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Thermal imaging to evaluate healing of burn wounds treated with colloidal nano silver hydrogel and decellularised bovine omentum in a rat model[#]

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Abstract

Infrared thermography is a safe, non-invasive and inexpensive imaging technique used for the assessment of depth of burn wounds. The present study was aimed at evaluation of burn wounds from the day of infliction till complete healing using thermography and compare it with the gross morphology and histopathology. Burn wounds were induced in Wistar rats under general anaesthesia followed by excision and grafting, 72 hours after the infliction of burn injury. Colloidal nano silver was used as the topical medication and decellularised bovine omentum was used as the scaffold. Thermography was performed on the day of infliction of burn injury and grafting, and on 3rd, 7th, 14th and 21st day thereafter. The thermal images were compared with the gross morphological changes and histopathological findings. The thermal images gave a different perspective of the burn wounds on the day of infliction and after 72 hours. Though the changes were comparable with gross morphology and histopathology from day '0' (after grafting) till complete healing, thermography seemed to be over-representing the inflammatory changes. The increased peri-wound temperatures indicated a healing wound. It could be concluded that infrared thermography could be effectively used for burn wound monitoring in the clinical scenario and as a complimentary tool for burn wound research.

Keywords: *Infrared thermography, gross morphology, histopathology, burns, healing, rats*

Infrared thermography is a safe, non-invasive and relatively inexpensive diagnostic modality which has applications in various medical fields, including, traumatology, orthopaedics, sports medicine, endocrinology, neurology, neonatology, vascular diseases and oncology (Kozhevnikova *et al*., 2017). It has various applications in engineering including material characterisation using surface temperature mapping (Meola *et al*., 2004). Szrek *et al*.(2021) reported that infrared thermography could be used for search and rescue operations during natural calamities. Thermal imaging has been considered as a non-invasive method to assess the depth of burn wounds. This can be helpful in triaging the burn patient as well as predict the progression of the wound (Xue *et al*., 2018; Ponticorvo *et al*., 2019; Carrière *et al*.,

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2020). It was suggested by Ye and De (2017) that it was most useful within three days of the thermal injury, since its accuracy reduced in the presence of granulation tissue. Thermographic evaluation of burn wounds during the entire course of healing has not been performed. Hence, the present study was aimed at thermographic evaluation of the healing of burn wounds from the day of burn infliction till complete healing. This was compared with the gross appearance of the wounds and its histopathological appearance on the corresponding days. The treatment adopted was topical application of colloidal nano silver hydrogel with decellularised bovine omentum used as a scaffold.

Materials and methods

The study was conducted on 18 Wistar rats of either sex, weighing 200-250 g. The study was approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University, as per order number CVAS/MTY/IAEC/23/41 dated13.09.23. Burn wounds measuring one centimetre square was induced on the dorsal thoraco-lumbar region of the rats under general anaesthesia. They were anaesthetised using intraperitoneal injection of a cocktail of Inj Xylazine HCL (5 mg/kg) and Inj Ketamine HCL (50 mg/kg). Inj. Buprenorphine was injected subcutaneously at the dose rate of 0.05 mg/kg for analgesia. The site was prepared aseptically and the burn wound was induced by placing a pre-heated (100°C) end plate perpendicular to the skin for 20 sec, using a burn inducing device designed by Anjana *et al*. (2022). Soon after burn induction, 10 mL Ringer's lactate was administered intraperitoneally for resuscitation. Thereafter, Meloxicam drops were given orally at the dose rate of 1 mg/kg for three consecutive days. The necrotic eschar was removed under general anaesthesia, 72 hours after burn infliction. The colloidal nanosilver hydrogel was applied on the wound bed and grafted with decellularised bovine omentum. This day was considered as day zero. The hydrogel was applied on the wound, over the graft, once daily till complete healing.

