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# Novel imaging methods reveal positive impact of topical negative pressure application on tissue perfusion in an in vivo skin model

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## Abstract

The influence of topical negative pressure application (TNPA) on tissue perfusion still remains controversial. TNPA was applied for 30 minutes on intact skin of 21 healthy participants. Measurements of tissue oxygen saturation and tissue temperature as signs of tissue perfusion were performed before application of the TNPA, directly after removal of the TNPA and 5, 10, 15, 20, and 30 minutes after removal of the dressing using the near infrared imaging (NIRI) and a thermal imaging camera. Tissue oxygen saturation showed an increase from 67.7% before applying the TNPA to 76.1% directly after removal of TNPA, followed by a decrease of oxygen saturation 30 minutes after removal of TNPA. The measured temperature of the treated skin area increased from 32.1°C to 36.1°C after removal of TNPA with a consecutive decrease of the temperature 30 minutes after removal. TNPA resulted in both a higher tissue oxygen saturation and a higher skin temperature after 30 minutes compared to the beginning. TNPA increases both tissue oxygen saturation and skin temperature as sign of an increase of tissue perfusion. NIRI and thermal imaging proved to be useful for measuring changes in tissue perfusion.

**KEYWORDS**

near infrared imaging, thermal imaging, tissue oxygen saturation, topical negative pressure application

## Key Messages

- the influence of topical negative pressure application (TNPA) on tissue perfusion still remains controversial
- TNPA was applied for 30 minutes on intact skin of 21 healthy participants
- near infrared imaging showed an increase of superficial tissue oxygenation after removal of TNPA
- thermal imaging showed an increase of skin temperature after removal of TNPA

## 1 | INTRODUCTION

Topical negative pressure application (TNPA) has become a standard tool in the treatment of complex and chronic wounds.[1-4] As a temporary wound closure TNPA reveals a positive impact on oedema and seroma formation, enables formation of granulation tissue, and reduces bacterial load. In acute wounds or after tumour resection, it is frequently used to bridge the time until definite reconstruction is possible.

The creation of a sub-atmospheric pressure is accompanied by a multitude of changes on a cellular and molecular biological level.[5] In spite of huge clinical experience and clinical research mainly based on case series and clinical trials, the exact underlying mechanisms are still not fully understood.[2,5-10] The influence of TNPA on the perfusion of a wound and the flow of adjacent vessels and capillaries is part of an ongoing controversial discussion. Many experimental and clinical studies indicate an increase of blood flow across the wound and/or close to the wound margins. By creating microdeformations at the wound–foam interface, molecular pathways for angiogenesis are activated.[11] Recent studies have shown a higher density of microvessels as well as an improvement of blood flow after applying continuous or intermittent TNPA.[11-17] According to Kairinos et al, TNPA can lead to an increase or decrease of perfusion at different anatomical locations.[18] This is challenged by results stating an impairment of blood circulation with regard to the application of negative pressure.[19] There is a wide range of study models that have been used to assess the influence of TNPA on blood flow, perfusion, and oxygenation such as a peripheral wound model in domestic pigs,[20] in vivo studies on intact human skin,[17] or clinical studies in wounds of vascular patients.[21] We focused on TNPA on intact human skin to exclude any influence of patient or disease characteristics such as diabetes or peripheral vascular perfusion disorder.

This study investigates the influence of TNPA on tissue oxygen saturation and skin temperature as indirect signs of perfusion. Therefore, two novel imaging methods, the near infrared imaging (NIRI) and a thermal imaging camera, were applied not yet assessed in this context.

## 2 | METHODS

The study was approved by the institutional ethics committee (registration number 310_19 B). Written informed consent was obtained from all participants. TNPA (KCI—an Acelity company, San Antonio, Texas) was applied on intact skin of 21 healthy humans (Figure 1).

