Biosensors and Bioelectronics 280 (2025) 117442

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

New sensor options for smart fracture implants and wearable devices: Laser-Doppler and white-light spectroscopy allow monitoring of bone regeneration via perfusion measurement

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ARTICLE INFO

Keywords:
Bone fracture
Digital health
Microcirculation
Nonunion
Perfusion
Rehabilitation
Tibia

ABSTRACT

The diagnostic options for monitoring fracture healing are currently limited to methods that expose patients to ionizing radiation, i.e. X-rays or computed tomography. The development of new methods that ideally allow continuous monitoring via smart implants or wearables is urgently needed. Laser-Doppler and white-light spectroscopy, non-invasive light-based methods, could allow to monitor fracture healing via changes in perfusion, but this has never been investigated. It was hypothesized that 1) blood flow (BF) increases before a linear increase in oxygen saturation (SO_2) and that 2) SO_2 in nonunion cases remains as low as the minimum in union cases. A longitudinal observational cohort study with tibial fracture patients was conducted with additional cross-sectional measurements in nonunion patients and healthy controls. To assess SO_2, relative haemoglobin amount (rHb), and BF in the fracture gap, the 'Oxygen to see' (O2C) device was used. Thirty-five patients (20 longitudinal, 15 nonunion) and 28 controls were included. In the longitudinal group, SO_2 decreased, reaching a minimum (10 mm: 17.96 days, 16 mm: 15.50 days), and subsequently increased. BF increased to a maximum (10 mm: 12.90 days, 16 mm: 33.51 days), followed by a decrease. The SO_2 values in the nonunion group were similar to the minimum values in the longitudinal group. Findings in nonunion patients vs. controls differed only in SO_2 (10 mm: $p < 0.001$, 16 mm: $p = 0.038$), not in rHb or BF. Laser-Doppler and white-light spectroscopy provide characteristic SO_2 and BF trajectories that may serve to monitor fracture healing.

1. Introduction

In routine clinical practice, fracture healing must be monitored to detect implant failure and to determine whether healing is progressing. Fracture healing is usually monitored by the clinical appearance combined with X-ray-based imaging, which has multiple disadvantages. These include the exposure to ionizing radiation that is associated with a risk of cancer (Pacheo and Stock, 2013) and a delayed representation of healing progress (Blokhuis et al., 2001). This delay is caused by the fact that X-ray-based imaging only shows the calcium phosphate content of the bone or fracture tissue, which is not the only factor that determines bone stiffness (Hart et al., 2017). In addition, due to the exposure to ionizing radiation and the associated risk of cancer, X-ray based methods are neither suitable for continuous monitoring with a wearable device nor a smart implant. As it is often uncertain whether a fracture will heal or not, a common approach is to wait and observe it for several months (Özkan et al., 2019). Fractures fail to heal in 5–10 % of cases (Zura et al., 2016), resulting in nonunion, with high socioeconomic costs (Hak et al., 2014; Rupp et al., 2018). In addition to these aspects relevant for clinical practice, interventions used to treat or prevent nonunion, which are thought to improve or accelerate fracture healing, cannot be readily studied in human patients and animals *in vivo* owing to a lack of available methods that allow live monitoring and quantification of the fracture healing progress (Ganse, 2024; McCarthy, 2006). However, *in-vivo* studies on fracture healing delays and accelerating interventions are urgently needed to improve clinical practice (Ganse, 2024; Ganse et al.,

This article is part of a special issue entitled: Continuous Monitoring Biosensors published in Biosensors and Bioelectronics.

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https://doi.org/10.1016/j.bios.2025.117442
Received 15 November 2024; Received in revised form 17 March 2025; Accepted 2 April 2025
Available online 3 April 2025

2022). Instead, this research is often conducted in mice or sheep that are sacrificed to study fracture healing progress via methods such as mechanical testing, histology, and immunohistochemistry (Menger et al., 2023). Another method used *in vivo* is to measure changes in stiffness through instrumented implants (Augat et al., 2014). Such smart implants for continuous monitoring of fracture healing are currently also being developed to be used in clinical practice (Ganse et al., 2022). A noninvasive and easy-to-use method that allows to monitor fracture healing progress and to detect nonunion earlier in humans *in vivo* would be highly desirable for clinical practice and research alike. Currently, no such technology is available for clinical practice. The only study showing longitudinal *in-vivo* human changes in fracture oxygenation used near-infrared spectroscopy (NIRS) and was recently published by us (Nowicki and Ganse, 2024). In a cross-sectional ultrasound study in human patients with tibial fractures with and without union, nonunion was associated with lower blood flow than union (Fischer et al., 2020). Thus, combined laser-Doppler- and white-light-spectroscopy-based perfusion measurements may be capable of distinguishing between union and nonunion. Such measurements have been proposed for *in-vivo* perfusion monitoring of the fracture gap in patients (McCarthy, 2006; Shymkiw et al., 2001) and were recently shown to detect changes in fracture oxygen saturation (SO_2) in severely injured patients (Koch et al., 2023). However, longitudinal human data obtained with this technology over the course of healing have never been published.

