Photoacoustic imaging of real-time oxygen changes in chronic leg ulcers after topical application of a haemoglobin spray: a pilot study

• **Objective:** To use a non-invasive measurement of oxygen saturation in chronic leg ulcers after the application of a topical haemoglobin spray to investigate if photoacoustic tomography is able to measure the oxygen saturation and if the stimulated oxygen increase can be demonstrated.
• **Method:** We measured the oxygen saturation of the ulcer tissue in five patients with chronic leg ulcers before application and 5 and 20 minutes after application of the haemoglobin spray, using photoacoustic tomography as a new method to assess oxygenation in real-time.
• **Results:** The average oxygen saturation showed a significant increase from 56.4% before to 69% (p=0.042) after 5 minutes and 78.8% (p=0.043) 20 minutes after the topical haemoglobin application.
• **Conclusion:** The oxygenation status of chronic, hard-to-heal wounds is gaining increasing interest in modern wound therapy. Topical haemoglobin spray is a new and effective method to increase the oxygenation in the ulcer tissue, but until now the link between clinical results and the mode of action was unclear. We were able to show for the first time that the use of a topical haemoglobin spray leads to an increase in oxygen saturation *in vivo* using photoacoustic tomography.

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chronic venous leg ulcer; haemoglobin spray; photoacoustic imaging; ultrasound

Chronic wounds are difficult to treat due to little or no healing over prolonged periods of time. The exact mechanisms that prevent the healing of chronic leg ulcers are yet to be identified,[1] but local oxygenation is getting increasing attention in new wound care strategies.[2–5] It is known that up to 70% of chronic leg ulcers have a venous component, leading to impaired blood flow and oxygenation.[6] As oxygen is a prerequisite for cell proliferation and protein synthesis during tissue repair, this might be one of the key problems that impede healing of chronic venous leg ulcers.

Previous studies have shown improved wound healing through application of oxygen by various methods. Hyperbaric oxygen therapy (HBOT) effectively increases blood and tissue oxygen levels and promotes wound healing under specific conditions.[2,3] However, it is expensive, uncomfortable and can even be dangerous, due to the risk of fire.[4] The use of a topical haemoglobin spray is an emerging new treatment with promising effects in early clinical studies.[5] The haemoglobin is applied directly as a spray on the wound surface and works as a transport vehicle for the oxygen from the ambient air into the wound tissue, as the exudate alone acts as a diffusion barrier for oxygen. The method is easy, safe, less expensive than HBOT, and accessible for in-home use. Although an increase in the oxygen saturation is anticipated, real-time monitoring and systematic studies were difficult, due to the lack of appropriate non-invasive imaging tools.

Commonly used techniques are not able to provide 3Dimensional (3D) information on the heterogeneous tissue oxygenation in chronic leg ulcers. Photoacoustic imaging (PAI) could help overcome this limitation as a new, hybrid imaging modality that combines the advantages of optical and ultrasonic imaging. Pulsed laser light with a specific wavelength is applied to the tissue. The target molecules absorb the laser energy and the short temperature rise leads to a thermo-elastic expansion, which can be detected as ultrasound wave.[7] As oxygenated and deoxygenated haemoglobin have different optical absorption properties, multi-wavelength PAI can provide 3D information about the local oxygen saturation (StO_2) without the use of exogenous contrast agents.[8–11] PAI data can be used to create a 3D volume with exact information about the oxygenation status in the leg ulcer at resolutions equal to ultrasound imaging.[8–10]

The aim of the study was to investigate whether it is possible to assess the local StO_2 in the tissue of chronic leg ulcers in real-time and 3D using

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multiwavelength PAI and whether the application of topical haemoglobin spray leads to an increase in ulcer tissue StO_2 *in vivo*. We used PAI monitoring of StO_2 for the first time in chronic leg ulcers before, during and after treatment.

Materials and methods

Study design

This cross-sectional clinical trial was approved by the institutional review board at the University Hospital Essen (13-5687-BO) and registered at the German Clinical Trials Register (DRKS00005993). All experimental procedures were in accordance with the Declaration of Helsinki.