The wounds were monitored daily and the gross appearance was noted at sequential intervals from the day of burn induction to complete healing. The eschar tissue that was removed on day zero- and five-millimetre punch biopsies were taken from the wound edges of three animals each on the 3rd, 7th, 14th and 21st days and preserved in neutral buffered formalin for histopathological studies. The samples were processed, stained with haematoxylin and eosin and examined under light microscope (Leica trinocular research microscope, DM 2000 LED). Thermal imaging was carried out on the day of burn infliction and thereafter at sequential intervals, using a plug-and-play type smart mobile phone thermal imager (HT-301 version V1.1, HTI, Dongguan Xintai Instrument Co. Ltd., China), attached to an android smartphone, using type-C interface. Image processing was done by the smartphone application (HTI Image application, Dongguan Xintai Instrument Co. Ltd., China) (Fig. 1a and 1b). The thermal imager had a 13 mm lens with an infrared image resolution of 384 x 288 pixels. The rainbow palette was selected for the study as proposed by Vardasca and Gabriel (2014).

Results and discussion

The comparative observations of gross morphology, histopathology and thermal imaging at various points in the timeline of wound progression (Fig. 2) were as follows:

Gross morphology

The burn wound soon after infliction had a pale-yellow central zone of coagulation with an elevated pale margin. On the third day, the wound had a pale necrotic centre, surrounded by a hyperaemic region. This was in accordance with Cai *et al*. (2014) and Anjana (2020). After grafting, the decellularised bovine omentum placed over the hydrogel appeared glistening and red, due to the wound fluids mixed with the hydrogel. On the 3rd day, the wound appeared dry with a reddish-brown colour and the sutures were intact. On the 7th day, the wound appeared drier with a brownish colour and the sutures were intact.

Fig. 1a

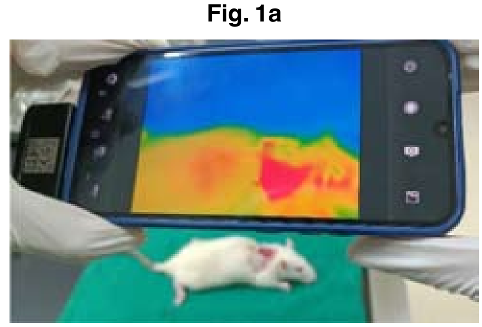

Fig. 1b

Fig. 1a. Thermal imager and smart phone application (in red square), **1b.** Image capture.