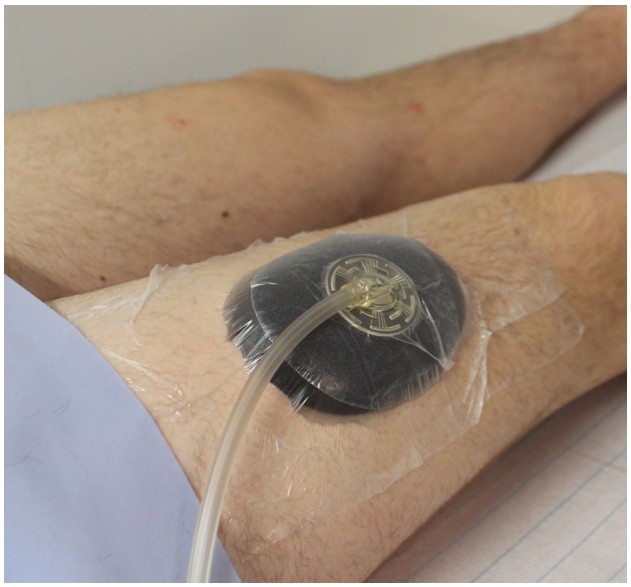

**FIGURE 1** Applied topical negative pressure application (TNPA) on the right thigh. The foam is placed on the right thigh, and the trackpad connects the foam to the pump

According to their own statement, none of them suffered from any disease affecting the skin, had perfusion disorders, or had any surgery of the leg previously.

All measurements were performed by one person in a room offering standardised conditions concerning room temperature, relative air humidity, and air pressure. After 10 minutes of cloth removal and resting, TNPA was applied on both thighs corresponding to the area of the anterolateral thigh flap.[22] As a landmark, the axis of the anterior superior iliac spine and the lateral superior patella was drawn. TNPA with an continuous pressure of −125 mmHg was applied according to the manufacturer's instructions for 30 minutes. NIR and thermal imaging were performed directly before applying the TNPA ($t0$), directly after removal of the dressing ($t1$) as well as 5 minutes ($t2$), 10 minutes ($t3$), 15 minutes ($t4$), 20 minutes ($t5$), and 30 minutes ($t6$) after removal of the dressing (Figure 2).

To measure tissue oxygenation in superficial tissue, near infrared reflectance-based imaging was performed at a standardised distance using Snapshot NIR (KENT Imaging Inc., Calgary, Canada). The distance was determined by two convergent laser points integrated in the device that overlap at a distance of about 32 cm. Near infrared light is transmitted onto the skin surface and reflected off the blood within the tissue. Due to the wavelength-dependent difference of oxygenated and deoxygenated light absorption of haemoglobin, the ratio from oxygenated to deoxygenated blood and therefore the viability can be determined by this method. Poorly

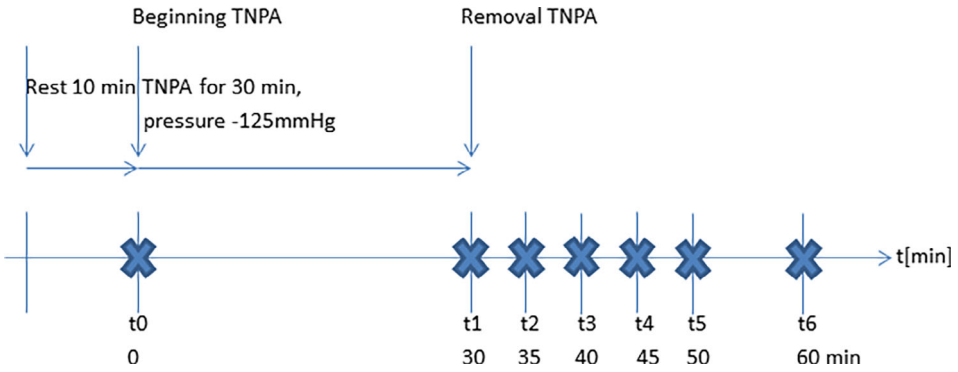


FIGURE 2 Study design

perfused skin has a lower percentage of oxygenated haemoglobin than that of well-perfused skin.[23] Within the marked area at the thigh, five circular regions of interest were defined with a single diameter of 9 mm. The averaged tissue oxygen saturation of the five regions of interest was calculated for each side.