All patients received application of topical haemoglobin spray (Granulox, SastoMed, Georgsmarienhütte, Germany) and PAI between March and August 2014. The PAI was performed before treatment, 5 minutes after application and 20 minutes after application of the topical haemoglobin spray. All patients provided written informed consent to participate in this trial. The topical oxygen spray is an approved medical device class III in Germany

Inclusion criteria

Patients over 18 years old with hard-to-heal chronic leg ulcers that persisted for more than 8 weeks, despite adequate treatment of underlying causes, were included. As adequate treatment we define sufficient diagnostics to find the underlying causes of the chronic ulcers and modern moist wound management as carried out by our certified wound clinic in the University Hospital Essen. A further inclusion criterion was a wound size larger than $4cm^2$.

Exclusion criteria

Exclusion criteria included acute wounds, patients with dependencies, allergies against any ingredient of the haemoglobin spray and those pregnant or lactating. An infection of the wound, sclerosis of the wound ground and severe systemic diseases with an American Society of Anaesthesiologists (ASA)>4, such as renal failure or cancer, led to exclusion as well. For all patients, severe sclerosis was diagnosed by a previously performed biopsy of the ulcer tissue. Sclerosis is a hardening of the tissue caused by chronic inflammation that leads to tissue alteration and necrosis of adipocytes. Sclerotic tissue is not able to absorb oxygen and working as barrier to oxgyen diffusion.

Topical haemoglobin solution

The purified haemoglobin used in this trial was produced from porcine blood and formulated as an aqueous 10% solution (10% carboxyhaemoglobin, 0.7% phenoxyethanol, 0.9% sodium chloride, 0.05% N-acetylcysteine, water (WFI, sterile). After the virus removal (heat inactivation and nano filtration) the solution was provided as a ready-to-use spray in bagon-valve canisters by the manufacturer. The haemoglobin was sprayed onto the wound surface from a distance of 5-10cm, after wound cleansing with sterile compresses.

Photoacoustic imaging

The PAI was performed by using the Vevo Lazr (FujiFilm VisualSonics) 21-MHz linear array transducer system (central frequency), integrated with a tunable nanosecond pulsed laser.[11] The laser light was passed to the ultrasound transducer through fibre optic cables. PAI were collected at a laser light wavelength of 750nm and 850nm, and the parametric maps of StO_2 were calculated based on a previously reported and tested algorithm.[12] All other parameters were constant during the different measurements, to guarantee the reproducibility of the data.

The system was calibrated precisely by the manufacturer before starting the data acquisition. The 3D data sets were collected by linearly translating the transducer with a stepper motor over a region of approximately 10mm length, while capturing two-dimensional (2D) images of the 3D stack. Altogether, 67 2D frames were recorded in approximately 2 minutes and put together to provide 3D images of the region of interest. The measurements were performed in the middle of the chronic ulcer and the starting point for the PAI transducer was marked for the following measurements. Ultrasound gel was applied to the wound area. The ultrasound transducer was used in conjunction with an Aquaflex gelpad to reduce the pressure on the wound. The stepper motor is used to create 67 consecutive images on a length of approximately 10mm and every measurement takes about 2 minutes. The first 3D measurement was conducted before treatment. Afterwards, the wound was cleaned from the ultrasound gel and the haemoglobin spray was applied topically, as stated in the manufacturer`s instructions. After 5 minutes and 20 minutes, we conducted another 3D measurement, as described before. Using the collected 3D data, the mean StO_2 was calculated in a superficial layer of about 1cm depth using the software provided by VisualSonics. The wound surface itself was excluded, to avoid an influence of the measurement by the topical haemoglobin spray.

During the measurements, the patient was lying down and advised not to move their lower extremities. The ulcer was not covered, except for the time of the measurements. The applied thin layer of ultrasound gel was removed after each measurement.

Adverse events

The subjects' safety was ensured during this cross-sectional trial according to the European standards for clinical trials on medical products in human subjects. All adverse events were documented and all

measurements and follow-up examinations were performed by a physician.