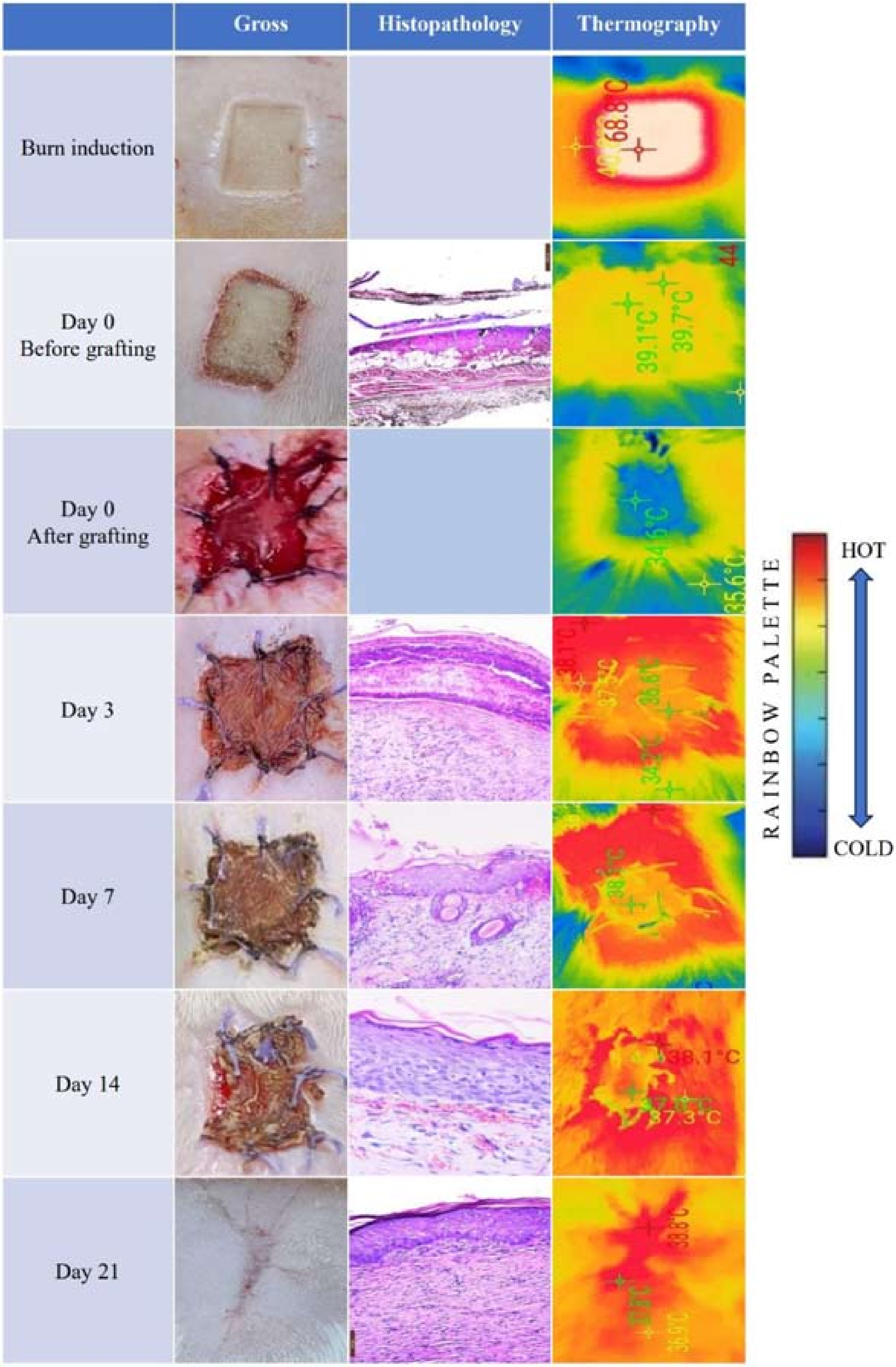

Fig. 2. Progression of a burn wound: From induction to complete healing- comparison between gross appearance, histopathology and thermal imaging.

On the 14th day, a few sutures were removed. The scaffold dried up and looked like a scab covering the wound. A healthy granulation tissue was visible in the area where sutures were lost. By the 21st day, the scaffold had fallen off and the epithelialisation of the wound was complete with a stellate pale coloured scar. Anjana (2020) observed a similar healing pattern in burn wounds. There was regrowth of hairs, which was shaved to visualise the scar and collection of biopsies.

Histopathology

On the third day after infliction of the burn wound, the necrotic eschar was surgically removed followed by wound dressing and grafting. The eschar, that was removed, was subjected to histopathology. When observed under 2x magnification, there was loss of epidermis with a carbonised layer noted on the epidermis and below the muscle layer indicating a full thickness burn. There was coagulation of epidermis and dermis appearing as a homogenous, basophilic region. On the third day, a punch biopsy was obtained from the wound edge, with practically no granulation tissue in the wound. Hence, the histopathology (20x) depicted the inflammatory changes in the wound margins. There was marked infiltration of leucocytes in the epidermis and dermis, homogenous eosinophilic oedema fluid in the superficial layers and hyperaemia in the dermis. The dermis had disorganised collagen fibres. On the seventh day, the signs of inflammation had subsided. A neo epidermis was being formed. Skin appendages and hyperaemic changes could be appreciated in the dermis with moderate deposition of collagen. On the 14th day, histopathology (20x) revealed a thin keratin layer over the neo epidermis. Hyperaemia persisted in the dermis. By the 21st day, the epithelialisation was complete with a pronounced basal layer of epidermis. A dense keratin layer was observed over the epidermis. There were more organised and denser collagens in the dermis. The histopathological findings of the present study were in accordance with that described by Ye and De (2017) and Anjana (2020).

Infrared thermography

Thermography was performed soon after infliction of burn wound. The central zone appeared as a bright spot with the maximum temperature reading, surrounded by a zone of hyperaemia, which had a red colour. The periphery had shades of yellow surrounded by green and blue. At the same time, the wound was observed in a video mode which revealed that the temperature in the central zone reduced in less than three minutes to match the temperature of the periphery (yellow). The reduction in temperature might be the result of evaporative cooling as well as dissipation of heat to the surrounding tissue. After 72 hours, before the removal of eschar revealed that the eschar had almost the same temperature as the surrounding inflamed tissue (yellow). On completion of grafting, thermography revealed a central area of hypothermia (blue), designating a deep wound. The surrounding tissue remained warm (yellow) indicating a zone of inflammation. On the third day, the wound area had a lower temperature (yellow) and the surrounding tissues were orange to red suggesting an active zone of inflammation around the wound. A similar picture was also observed on the seventh day. On the 14th day, the zone of inflammation had reduced and was confined to the immediate vicinity of the wound (orange to red). The temperature of the wound was still lesser than the surroundings (yellow). On the 21st day, the hyperthermia (orange to red) was restricted to the scar tissue which suggested an active inflammation at the scar tissue, possibly leading to remodelling of the wound. But that did not correlate with the histopathology or the gross appearance.

