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Topical negative pressure wound therapy enhances the local tissue perfusion – A pilot study

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ABSTRACT

Background: Topical negative pressure wound therapy (TNPWT) is a regularly used method in modern wound treatment with a growing and diverse potential for clinical use. So far positive effects on microcirculation have been observed and examined, although precise statements on the underlying mechanism appear unsatisfying.
Objective: The aim of our study was to extend the understanding of the effect of TNPWT on tissue perfusion and determine the time frame and the extent to which the tissue perfusion changes due to TNPWT.
Material and methods: TNPWT was applied to the anterior thighs of 40 healthy individuals for 30 min, respectively. Before and up to 90 min after the application, measurements of the amount of regional haemoglobin (rHb), capillary venous oxygen saturation (sO2), blood flow (flow) and velocity were conducted with spectrophotometry (combining white light spectrometry and laser Doppler spectroscopy) within two different depths/skin layers. A superficial measuring probe for depths up to 3 mm and a deep measuring probe for up to 7 mm were used.
Results: All parameters show significant changes after the intervention compared to baseline measurements. The greater effect was seen superficially. The superficially measured rHb, sO2 and flow showed a significant increase and stayed above the baseline at the end of the protocol. Whereas deeply measured, the rHb initially showed a decrease. The flow and sO2 showed a significant increase up to 60 min after the intervention.
Conclusion: The application of TNPWT on healthy tissue shows an increase in capillary-venous oxygen saturation and haemoglobin concentration of at least 90 min after intervention. A possible use in clinical practice for preconditioning to enhance wound healing for high-risk patients to develop wound healing disorder, requires further studies to investigate the actual duration of the effect.

1. Introduction

The principle of generating sub-atmospheric pressure on a wound area was commercialized as vacuum-assisted-closure® (V.A.C.®) therapy (KCI, San Antonio, Texas) in the 1990s (Argenta and Morykwas, 1997). This approach revolutionized the treatment of wounds. In recent years, the field of application for V.A.C.® therapy expanded through research and development. Current indications include the treatment of acute, subacute, chronic, dehiscent or traumatic wounds, and it can be used to treat radiation damage, burns and ulcers and is used post-operatively for open abdomen, mesh graft fixation, temporary soft tissue covering and for the preparation of skin transplants (Lima et al., 2017). Furthermore, in high-risk patients topical negative pressure wound therapy (TNPWT) is used prophylactically on closed incisional wounds preventing wound healing disorders und surgical site complications (Norman et al., 2020; Vries et al., 2016).

The positive effects are clinically confirmed by many practicing physicians and although TNPWT is a rapidly expanding method with widening, diverse indications, particularly regarding modern wound-healing treatment in everyday clinical practice. The mechanisms introducing these effects have not yet been fully discovered nor understood.

We hypothesized that the tissue perfusion would increase after the application of TNPWT on the skin and that the effect would last at least 90 min after treatment discontinuation.

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Abbreviations

NPWT	negative pressure wound therapy
TNPWT	topical negative pressure wound therapy
LDF	laser Doppler flowmetry
rHb	amount of regional haemoglobin
sO2	capillary venous oxygen saturation
flow	blood flow
V.A.C.®	vacuum assisted closure
O2C®	Oxygen to See®
BMI	body mass index
ANOVA	analysis of variance
Fig.	figure
Tab.	table
Str.	stratum

2. Materials and methods

This interdisciplinary and interprofessional research project was approved by the ethics committee of our institution (EK 495122018), in accordance with the Helsinki Declaration.

2.1. Setup

The measuring equipment, which is depicted in Fig. 1, consisted of an examination couch, the Oxygen to See® (O2C®) device (LEA Medizintechnik GmbH, Gießen, Germany), a V.A.C.® unit and the camera measuring stand. The data collected by the RGB camera will be evaluated and published in an additional paper.

2.1.1. Tissue photo spectrometry (O2C device)

The Oxygen to See® (O2C®) device (LEA Medizintechnik GmbH, Gießen, Germany) is an optical measuring device enabling the spatial and temporal detection of four different parameters characterizing microcirculation, namely, the capillary venous oxygen saturation (sO2), the amount of regional haemoglobin (rHb), the blood flow (flow) and velocity by using spectrophotometry (Krug, 2006; Walter et al., 2002). Spectrophotometry consists of a combination of two physical measurement principles, mainly the white light spectrometry and the laser Doppler flowmetry (LDF). In both cases light is emitted and detected using probes with different penetration depths, realized with different distances between emitter and detector. This study used a superficial measuring probe with a penetration depth of up to 3 mm and a deep measuring probe with a penetration depth of up to 7 mm (LFx70 and LFx37 from LEA Medizintechnik GmbH). Since the measurement is a cumulative result of all the information within the penetrated probe, information from more superficial skin layers is also included (Krug, 2006).

LDF is used to measure flow and velocity. Near-infrared laser light with a wavelength of 820 nm is brought into the tissue to be examined. The photons that are scattered or reflected on moving particles, mostly erythrocytes and mitochondria, experience a frequency shift, known as Doppler effect (Ghazanfari et al., 2002). The concentration of erythrocytes moving with a certain velocity can be determined by the intensity of the reflected laser light at a certain frequency. The Velocity is the average speed of the erythrocytes and the flow is the sum of the products of velocity and erythrocyte concentration of all velocities (Krug, 2006). Flow and velocity are dimensionless and thus given with arbitrary units (AU) (Krug, 2006).