Statistics

The tissue StO_2 before and 5 minutes as well as 20 minutes after the treatment was statistically compared using the Wilcoxon signed-rank test with a significance level of 0.05 using SPSS Statistics (IBM, Version 22) as a non-parametric test comparing two related samples.

Results

Patient characteristics

No patient was excluded due to exclusion criteria and five patients were consecutively recruited. All were female and aged between 48 and 89 years old (mean: 80.2 years old). The patients had been treated in our certified wound clinic for between 32–119 months (mean: 63.2 months) and the wound sizes were between 9 and 56 cm^2 (mean: 21 cm^2, Table 1). Of those included, three patients had diabetes mellitus.

Table 1. Acquired patient data

Patient number	Age (years)	Wound size in cm^2	Oxygen saturation in %		
			Baseline	5 minutes	20 minutes
1	57	56	65	90	96
2	48	15	74	84	85
3	89	10	36	37	50
4	79	15	52	78	76
5	38	9	55	56	87
mean	80.2	21.0	56.4	69.0	78.8
p-value compared with baseline				0.042	0.043

Adverse events

There have been no side effects due to our procedure in any patient, neither through the topical application of the haemoglobin spray nor the measurement procedure with the PAI device.

Photoacoustic imaging for StO_2

In all five patients we measured an increase of StO_2 in the tissue of the leg ulcer 5 minutes and 20 minutes after application compared with the initial values (Fig 1). The mean StO_2 was 56.4% (median: 55%, standard deviation (SD): 14.3%) before, 69% (median: 78%, SD: 22.0%, p=0.042) five minutes and 78.8% (median: 85%, SD: 17.6%, p=0.043) 20 minutes after application of the haemoglobin spray (Fig 2).

Discussion

Several methods have been used to measure tissue oxygenation. Direct measurements with pO_2 histography[13,14] using a sterile polarographic needle electrode have been performed, but this invasive technique can only provide data about the tissue adjacent to the electrode. Reflectance spectroscopy and diffuse optical tomography as non-invasive methods were used to monitor changes in blood oxygen saturation. However, this is an indirect method, only representing the tissue oxygenation status to a certain degree.[15,16] Luminescence dual sensors oxygen-imaging can provide non-invasive information about the oxygenation in leg ulcers as well,[1,17,19] but the data obtained provides only a 2D image of the wound surface. The PAI has the capability to overcome these limitations. It has been showing promising results as new imaging technique in various fields of application.[9,20,21] Regarding wound healing, the feasibility of photoacoustic imaging to monitor the healing process of burns in rats has already been shown.[22] The time-dependent recovery of blood perfusion in the healing wounds and the change in the peripheral haemodynamics was examined to gain additional information about the progression of the healing process. Although the feasibility of PAI in pre-clinical research has been demonstrated a number of times,[8,12,22] the transla-

Fig 1. 3D images of the leg ulcer of patient 2 with StO_2 visualisation. Dots represent 1mm. Before application (a), 5 minutes after application (b), 20 minutes after application of the haemoglobin spray (c). Blue pixels represent low oxygenation, red and white pixels represent high local oxygenation.