To summarise the findings, thermal imaging gave a different perspective to the evaluation of burn wound healing. On the day of burn induction, the hyperaemic zone seen as red in thermography, was observed grossly as a pale elevated margin of the burn wound. At 72 hours, the margins of the eschar were not differentiated in thermography, as the whole area had a uniform yellow colour. But the changes were obvious in the gross morphology and histopathology. The gross appearance on day 0 after grafting, correlated well with the thermal image. From the third to twenty first day, the three methods were comparable, which suggested a progression in wound healing and a subsiding inflammation.

Pereira *et al*. (2012) stated that Wistar rats could be used as experimental models for studying deep second-degree thermal wounds as these animals showed great ease of handling, accommodation and resistance to surgical aggressions and infectious processes, with low mortality. Ye and De (2017) reported that inducing burn wounds by exposing to 100 °C for 20 sec. can create full-thickness burns in rats. These parameters were used in the present study for the induction of burn wounds in Wistar rats.

Ye and De (2017) reviewed the different imaging techniques used to assess depth of burn wound based on surface temperature. They observed that there is an inverse correlation between temperature and burn depth since deeper wounds were colder than more superficial one due to decreased vascular perfusion. They suggested that thermal imaging was most useful within three days of the thermal injury. Ponticorvo *et al*. (2019) opined that the decreased vascular perfusion in deep wounds led to a reduction in thermal emission. In this study we used thermal imaging to monitor the wounds throughout the healing period. On day 0, the thermal image after grafting indicated a hypothermic zone (blue) indicating a deep wound after removal of the eschar. During the third day to twenty first day, the peri-wound temperatures were high. Mota-Rojas *et al*. (2024) opined that higher peri-wound temperatures were associated with a better healing process.

The gross inflammatory changes in the current study correlated well with the thermal images. But the cellular details obtained in histopathology were not matching with thermography. However, the only advantage was its non-invasiveness.

Mota-Rojas *et al*. (2024) suggested that high-resolution thermography (320 × 340 pixels) could detect minimal changes in surface temperature. The resolution of the infrared camera used in this study fell in this range and yielded good images. The other factors that influenced the thermal imaging were the presence or absence of hair, environmental factors such as wind and direct solar radiation. In this study, thermal imaging was not affected because the hairs were removed to create burn wounds. The study was conducted under controlled ambient temperature and uniform lighting conditions in an operation theatre to avoid such variables.

Ye and De (2017) suggested that thermal imaging had 90% accuracy in clinical trials. They also opined that the accuracy of thermal imaging reduced with the appearance of granulation tissue and hence it is best suited for the initial period of burn wound healing. Giggin (2023) used infrared thermography to detect persistence of subclinical inflammation and thus chronicity of a wound in elephants. In the present study, the wound temperatures (yellow to orange) were lower than the periphery (orange to red) as observed by Giggin (2023) in some of the wounds, but the increased peri-wound temperatures observed in the current study were associated with a healing process, rather than chronicity of the wound. However, several authors have reported that thermal imaging is less accurate compared to other imaging modalities (Jaspers *et al*., 2019 and Han *et al*., 2020).

Conclusion

Thermography seemed to be over-representing the zone of inflammation compared to the gross appearance and histopathology. Infrared thermography currently lacks sufficient automation and relies on subjective interpretation. The accuracy of thermography could be affected by the technical aspects of the equipment, the species under consideration, stage of wound healing and environmental factors. Hence it could be concluded that infrared thermography is a safe, non-invasive imaging modality for the assessment of burn wounds, which can be effective in the clinical scenario, but can be considered only as a complementary tool when it comes to burn wound research.

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Conflict of interest

The authors declare that they have no conflict of interest.

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