White light with a wavelength spectrum of 500–850 nm is used to measure the sO2 and the rHb. To determine oxygen saturation, the colour of reflected light, which changes depending on the degree of haemoglobin being saturated with oxygen molecules. Emitted light is modulated in the tissue through scattering, reflection and absorption. Due to the characteristic absorption properties of haemoglobin in the saturated and unsaturated state, the oxygen saturation can be estimated from the spectrum on the photo sensor. For the calculation of the rHb, the wavelength dependent proportion of absorbed light is considered. Haemoglobin absorbs light relatively strongly and as said before, depending on the oxygen saturation, with a certain wavelength-dependent characteristic. Consequently, the amount of haemoglobin in the measuring volume influences the amount and spectral composition of absorbed light and thus also the wavelength-dependent ratio of reflected light measured by the detector in relation to the emitted light. With the help of this ratio, the regional amount of haemoglobin can be calculated with the help of pattern recognition. The composition of the

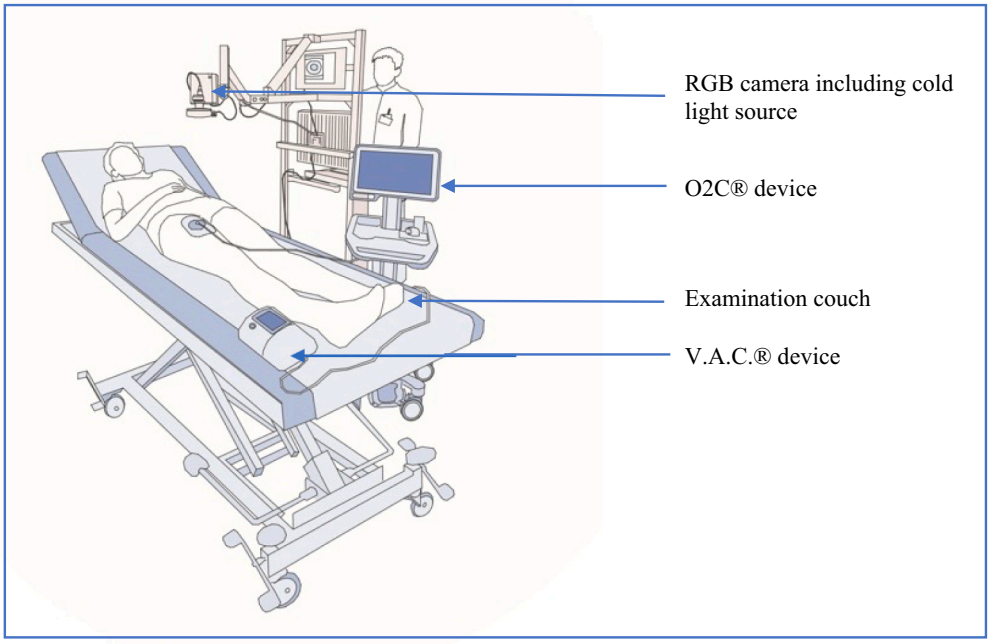

Fig. 1. Display of the measuring structure with arrangement of the measuring instruments.

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blood in the microcirculation consists of 75% venous, 14% capillary and 11% arterial blood (Krug, 2006). As the amount of venous blood in the vascular system is significantly more, the measured values for sO2 and rHb are dominated by venous proportions (Kroger et al., 2012).

The sO2 corresponds to the absolute loading of haemoglobin with oxygen and is given in percent. The rHb refers to the haemoglobin concentration per tissue volume and depends on vascular density, vascular volume and haemoglobin quantity (Kroger et al., 2012). It is a parameter that represents the total amount of blood in the investigated area, regardless of the erythrocyte functionality. Because rHb is a relative measure, the parameter is dimensionless and therefore given as AU.

2.1.2. Topical negative pressure wound therapy (TNPWT; V.A.C.® system)

In general, to produce and apply sub-atmospheric pressure on a wound surface a sealed and closed system is needed. The V.A.C.® system consists of four main components: an open-pored sponge that is inserted into or onto the surface, a semi-occlusive drape, a connecting suction tube, and a suction device with a connected collecting canister for generating the sub-atmospheric pressure. The standard range of negative pressure are 50 to 125 mm Hg compared to the ambient pressure and the application mode can be chosen between continuous or intermittent. In this study the V.A.C.® system ACTIV.A.C.™ (KCI Medizinprodukte GmbH, Wiesbaden, Deutschland) and a polyurethan sponge (V.A.C.® GRANUFOAM™) were used, with an application of continuous negative pressure of 125 mm Hg compared to the ambient pressure.

2.2. Data

This is a prospective clinical-experimental pilot study, which was conducted between January 2020 and June 2020.

As depicted in Table 1, a total number of 40 healthy individuals participated in this study, including 20 female and 20 male subjects, with an average age of 27 ± 4.06 years and average body mass index (BMI) of 23.72 ± 4.1 kg/m^2. None of the subjects suffered from cardiovascular diseases, two subjects had neurodermatitis (one in combination with cutaneous mastocytosis) and three suffered from asthma. Alcohol was consumed on an average of 1.16 ± 1.08 times per week, only one subject stated to smoke occasionally.