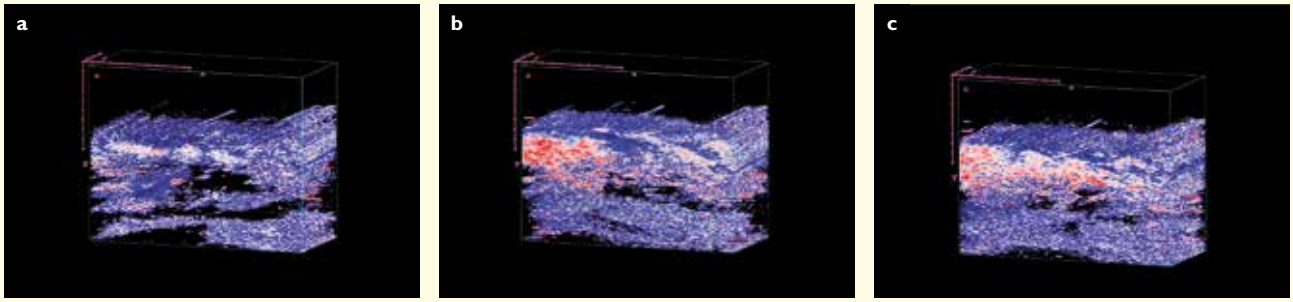
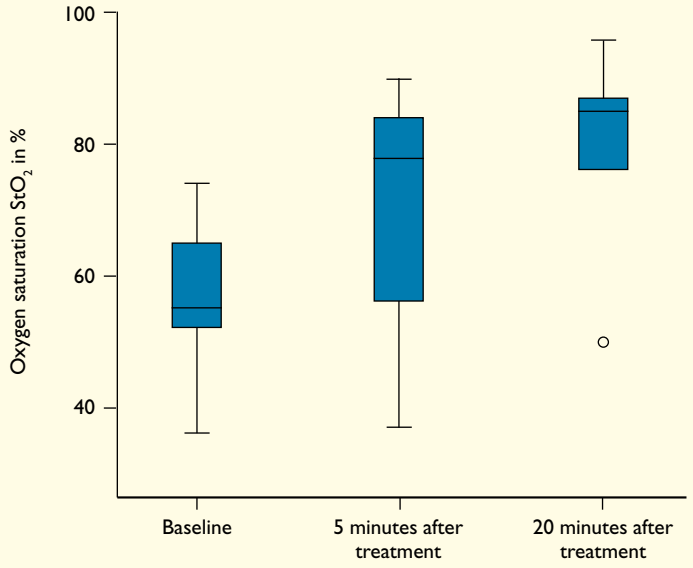


Fig 2. Mean oxygen saturation before and after application of haemoglobin spray. Boxplot with whiskers for minimum and maximum values.



tional aspect of the technology was limited until recently, due to the shape and size of the systems.

Our results show the effects of the topical haemoglobin spray in a clinical setting. Furthermore, the oxygen-increasing effect of the spray is not only assessable on the surface, but also in the deeper tissue (Fig 1). These results proved that topical haemoglobin acts as a transporter vehicle for oxygen from the ambient air to the wound ground *in vivo*. This local increase of oxygenation in the ulcer tissue may improve the healing process, as an explanation for positive results shown in clinical trials.[5]

The initial results of this pilot study indicate the great potential of PAI to assess the healing process potential of wounds by detecting the early changes in oxygenation and improving the patient care path by enabling personalised medical decisions. The non-invasive PAI measurements did not lead to any discomfort or side effects, making this technique highly accepted by the patients. The new possibility to study the effects of treatment options that promote a local increase in StO_2 in real-time and 3D in chronic wounds in particular are unmatched by existing techniques.

Even the likelihood of a successful healing process under a certain therapeutic modality might be predicted through PAI measurements in the future. If adequate surrogate markers can be found, prolonged conservative wound therapies without any benefit for the patient could be avoided, with a substantial benefit for the patient and lower treatment costs.

Limitations of the study

For technical reasons, the measurements were limited to 20 minutes after application; thus we have no information over longer time periods. As the haemoglobin spray is usually used only once a day, further studies about the oxygenation change should be considered, to evaluate the effectiveness of the haemoglobin spray after several hours and possibly days. Also the low number of included patients is a limitation and further measurements will be conducted to verify our findings. Finally, the age and wound situation of each patient may lead to a varying oxygenation increase, which may limit the interpretation of our results in such a small population.

Conclusion

In the present investigation, we were able to show the clinical application of PAI in the real-time monitoring of the oxygenation status in chronic wounds. Our data indicate that the topical application of a haemoglobin spray leads to a distinct increase of the local oxygen saturation in patients with chronic ulcers without severe sclerosis. Even in this limited study population of 5 patients, the increase of local oxygen saturation was significant after 5 minutes as well as after 20 minutes. To our knowledge, this is the first time that the propagated oxygen-increasing effect of topical haemoglobin spray was measured in chronic leg ulcer patients *in vivo*. Trials with more participants will be needed to confirm our preliminary findings. ■

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