Thermal images were performed by using a smartphone-compatible thermographic camera (FLIR ONE Pro, FLIR Systems, Inc., Wilsonville, Oregon), which uses a long-wave infrared sensor. The provided effective temperature range is set from −20°C to 400°C with a resolution of 0.1°C and with a sensitivity that detects temperature differences down to 70 mK. Image processing enables merging of a photograph with a thermal image. Besides the aforementioned standardisation for imaging, a distance of 70 cm was defined. Identical regions of interest were used as for near-infrared spectroscopy and the average temperature was calculated.

Data from timepoint *t*0 were compared with data from timepoints *t*1 and *t*6 as well as those from *t*1 to *t*6.

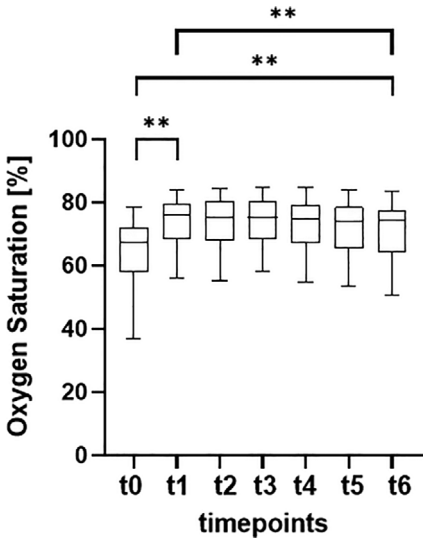


FIGURE 3 Tissue oxygen saturation measured at the different timepoints. Tissue oxygen saturation showed an increase after 30 minutes of topical negative pressure application (TNPA) (*t*1) and decreases 30 minutes after removal of the TNPA (*t*6) ending up in higher tissue oxygen saturation compared to the beginning (*t*0)

### 2.1 | Statistics

The statistics were performed using Microsoft Excel (Microsoft, Redmond, Washington) and Prism 8 (GraphPad Software, San Diego, California). The median values of each timepoint were compared. The Wilcoxon signed-rank test was used for the comparison of not normally distributed, depending samples. The level of significance was set at 0.05.

## 3 | RESULTS

### 3.1 | Participant characteristics

All 21 participants (n = 42 thighs) were included for thermal imaging (10 female, 11 male). Three patients were excluded from the NIRI because of irregular skin pigmentation, and this might bias spectroscopy results according to the manufacturer. The participants had an average age of 27 years (21-32 years), with an average body mass index of 23 kg/m$^2$ (20-30 kg/m$^2$).

### 3.2 | Tissue oxygen saturation

Tissue oxygen saturation showed an increase of 8.4% from 67.7% (SD 10.68; range 37.00%-78.60%; mean 64.83%) before applying the TNPA (*t*0) to 76.1% (SD 7.48; range 56.00%-83.80%; mean 73.83%) ($P < .0001$) directly after removal of the TNPA (*t*1). Furthermore, a decrease of oxygen saturation ($P < .0001$) of 0.9% 30 minutes after removal of the TNPA (*t*6) compared to the timepoint directly after removal of the TNPA (*t*1) could be shown.

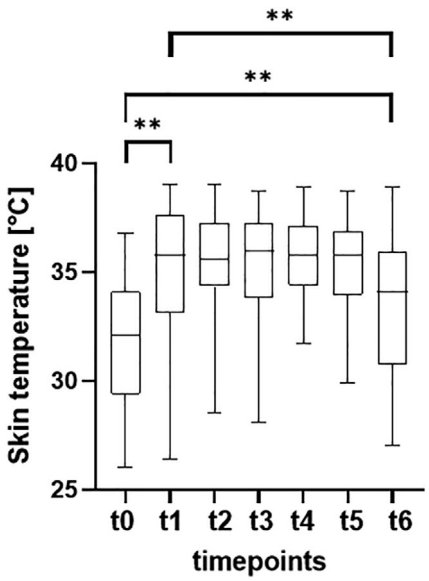


**FIGURE 4** Measured skin temperature of the timepoints *t*0 to *t*6. The measured skin temperature increases after 30 minutes of topical negative pressure application (TNPA) (*t*1) and decreases 30 minutes after removal of the TNPA (*t*6), resulting in higher skin temperature compared to the beginning (*t*0)