2.3. Protocol

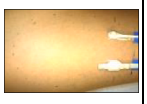
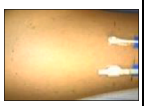
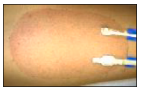
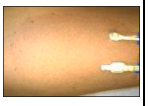
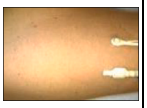
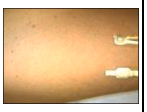
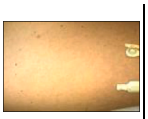
The temporal sequence of our study can be seen in Table 2. The subjects were placed on the subject couch with bare, shaved thighs, before a 15-minute rest phase was initiated. Meanwhile the measuring area was drawn in the shape of the sponge, the probes were fixed with double-sided tape and the camera (not included in this study) was raised and adjusted.

After completion of the rest phase, two measurements (preVAC1 and preVAC2) of 1,5 min each with a time gap of 4.5 min were carried out to determine the initial values. Subsequently, the sponge was fixed to the anterior thigh with a hermetic dressing (known as V.A.C.® dressing) and

Table 1
Characteristics of the participants group including the minimum, maximum and average age in years and BMI in kg/m^2, the number of smokers and the average number of days per week where alcohol is consummated.

	Total	Male	Female
Age [years]	Max. 41 Min. 22 Average 27 ± 4.06	28.8 ± 4.58	25,2 ± 2.44
BMI [kg/m^2]	Max. 42.1 Min. 18.7 Average 23.72 ± 4.1	23.99 ± 4.92	23.46 ± 3.18
Smoker	1 (occasionally)		
Alcohol [per week]	1.16 ± 1.08	1.43 ± 1.23	0.9 ± 0.85

Table 2
Schematic representation of the protocol. Each measurement lasted 90 s. In total 7 measurements per subject.

	Rest	Pre VAC1	Pause	Pre VAC2	VACON	VAC OFF0	Pause	VAC OFF15	Pause	VAC OFF30	Pause	VAC OFF60	Pause	VAC OFF90
Duration	15′	1,5′	4.5′	1,5′	30′	1,5′	15′	1,5′	15′	1,5′	30′	1,5′	30′	1,5′
Instant of time	0′	15′	16.5′	21′	22.5′	52.5′	54′	69′	70.5′	85.5′	87′	117′	118.5′	148.5′
														

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a continuous negative pressure of 125 mm Hg compared to the ambient pressure was induced for 30 min. This is marked with the abbreviation 'VACON'. Immediately after removal of the V.A.C.® dressing, a measurement was conducted, denoted by 'VACOFF0'. All subsequent measurement points refer to the time 0. The next measurement was taken 15 min after the end of the intervention and was labelled 'VACOFF15'. The following measurements took place 30, 60 and 90 min after the end of the TNPWT and were continued according to the designation scheme, i. e. with the abbreviation 'VACOFF30', 'VACOFF60' and 'VACOFF90' in the measurement protocol as depicted in Table 2. For a subject's measurement the continuous monitoring function by the O2C® device was started, during the V.A.C.® intervention it was interrupted. Marker points were set at the defined measuring points.

2.4. Statistics

Statistical analyses were performed using the computer software JASP Version 0.14.1 (University of Amsterdam, The Netherlands). As data were dependent samples with multiple testing with more than two steps (seven measurement times), the analysis of variance (ANOVA) for multiple testing was chosen to assess the parameter wise changes in the collected data over the course of time. Although the collected data is not normally distributed, the ANOVA can be applied assuming the central limit theorem. This theorem states that a superposition of independent random effects in large samples, usually assumed as $n > 30$, leads to an approximate normal distribution overall. Per measurement (lasting 1.5 min) a total of 9 average values à 10 s were calculated for each parameter. This resulted in a sample size of $n_{(t)}$ 360 and $n_{(T)}$ 2520 (respectively for velocity $n_{(t)}$ 189 and an $n_{(T)}$ 1323). Furthermore, according to actual findings the ANOVA is robust against violation of normal distribution (Blanca et al., 2017; Schmider et al., 2010). Sphericity is a necessary requirement of ANOVA with repeated measures and hence the Mauchly's test was chosen showing that the assumption of sphericity was violated. Therefore, the Greenhouse-Geisser correction was used to correct the degrees of freedom downwards. The post hoc tests provide information on whether the parameters' values at the specific measurement times differ significantly from the baseline (preVAC2). With the multiple application of statistical tests on the same data set, the probability of accepting a wrong hypothesis (α error) increases (Bortz and Schuster, 2010). Therefore, we applied a Holm correction (sequential Bonferroni) for multiple testing. Differences were considered significant at $p < 0.05$.

3. Results

There were no adverse events during or after the investigations.

Figs. 2 to 5 depict the changes of parameters detected with white light spectrometry namely rHb (superficial and deep) as well as sO2 (superficial and deep) over the period of time in boxplots.

3.1. Amount of regional haemoglobin (rHb)

Fig. 2 depicts the changes of rHb (superficial) over the period of time. The rHb (superficial) started at a baseline of 65.8 AU (standard deviation ± 10.0 AU) and increased to 84.2 AU (±11.3 AU) after a 30-minutes TNPWT which equals an increase of 27.9%. During the following 90 min of measurement the values dropped down to the end value of 73.5 AU (±9.5 AU). With $p < 0.001$ the changes before compared to after the intervention are significant.

In average, the initial value of rHb (deep) with 23.9 AU (±3.2 AU) was lower in comparison to the superficially measured values. It even decreased after the removal of the V.A.C.® dressing to 20.3 AU (±3.0 AU) which is a decrease of 14.7%. During the following 90 min the rHb went up to 25.2 AU (±3.1 AU), after 60 min it had exceeded the initial value. Fig. 3 depicts this trend. The changes from preVAC2 to VACOFF0 to VACOFF30 showed a significant decrease with $p < 0.001$ and preVAC2 to VACOFF90 showed a significant increase (overshot).

