not significantly different from the basal ones at day 21. These results are stated useful to provide reference values for daily use of the IRT in animal lab facilities [26]. In our study, it was determined that the maximum temperatures in the intra-wound area and the minimum temperatures in the wound edge area on the 1st day of wound treatment were significantly higher in the treatment group. Also, the minimum temperatures in the wound edge area on the 14th day were significantly lower in the treatment group. The differences in other measurements were not statistically significant. Based on these, it can be said that the local temperature increases in the areas where appropriate treatment is applied during the wound healing process. The more distance from the wound center or the more wound treatment progresses, the local temperature increases less. The reason for this was thought to be capillary hyperemia in the wound as a result of topical stimulation with the first treatment application. In addition, time/local temperature correlation was examined in terms of wound treatment in our study. Our results indicate that there was less local temperature increase and therefore less inflammation in the treatment group as the treatment progressed, while more

inflammation was formed in the untreated group. The limitations of our study are the inability to perform further methods that can examine vascularization, such as histopathological and immunohistochemical examinations. More detailed plans can be made in this respect in future studies.

Thus, with the researches in recent years, scientific data and consensus based on the mechanisms, properties and effectiveness of IRT have been increasing. Thermography is considered an effective and convenient method with many advantages in both laboratory animals and domestic animals [6, 30]. In our study, in addition to the usefulness of IRT, the results showing the thermographic changes of the wound tissue in terms of intra-wound, wound edge and non-wound areas in the wound healing process provided original data.

CONCLUSION

In conclusion, this study determined the reference values for the use of IRT in wound healing studies in the full-thickness excisional skin wound model in rats. Further research is needed to standardize the methodology for using IRT in other wound models, such as infected wounds and diabetic wounds.

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Conflict of Interest. “The authors declared that there is no conflict of interest.”

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