Nevertheless, TNPA resulted in a higher oxygen saturation of 75.2% (SD 9.16; range 50.80%-84.40%; mean 71.74%) 30 minutes after removal of the TNPA (*t*6) compared to the beginning (*t*0) ($P < .0001$) (Figures 3, 5-7).

### 3.3 | Skin temperature

The measured temperature of the treated skin increased 4°C from 32.1°C (SD 2.98; range 26.00°C-36.80°C; mean 32.00°C; *t*0) to 36.1°C (SD 3.04; range 26.40°C-29.00°C; mean 35.20°C; *t*1) ($P < .0001$) (Figure 4). Besides, a decrease of 1.9°C of the temperature between *t*1 and *t*6 (34.2°C; SD 3.28; range 27.00°C-38.90°C; mean 33.32°C) could be shown ($P < .0001$). TNPA resulted in a 2.1°C higher skin temperature after 30 minutes (*t*6) compared to the beginning (*t*0) ($P < .0001$) (Figures 5-7).

## 4 | DISCUSSION

Different theories exist about the underlying mechanisms of TNPA and the effects on the surrounding tissue.

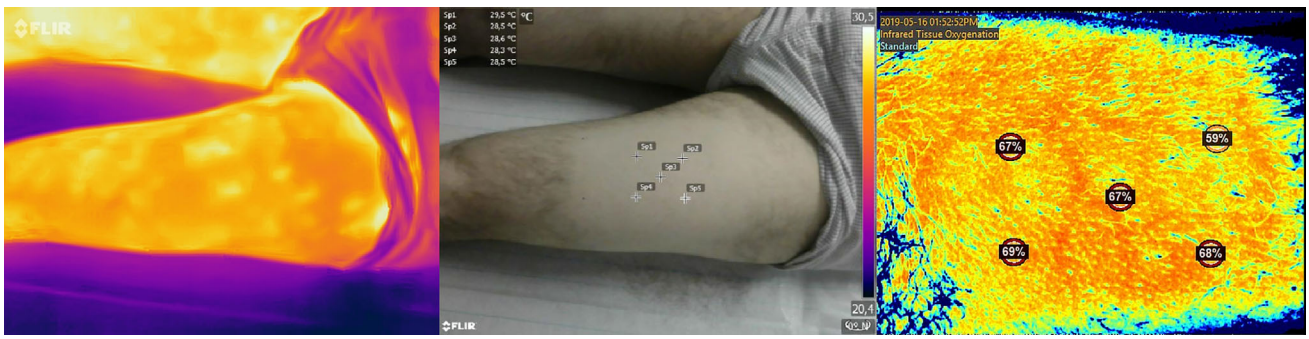


**FIGURE 5** Skin temperature and tissue oxygen saturation at *t*0. Before application of the topical negative pressure application (TNPA), the skin temperature using thermal imaging (A,B) and tissue oxygen saturation using near infrared imaging (NIRI) (C) were measured. Five circular regions of interest were defined both for the thermal imaging and the NIRI