3.2. Capillary venous oxygen saturation (sO2)

Figs. 4 and 5 depict the changes sO2 (superficial and deep) over the period of time in boxplots.

Before the application of TNPWT the value of the sO2 (superficial) stated 49.8% (±10.7%). A significant increase was measured after TNPWT with the highest value of 79.0% (±11.3%) at VACOFF0 which is an increase of 58.9%. The value dropped but remained above the initial baseline with 53.0% (±10.4%). The change of sO2 (superficial) over the whole observation period was calculated as significant with $p < 0.001$.

A slightly higher value was measured for the sO2 (deep) before the intervention with 56.1% (±11.4%) compared to the superficial sO2. Differing from the superficial measured sO2, the maximum measurement was reached 15 min after the intervention with a value of 61.2% (±10.6%), then it decreased and finally dropped below the baseline at the final measuring point with 55.9% (±8.7%). Up to 60 min after TNPWT a significant ($p < 0.001$) increase of sO2 (deep) was found.

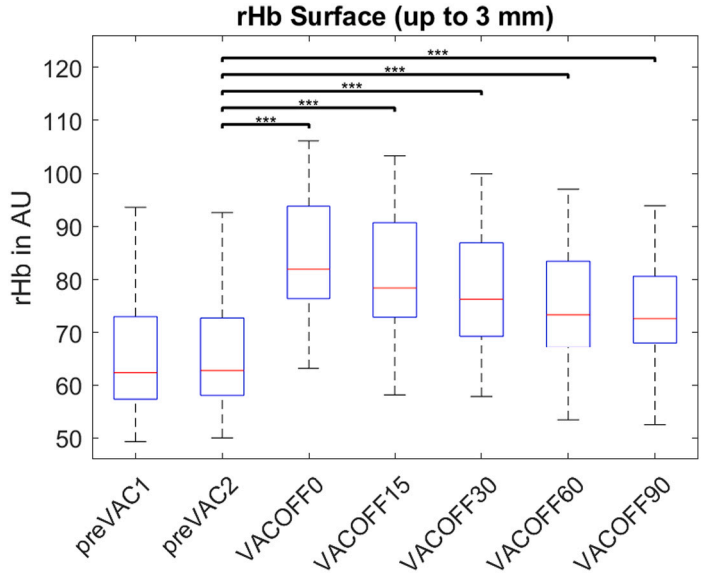

Fig. 2. Box plot depicting changes of the amount of regional haemoglobin over course of time superficially.

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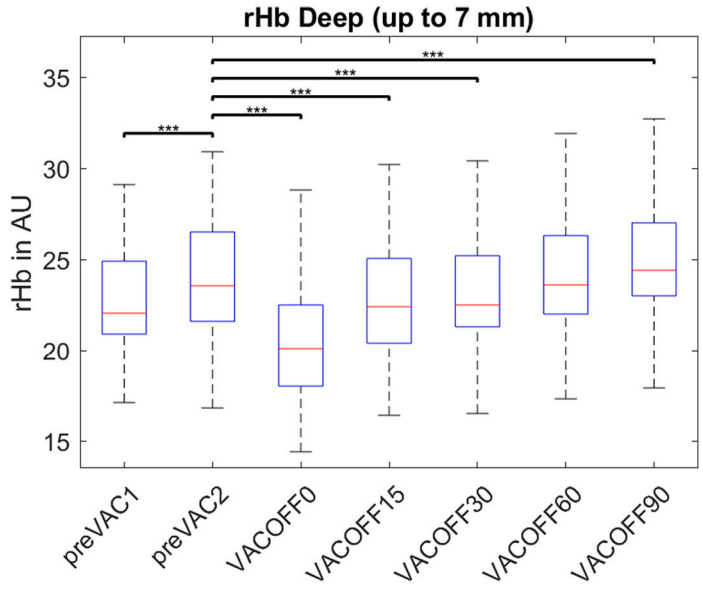

Fig. 3. Box plot depicting changes of the amount of regional haemoglobin over course of time deep.

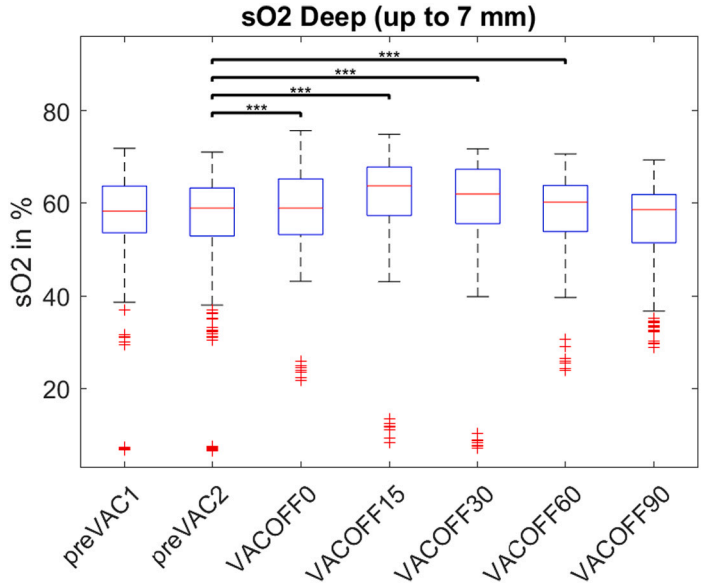

Fig. 4. Box plot depicting changes of capillary venous oxygen saturation over course of time deep.