Laser-Doppler spectroscopy relies on the Doppler effect, where light undergoes a frequency shift when scattered by moving particles such as red blood cells in microcirculation. The shift in light frequency is proportional to the velocity of the moving particles, here the red blood cells. By analysing the interference between shifted and unshifted light, laser-Doppler spectroscopy provides real-time measurements of blood flow and perfusion dynamics (Jayachandran et al., 2016). Among other indications, laser-Doppler spectroscopy, also called Laser-Doppler flowmetry in the clinical literature, is widely used in plastic surgery to monitor free skin flaps after transplantation (Shiue et al., 2025) and to diagnose and monitor peripheral artery disease (Chen and Rosenson, 2018). High-definition white-light spectroscopy utilizes broadband white light to assess tissue composition through absorption and scattering (Wunder et al., 2005). Absorption follows the Beer-Lambert Law, where chromophores like haemoglobin and oxyhaemoglobin exhibit distinct spectral signatures, enabling quantification of oxygenation and metabolic activity. Scattering is governed by Mie and Rayleigh theories, with wavelength-dependent scattering coefficients providing structural information about tissue organization and mineralization (Fox et al., 2013; van der Laan et al., 2021). It is frequently used to assess soft tissue oxygenation, including the skin, subcutaneous and muscle tissue (Brandl et al., 2024; Forst et al., 2008; Ganse et al., 2019). In summary, laser-Doppler spectroscopy provides dynamic blood flow measurements, while white-light spectroscopy gives static tissue composition data. This dual approach offers a non-invasive comprehensive assessment of fracture healing by simultaneously monitoring vascularization and biochemical changes.

Blood flow (BF) correlates with the extent of endosteal new bone formation (McInnis et al., 1980). The vascular system in bone comprises afferent and efferent vessels, as well as a microvascular network with anastomoses, including a central artery in the bone diaphyseal marrow, two capillary networks and the lacunar-canalicular system in compact bone, and sinusoids in the bone marrow (Kiaer, 1994; McCarthy, 2006; Stegen and Carmeliet, 2018). Bone fracture leads to hypoxia via disruption of the bone vasculature combined with further disturbance of the oxygen supply by injury to the surrounding soft tissues (McCarthy, 2006). In addition, surgery may further diminish the blood supplied to the fractured bone (Greksa et al., 2021). The onset of hypoxia is thought to occur within the first day after fracture or osteotomy (Epari et al., 2008). In Wistar rats, transverse femoral osteotomies lead to an approximately 50 percent reduction in total bone BF and an approximately 40 percent reduction in cortical BF in the diaphysis (Grundnes and Reikerås, 1992). The total bone BF then doubled after four weeks into the healing process. Several other longitudinal animal studies in rabbits and dogs confirmed increases in bone BF several weeks into healing, which showed the effects of revascularisation (Hackenbroich et al., 2008; Jain et al., 2000; Laurnen and Kelly, 1969). All these studies were conducted in animals. In the development of smart implants, longitudinal human data on changes in oxygenation, haemoglobin and BF in the fracture gap are required to determine whether or not these measurements may be of interest for continuous monitoring via an implant or wearable device.

Several types of nonunion with differing underlying causes exist, including atrophic and hypertrophic nonunion caused by either physiological (e.g., lack of blood supply) or biomechanical (e.g., instability and excess forces) factors (Rupp et al., 2018). Based on the current diagnostic methods, it can take months before it is certain a fracture is not healing, which becomes obvious when callus mineralization of the fracture gap is delayed (Bowers and Anderson, 2024). Therefore, nonunion is usually not diagnosed until six to nine months after the injury (Özkan et al., 2019; Qvist et al., 2021). Several definitions of nonunion exist (Cunningham et al., 2017; Özkan et al., 2019; Qvist et al., 2021). In daily clinical practice, just as in this study, the authors diagnose and define a nonunion for study inclusion if no union is evident based on the X-ray imaging and/or computed tomography after 6 months.

This study presents the first longitudinal clinical observational laser-Doppler and white-light spectroscopy perfusion data on tibial fractures in human patients. In the present investigation, longitudinal findings were compared with cross-sectional data from diagnosed nonunion patients and healthy control participants (control group). On the basis of the described studies from animal and human research, the following hypotheses were proposed: 1. BF increases preceding a linear increase in SO_2 during fracture healing following tibial fractures in human patients, and 2. The SO_2 readings in nonunion cases remain as low as the minimum values in the trajectory of fractures with union.