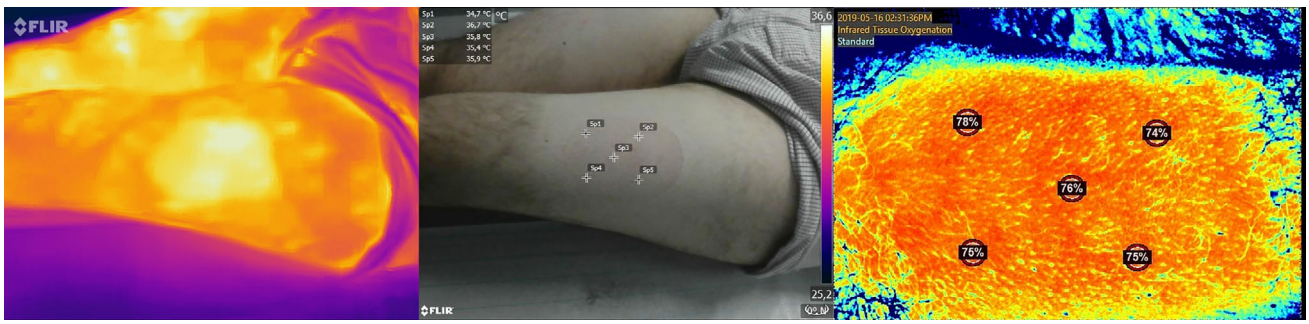


**FIGURE 6** Skin temperature and tissue oxygen saturation at *t*1. Directly after removing the topical negative pressure application (TNPA) after 30 minutes, an increase in skin temperature as well as a higher skin temperature of the treated skin compared to the untreated skin is seen on thermal imaging (A,B). Furthermore, higher tissue oxygen saturation is measured by near infrared imaging (NIRI) (C)

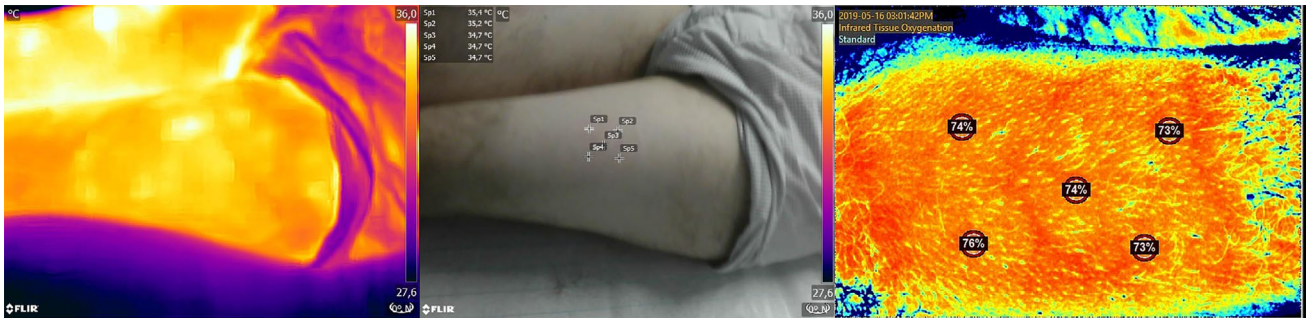


**FIGURE 7** Skin temperature and tissue oxygen saturation at *t*6. 30 minutes after removal of the topical negative pressure application (TNPA), a still higher skin temperature was measured compared to the beginning (*t*0). The higher skin temperature of the treated skin compared to the untreated skin is still seen on the thermal imaging (A,B). A higher tissue oxygen saturation was measured compared to *t*0 using the near infrared imaging (NIRI) (C)

Controversial findings are described throughout the literature supporting the hypothesis of either an increase or a decrease of tissue perfusion. But the exact influence regarding the perfusion of wounds or skin still remains unclear. Beside the treatment of chronic or complex wounds, positive effects of hyperaemia or the modulation of perfusion are highly relevant in different therapeutic fields. Therefore, the purpose of this study was to contribute to the insight of the underlying mechanisms of TNPA concerning the impact on perfusion by measuring changes in tissue oxygen saturation and skin temperature as indirect signs of perfusion changes.In general, molecular pathways for angiogenesis are activated by applying TNPA leading to beneficial effects on wound healing such as less wound dehiscence and less wound infection.[11,24-26] An increase of perfusion is associated with an improved wound healing. Furthermore, improving perfusion of tissue could be an option of flap preconditioning.