Figs. 6 to 9 depict the changes of parameters detected with laser Doppler spectroscopy namely flow (superficial and deep) as well as velocity (superficial and deep) over the period of time in boxplots.

3.3. Local blood flow (flow)

The local blood flow (superficial) depicted in Fig. 6, showed a significant increase from 36.7 AU (±19.4 AU) to 99.3 AU (±52.6 AU) comparing before and immediately after TNPWT. This represents an increase of 170.9% compared to the baseline. Over the course of 90 min after removing the V.A.C.® dressing, the values dropped continuously, reaching a value of 45.0 AU (±25.8 AU) for the last measurement which accordingly remained above the baseline. The change in flow (superficial) from preVAC2 to each defined measuring points after the V.A.C.® intervention showed a significant change with $p < 0.001$.

The local blood flow (deep) exhibited similar results, depicted in Fig. 7 The initial value was higher starting with 75.1 AU (±25.7 AU) and increasing to 133.3 AU (±65.5 AU) after the intervention, representing an increase of 77.5%. Over the observation period the flow dropped continuously reaching its final value of 78.0 AU (±29.0 AU) after 90 min. Comparing the values of the local blood flow (deep) after TNPWT with the measured initial suction-free value (preVAC2) showed a significant change up to 60 min after the intervention with $p < 0.001$.

3.4. Velocity

This parameter was collected for 21 subjects giving additional information. Fig. 8 shows the changes of velocity (superficial) over the period of time. Superficially the baseline was measured at 15.9 AU (±3.1 AU), increased up to 18.5 AU (±4.0 AU) after TNPWT and stayed above the baseline until 60 min after the removal of the V.A.C.® dressing with 16.0 AU (±2.8 AU). Only the first three measured values after the intervention showed a significant increase compared to the baseline with $p < 0.001$ and $p < 0.05$.

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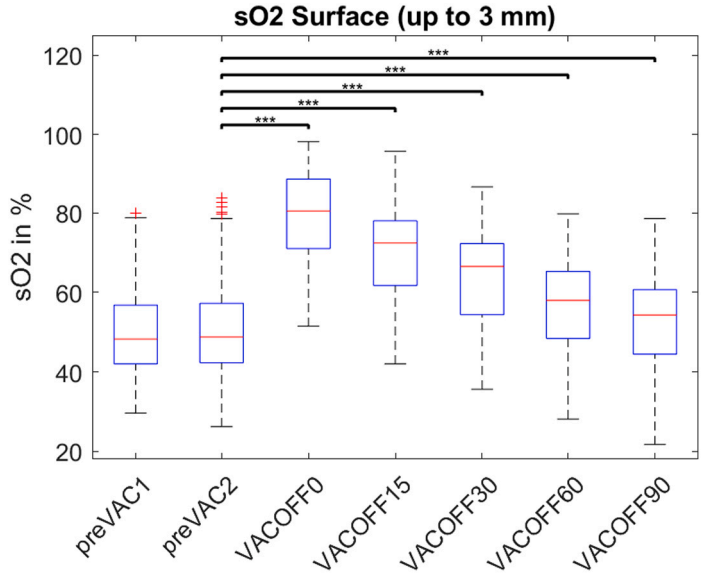

Fig. 5. Box plot depicting changes capillary venous oxygen saturation over course of time superficially.

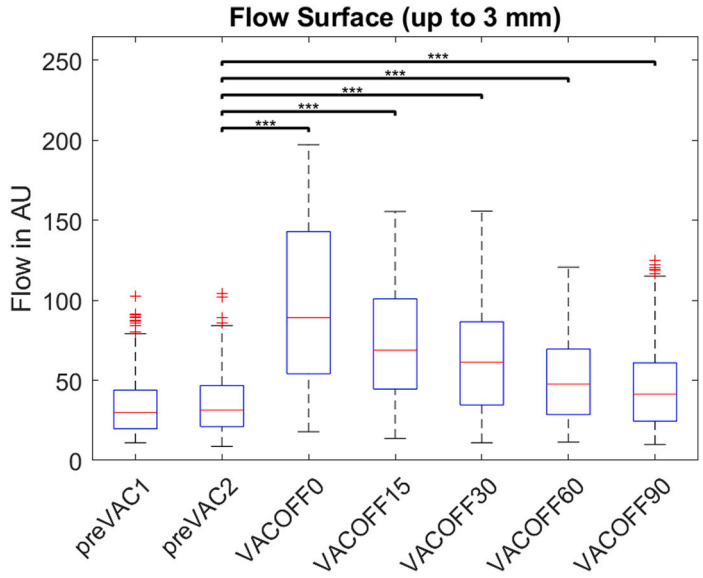

Fig. 6. Box plot depicting changes of flow over course of time superficially.

The temporal course of the deep-measured values for the velocity showed a similar behaviour (Fig. 9). The initial value was 19.6 AU (±2.6 AU). After the V.A.C.® dressing intervention there was an increase, which reached its maximum point at VACOFF15 with a value of 22.3 AU (±3.4 AU). The values remained significantly above the baseline level over the entire measurement period, with $p < 0.001$ and for the final value with $p < 0.05$.