2. Materials and methods

Ethical approval was obtained from the institutional review committee of the Saarland Medical Board (Ärztekammer des Saarlandes, Germany, application number 127/22). Written informed consent was obtained according to the Declaration of Helsinki prior to the start of the experiments. This study was registered with the German Clinical Trials Register (DRKS00031942). Participation in this purely observational study did not affect the patients' treatment.

2.1. Patients and healthy control participants

Fig. 1A shows an overview of the three groups measured. Patients 18 years of age and older who had tibial shaft and distal tibial fractures were recruited for the longitudinal cohort during the first days of their inpatient stay after surgery at Saarland University Hospital between October 2022 and April 2024. The inclusion criteria were the ability to provide written and verbal informed consent, age 18 years and older, a newly obtained fracture of the tibia, and no additional more serious fractures or injuries. The exclusion criteria were the inability to give written and verbal informed consent, age under 18 years, and the impossibility of carrying out the measurements in the fracture gap. Follow-up measurements were conducted repeatedly during the patients' in-hospital stay and every time they returned to the outpatient clinic. Outpatient visits were usually scheduled at approximately two, three, and six weeks after surgery, but the exact duration of visits varied among patients. The authors considered this frequency of measurements sufficient for the hypothesized SO_2 and BF trajectories following fracture (Fig. 1B). This pattern and the frequency of regular visits to the outpatient clinic resulted in the time intervals used for data analysis illustrated in Fig. 1C. The fracture patients were included independent of the

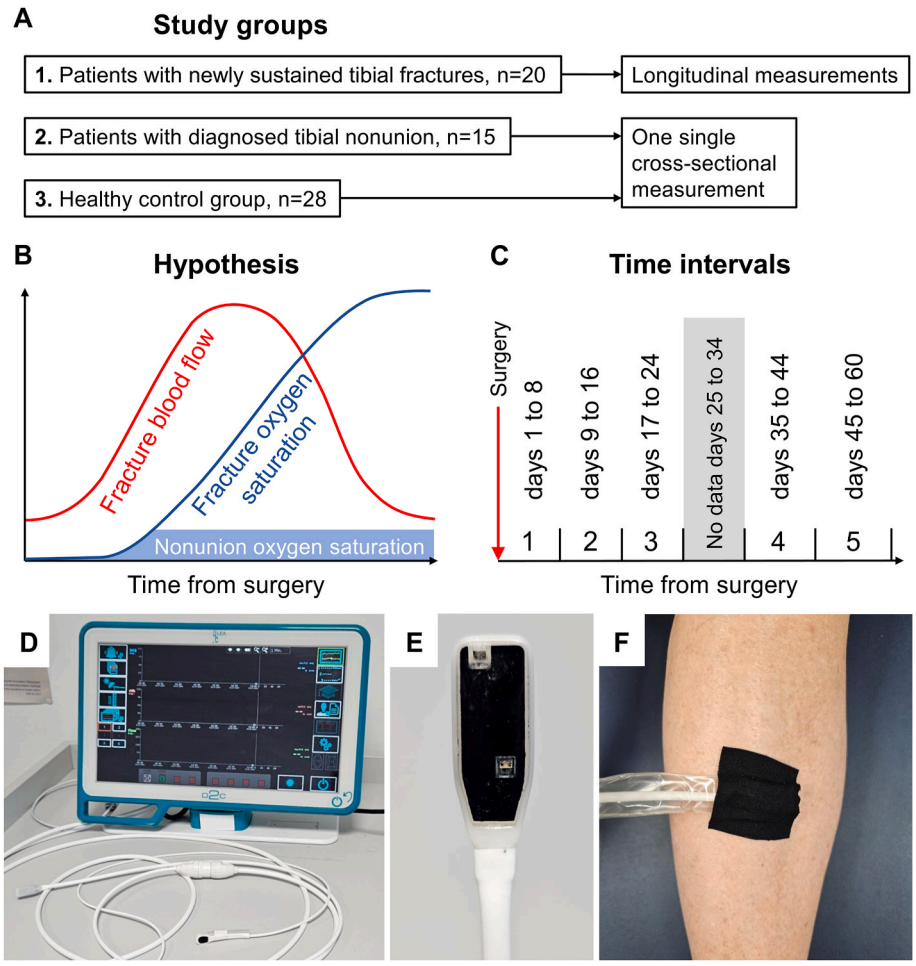

Fig. 1. Methods overview. (A) Overview of the three study groups. (B) Hypothesized trajectories of the fracture BF and SO_2 and the nonunion SO_2. (C) Time intervals for the longitudinal analyses. While the patients were in the hospital (with different lengths of stay), measurements were conducted every 2–3 days. After discharge, the patients returned for outpatient visits approximately two, three and six weeks after surgery, which caused a gap in measurements between days 25 and 34. (D) The O2C device with light fibres and (E) probes including a laser and a white light source that were (F) fixed on the skin over the fracture in a polyurethane cover and with black kinesiology tape for ambient light protection and to standardize the contact pressure.

implant type used.