Sogorski et al showed that intermittent TNPA of 10 minutes applied on the anterolateral thigh of seven healthy humans lead to a statistically significant increase of the postcapillary tissue oxygen saturation under the foam, using combined laser Doppler spectrophotometry (CLDS) as well, that determines postcapillary oxygen saturation and relative haemoglobin content.[13]

It has already been shown by using a laser Doppler that perfusion in healthy human skin is increased 5-fold by applying pressure of 300 mmHg to the lateral forearm for 20 minutes.[27] Other studies have measured an increased blood flow in healthy humans after TNPA with a combined CLDS.[13] In contrast, Sundy et al found that intermittent TNPA of 40 mmHg over a period of 2 minutes led to an increase of skin temperature of the lower extremity while continuous TNPA resulted in a decrease of skin temperature measured by a temperature transducer, which produces an output proportional to absolute temperature.[16] Kairinos et al measured the blood flow with a laser Doppler and showed that application of TNPA with 125 mmHg for 2 minutes could lead to an increase or decrease of perfusion in different areas of the body in healthy persons.[18] In another study, continuous TNPA increased the perfusion after sternotomy compared to a control group, shown by laser Doppler flowmetry.[19] In our study, 30 minutes of TNPA resulted in a statistically significant increase of skin temperature and of oxygen saturation. After removal of TNPA, a gradual decrease of skin temperature as well as of oxygen saturation was observed. Measurements of both parameters were still higher compared to the baseline 30 minutes after removal. This can be interpreted as an indirect sign for an improved perfusion of the tissue treated with TNPA.

In addition to the use of the TNPA on open wounds, the application on closed incisions (closed incisional TNPA [ciTNPA]) has shown multiple beneficial effects. ciTNPA leads to lower infection rates after wound closure and lower rates of wound dehiscence in different fields of surgery.[19,24,28-31] Shah et al reported that ciTNPA leads to a higher expression of vascular endothelial growth factor compared to the untreated control group.[32] Improved angiogenesis resulting in higher perfusion might be a reason for the optimising effect of ciTNPA on closed incisions. Furthermore, ciTNPA increases the microcirculation and improves the oxygen saturation levels leading to optimised wound healing.[33] The results in our study support these findings. In contrast to accurately measuring the blood flow with a laser Doppler directly at four different areas, examination of the skin with the NIRI and thermal images showed higher oxygen saturation and higher skin temperature as sign of higher perfusion after applying TNPA.[19,27]

The beneficial effect of TNPA on microcirculation observed in our study might be a potential instrument for non-invasive flap preconditioning (NIFlaP). Improvement

of microcirculation occurred after postoperative ischaemic preconditioning by using a tourniquet resulting in higher blood flow and higher tissue oxygenation compared to a control group.[34] Instead of improving microcirculation postoperatively, direct NIFlaP by applying TNPA before flap surgery might lead to similar effects of the classic flap preconditioning through a delay procedure.[35,36] Several authors showed that augmenting the arterial blood inflow to the flap appears to improve skin flap survival area.[37-39] Comparing the effects of preoperative TNPA prior to flap harvest and of surgical delay in a rabbit model, both methods are equivalent in rates of total flap necrosis and necrotic flap areas and superior to non-delayed flaps.[40] Preconditioning of fasciocutaneous flaps with non-invasive suction using a cupping device improved flap survival compared to the non-treated control group in rats.[41] Specifically in perforator flaps, increased perfusion initiated by TNPA over the flap area might lead to an opening of direct and indirect linking vessels, which connect adjacent perforasomes.[42] This could improve perfusion across adjacent perforasomes resulting in an enlarged flap volume/surface perfused via a single perforator. This is similar to the mechanism of flap delay where linking or so-called choke vessels dilatate and reorientate due to increased blood flow because of opening of arteriovenous anastomoses.[35,36] Transferred to the clinical application, NIFlaP might potentially reduce operative procedures and enable individualised delay procedures dependent on the required flap size.