4. Discussion

Although the clinical value of TNPWT cannot be argued and the application spectrum keeps widening, the scientific community has failed so far in describing the exact impact of negative pressure on tissue perfusion. While several studies advocate the increase of pressure in the tissues with a narrowing of the vessel diameter, other studies have described the exact opposite. Kairinos et al. (2014) first proved that the laser Doppler measurement of velocity may not be appropriate in the research of TNPWT, as the narrowing of the vessel diameter would result in increased velocity, while the actual tissue perfusion would be decreased. The same author proved in several in vitro and in vivo studies, that tissue perfusion increases during TNPWT (Kairinos et al., 2009b, 2009a).

By using the white light spectrometry in addition to laser Doppler (O2C Device), Sogorski et al. (2018) investigated the effect of intermittent TNPWT on the antero-lateral thighs of seven healthy volunteers for cycles of 10 min with a negative pressure of 125 mm Hg related to the ambient pressure alternating with 10 min without pressure. The results showed an increase in velocity and blood flow with constant sO2 during the suction intervals as well as increasing sO2 between suction intervals and a constant increase of rHb. The probes were placed under the TNPWT foam and next to it, with the probes outside the suction area showing similar changes, although not as significant as the ones under the foam. The authors discussed that their results are concurrent with the ones of (Kairinos et al.), showing an expected increase in velocity and blood flow measured with the laser Doppler, while having constant sO2 values. These results may though be interpreted differently, as an

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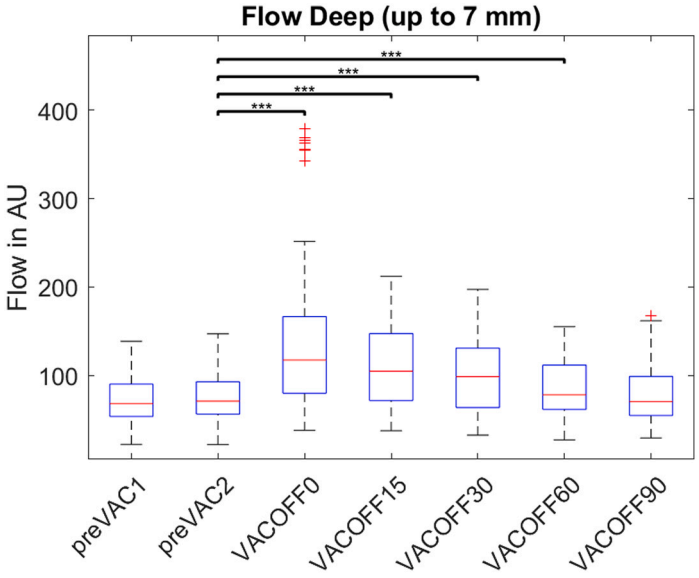

Fig. 7. Box plot depicting changes of flow over course of time deep.

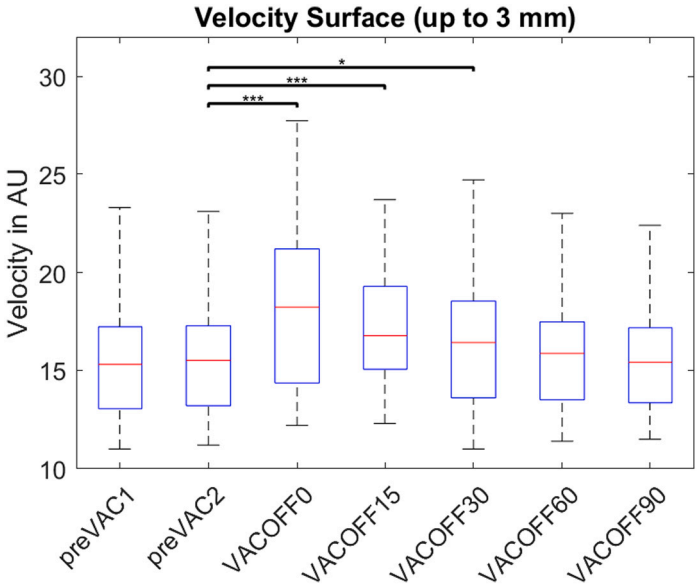

Fig. 8. Box plot depicting changes of velocity over course of time superficially.

increase in velocity and blood flow with constant sO2 would imply an increase in oxygen availability to the tissues (Zwanenburg et al., 2019). The increase in rHb in the same study was interpreted as a sign of vasoconstriction and increase in blood cell viscosity. The rHb shows though the capillary bed filling, which corroborated with the constant sO2 could also demonstrate an increase in oxygen availability to the tissues.

In another study using the O2C device, Muenchow et al. (2019) applied a probe measuring 8 mm deep under the TNPWT dressing and used two protocols to apply negative pressure for 30 or 60 min on the healthy skin of the lateral thigh of 30 healthy volunteers. This study showed that a 30-minute TNPWT interval leads to an increase of rHb, sO2, blood flow and velocity, which lasts for at least 60 min after the suction interval. Here again the O2C device has shown an increase in oxygen availability to the tissues during the suction interval. The prolongation of the TNPWT to 60 min did not show a further increase in blood flow, but rather a slight decrease.

Recent in vitro studies showed an increase in peak pressure within the foam surface of TNPWT (Biermann et al., 2020), while the Oxygen levels within the foam under negative pressure appeared to be decreasing (Biermann et al., 2019). Another novel in vitro study from (Livingstone et al., 2021) showed different results, in which the application of circumferential and near-circumferential TNPWT around a water ball decreased the pressure inside the ball. The crossover point in which the pressure in the ball started to increases under TNPWT was in experiments above the therapeutical 125 mm Hg.

Sundby et al. (2016) used an airtight chamber to induce intermittent negative pressure (40 mm Hg) to the lower leg of 23 healthy volunteers and measured the velocity with LDF. The results showed an increase in velocity with shorter suction intervals (up to 30 s) with a decrease in velocity with a 2-minute suction interval. These outcomes are inconsistent with the results of (Kairinos et al., 2014) regarding an increase in velocity with an increase in tissue pressure when applying negative pressure, but show once more that the single use of LDF in the evaluation of NPWT may be insufficient.

Our study, which included 40 healthy volunteers, aimed to

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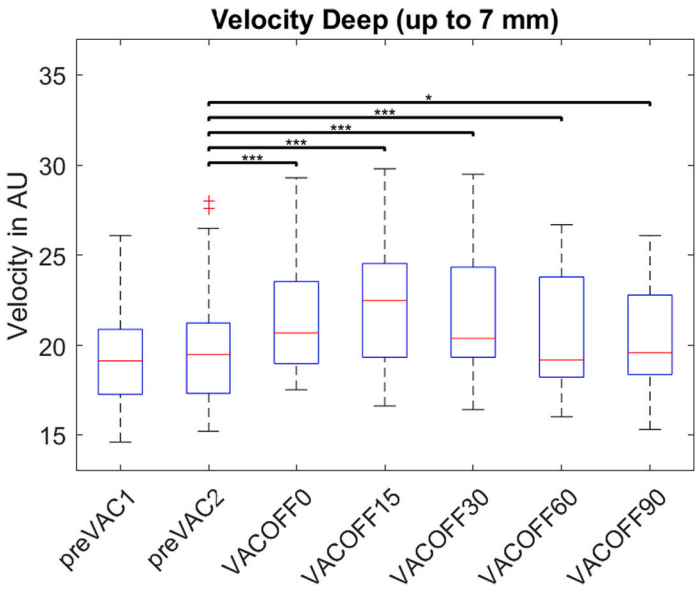

Fig. 9. Box plot depicting changes of velocity over course of time deep.

determine the time span in which TNPWT changes the tissue perfusion. As the study design has been employed before without any adverse effects (Muenchow et al., 2019; Sogorski et al., 2018), we had no concerns about a possible disturbance of the skin tissue perfusion during the treatment. Even when accepting the hypothesis that TNPWT produces pressure in the tissues, it seems that light pressure exerted on tissues produces enhanced skin circulation. In the case of tissue expanders, which are being inserted to gain an extended surface area, pressure is put on the overlying tissue. Nevertheless, an increased capillary flow was measured, as argued by (Müller-Seubert et al., 2021). By using two probes, which capture different depths of the tissue and measure four different parameters, we were able to describe more accurately the effect of sub-atmospheric pressure on healthy skin and subcutaneous tissues. As our measurements were not performed during the TNPWT, our results are not able to bring further evidence in the dispute about tissue perfusion during negative pressure treatment. The investigation aims to further clarify the changes which occur in the tissue perfusion after TNPWT as well as the time frame. Moreover, the simultaneously performed tissue perfusion measurements using camera based photoplethysmography, which will be published in a separate paper, will

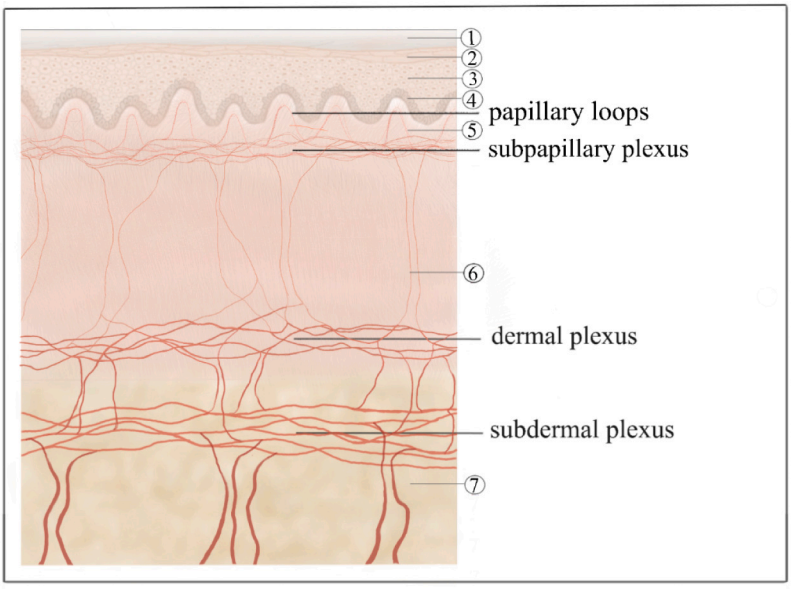

Figure 10:Schematic representation of blood flow in cutis and subcutis according to (Imanishi et al. 2008);

epidermis:

1 str. corneum

2 str. granulosum

3 str. spinosum

4 str. basale

dermis:

5 str. papillare

6 str. reticulare

7 subcutis

Fig. 10. Schematic representation of blood flow in cutis and subcutis according to (Imanishi et al., 2008); epidermis:
1 str. corneum.
2 str. granulosum.
3 str. spinosum.
4 str. basale.
dermis.
5 str. papillare.
6 str. reticulare.
7 subcutis.

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further try to describe the perfusion changes in the superficial skin layers.