Owing to our findings another potential use of TNPA on intact skin and its positive influence on perfusion could be the treatment of different types of pain usually sensitive to increased vascularity and warmth of the affected tissue. Increased local perfusion that is usually produced by applying warmth on the painful area among others relaxes the painful muscles and reduces the pain.[43,44] Furthermore, the stimulation of blood flow induced by negative pressure suction present a known effect of cupping, which is nowadays used for conditions involving pain such as back pain or arthritis.[45,46] Cupping produces a visible vasodilatation of the superficial capillaries that produces a localised hyperaemia.[46] The skin surface temperature measured with an infrared camera after 5 minutes of cupping therapy increases in comparison with the non-treated control group.[47] Similar effects after applying TNPA were observed in our study. Different modes of negative pressure levels as well as continuous and intermittent protocols, and the possibility of treating large areas by using large foams could be of interest in this context. Further studies could deal with this new field of application to analyse possible beneficial effects.

Both the NIRI and thermal imaging proved to be useful tools to measure changes in tissue oxygen saturation and temperature as indirect signs of changes in perfusion. In contrast to the measurement with a laser Doppler, which assesses just a small volume of tissue (1 $mm^3$),[19] the NIRI can measure larger areas of skin of approximately 150 $cm^2$.[23] Instead of collecting data from just one single point, the NIRI uses an array of infrared emitters and evaluates the complete examined area.[23] The thermal imaging has been shown to assess an area up to 300 $cm^2$.[48] Another disadvantage of the measurement of tissue perfusion with a laser Doppler is that the applied pressure can cause microcirculatory disturbances and influence the measured results.[49] Furthermore, the measured oxygen saturation might be higher when the laser Doppler is directly applied over a vessel. Therefore, to evaluate a specific area of tissue properly, nearly limitless measured points had to be assessed. The thermal imaging as well as the NIRI assesses larger areas in one and without skin contact, which might result in more accurate results.

Some potential study limitations can be discussed. The tissue oxygen saturation of our participants before applying the TNPA was 67.7%. We focused on showing the increase and decrease of tissue oxygen saturation and not on exact measurement values. This study assessed TNPA on skin of healthy humans; hence, the application on different wounds in the clinical setting is not fully reflected. On the other hand, the applied measuring tools are designed for the detection of changes in temperature and oxygen saturation in skin and cannot simply be applied to open wounds. This is due to the abundance of red light in a wound vs the typical skin colour that does interfere with the red light emission of erythrocytes. The improvement of perfusion measured as higher temperature and higher oxygen saturation after applying TNPA was seen on principally healthy and well-perfused skin. Therefore, it is expected that TNPA increases the perfusion in similar well perfused tissue like muscle, while our results are not readily transferable to poorly perfused tissue like bone or tendons. There might be a possible effect of the dressing itself leading to an increase of the skin temperature at the assessed area. However, different studies have shown an increase in perfusion after ciTNPA compared to a standard dressing.[19,50] Nevertheless, the NIRI as well as the thermal imaging in our study have been proven to be appropriate tools for measuring the influence of TNPA on skin.

## 5 | CONCLUSIONS

TNPA leads to higher skin temperature and higher oxygen saturation. Both parameters are indirect signs of increased perfusion. The used NIRI as well as thermal imaging are new and appropriate devices to measure the influence of TNPA on skin.

The presented results contribute to a better understanding of the underlying mechanisms of TNPA and reveal possible new application fields. In future, new scopes of applications for TNPA are conceivable and have to be assessed in further studies.

## CONFLICT OF INTERESTS

R. E. Horch has received third-party funding for scientific research on NPWT from KCI—an Acelity company in the past and has served as a member of a Scientific Advisory Board of KCI-Acelity in the past. R. E. Horch and A. Arkudas served as speakers on scientific symposia of KCI-Acelity in the past. The authors have no other relevant affiliations or financial involvement with any organisation or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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