The two probes used in our study are measuring the whole tissue from the skin surface downwards, measuring 3 and 7 mm respectively. The skin thickness at the anterolateral thigh measures 1.220 ± 0.257 mm and contains the subpapillary and the dermal plexus (Imanishi et al., 2008) (Fig. 10). The subdermal plexus is located at the junction between the skin and the subcutaneous tissue (Braverman, 2000), therefore the main blood supply of the anterolateral thigh skin is located within 3 mm of the surface.

The value of rHb in the superficial tissue showed a rapid significant growth with a continuous drop during the 90 min after treatment, without reaching the baseline. On the other hand, the deep probe showed first a significant drop in rHb after the intervention compared to the baseline. Afterwards it showed a growth, which exceeded the baseline after 60 min, remaining significantly above the baseline after 90 min. While the superficial probe measures only the highly vascularized skin tissues, the deep probe encompasses in its measurements the skin and the deeper, less well-vascularized, fatty tissue. The increase in rHb in the superficial probe could be explained by an increased concentration of blood in the dermal plexuses on account of depleting the blood from the subcutaneous tissue. Subsequently a local superficial hyperaemia might occur, which increases the overall blood supply, maintaining the rHb above the baseline over the measured 90 min.

The sO2 showed a highly significant increase of 58.9% in the superficial probe, remaining significantly above the baseline after 90 min. At the same time, the deep probe showed a slightly higher baseline value, which could be explained by the slower basal metabolism and oxygen uptake rate of fatty tissue in the femoral region. Frayn and Karpe (2005) have shown that the local blood flow in the fatty tissue is dependent on the metabolic activity, increasing with the need for fat storage after a meal ingestion or lipolysis like in fasting or with high physical activities. There are regional differences concerning fat tissue blood flow, the thigh fatty tissue having less blood flow compared to the abdominal fat tissue, which is also more susceptible to hormonal influences. In our study the intervention in the deep probe showed a significant increase in sO2 after 15 min which lasted for only 60 min, after which it dropped below the baseline. These measurements indicate a lasting hyperaemia in the skin, while the less vascularized and less metabolic active fatty tissue appears to return faster to physiological values.

On the one hand, the flow in the superficial probe showed an increase of 170.9% directly after the intervention, remaining significantly above the baseline for at least 90 min. On the other hand, the deep probe showed a significant increase in blood flow for only 60 min, before dropping back to the baseline. The increased baseline values of the deep probe compared to the superficial probe may be explained by the larger vessel calibre in the subcutaneous tissue, with higher flow compared to the skin plexuses, where the individual vessel calibre progressively drops simultaneously with the flow. In 21 subjects a fourth parameter, the velocity was measured. In the superficial probe the velocity increased significantly for the first 60 min, while the deep probe showed a significant increase over 90 min. As the baseline shows, the velocity is higher in the deeper probe, where larger vessels are being measured. While the velocity in the skin vessels also increases, it does not last as much as the increase in sO2 and rHb. As the measurements were made before and after the TNPWT, there is no expected increase in tissue pressure and therefore the LDF measurements ought to be accurate.

(Jani et al., 2021) showed in hamsters in a dorsal window chamber with a negative pressure application device using intravital microscopy, that functional capillary density and venular outflow improve under 4 mm Hg constant negative interstitial pressure in normovolemia and in haemorrhagic shock. In both situations, the mean capillary perfusion was improved by increasing the venular outflow while maintaining the arterial inflow. This confirms the results of our study, which showed improved oxygen availability to the cells in the skin as well as in the superficial subcutaneous tissues.

The study has several limitations. By performing the measurements before and after the TNPWT, the research does not bring additional information to the dispute concerning tissue perfusion during TNPWT. The two probes, while offering valuable information from two tissue depths, measure the sum of all tissues encountered and not a separate analysis of the skin and subcutaneous tissue perfusion. As the measured parameters were partly still increased at the end of the investigation, we cannot state how long do the changes in perfusion last.

In conclusion, our study showed that sub-atmospheric pressure significantly increases the tissue perfusion, expressed in rHb, blood flow, sO2 and velocity, over 90 min after a treatment of 30 min. While rHb and sO2 remain increased above the physiological values for at least 90 min, the blood flow and the velocity tend to return to the baseline values within 60 to 90 min. Using the two different depth measuring probes we could show significant differences between the baseline values and in reaction to the intervention. This could be attributed to the particularities of skin and subcutaneous tissue anatomy, with superficial sO2 and rHb showing the most prominent increase over time.

The clinical application of this local increase in blood flow could be the implementation of the TNPWT in the preconditioning of the skin before surgery in high-risk patients and the adjuvant treatment of chronic wounds with sub-atmospheric pressure. Further measurements are needed to investigate the local perfusion for longer periods, in order to determine the complete duration of increase in tissue perfusion.

CRediT authorship contribution statement

Olimpiu Bota: Conceptualization, Methodology, Investigation, Project Administration, Funding acquisition, Writing – Original Draft, Review & Editing.

Judy Martin: Investigation, Validation, Formal analysis, Visualization, Writing – Original Draft.

Alexander Hammer: Investigation, Validation, Formal analysis, Writing – Original Draft.

Matthieu Scherpf: Methodology, Investigation, Validation, Writing – Original Draft.

Klaus Matschke: Conceptualization, Resources, Funding acquisition, Validation, Writing – Original Draft.

Adrian Dragu: Conceptualization, Methodology, Resources, Funding acquisition, Validation, Writing – Original Draft.

Hagen Malberg: Conceptualization, Methodology, Resources, Funding acquisition, Validation, Writing – Original Draft.

Declaration of competing interest

We have no conflict of interest to declare.

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