In addition, a cross-sectional cohort of patients with a diagnosed tibial nonunion was recruited at least six months after the fracture for a single measurement when they presented to the outpatient clinic. These patients received one measurement to compare their findings to the trajectories of the longitudinal group and to assess whether it is possible to predict nonunion from the data. In detail, Saarland University Hospital provides an outpatient clinic specifically for patients with fracture nonunion to plan and conduct their further treatment, usually revision surgery. The patients are mostly referred from other hospitals or local health care providers to this outpatient clinic. The inclusion criteria for the nonunion group were the ability to provide written and verbal informed consent, age 18 years and older and a diagnosed tibial nonunion at least 6 months after the fracture occurred, that has not yet been revised in a revision surgery. The exclusion criteria were the inability to give written and verbal informed consent, age under 18 years, and the impossibility of carrying out the measurements in the fracture gap.

As a third group, for reference purposes, an age-matched group of healthy control participants received measurements of their tibia. The inclusion criteria of this healthy control group were the ability to provide written and verbal informed consent, age 18 years and older, no previous fracture or surgery of the legs, and no current participation in another study. The exclusion criteria were the inability to give written and verbal informed consent, and age under 18 years. In each of the figures showing the results, the average value of the control group is indicated as a dashed line.

2.2. Measurements

A CE-certified medical device that functions via laser Doppler and white light spectroscopy ('Oxygen to see', O2C, LEA Medizintechnik, Winchesterstr. 2, D-35394 Gieβen, Germany) was used to measure the SO_2, relative haemoglobin amount (rHb), and BF in the fracture gap. The O2C operates with a laser (wavelength of 820 nm) and an emitter/detector within the white-light spectrum range (wavelength of 500–800 nm) (Forst et al., 2008). The wavelength-settings cannot be changed. The device determines the SO_2 and rHb by the white-light spectral shift (changes in blood colour) and the BF by the laser Doppler shift. Probes were noninvasively fixed onto the skin with black kinesiology tape to standardize the contact pressure and control for ambient light (Fig. 1D–F). The probes were fixed in a standardized manner by only applying pressure to the kinesiology tape next to the probe and not to the probe itself. The probes were covered in single-use 'Ultracover for TEE Endocavity Probe Cover' (ECOLAB, Microtek Medical B.V., Hekkehorst 24, 7207 NL-Zutphen, The Netherlands), 25 × 11 × 1000 mm, made of polyurethane. If needed, the fracture location was confirmed by ultrasound imaging and photos of the probe position were taken to increase reproducibility. If a metal plate had been implanted, adjacent measurements were conducted to guarantee that measurements would be

conducted in the actual fracture. The O2C device gives an alarm and does not record data when the metal is within the measurement volume. In the control group, measurements were conducted over the midshaft of the tibia. The measurements were performed with two separate probes at depths of 10 mm and 16 mm. Among the available probes for this device, the 10 mm probe was selected, as it measures in the more superficial (cortical) bone fracture with the skin and periosteum cover likely being around 5 mm thick. The 16 mm probe is the deepest provided with the O2C device and it was selected to get readings from a deeper region of the (spongious) bone fracture. For each depth, three measurements were taken at slightly different positions across the fracture gap, and these three values were averaged. Each measurement was recorded for 10 s and averaged by the device. Only these processed values were exported and used for further analyses. As the measurement volume depends on the tissue density and device wavelength, it cannot be reported precisely, which is a well-known weakness of this measurement technology. Laser-Doppler-based systems for BF measurements, such as photoplethysmography, have been shown to be capable to measure in bone (Hanne et al., 2019; Howden et al., 2017). Significantly greater depths of penetration were found for trabecular bone (3.5 ± 0.2 mm) than for cortical bone (2.9 ± 0.2 mm) by laser-Doppler flowmetry (Nötzli et al., 1989). This finding is relevant because the fractures were mostly reduced during surgery, resulting in a very small fracture gap.

2.3. Statistics

All the statistical tests were executed with IBM SPSS Statistics version 30 (IBM SPSS Statistics, Armonk, NY, United States). Significance was defined as a p value of <0.05. The normality of the data was tested with the Kolmogorov–Smirnov and Shapiro–Wilk tests. In the case of nonnormality, the data were transformed to reach normality. Linear mixed effect models were fitted to analyse time effects, with TIME as a fixed effect and PATIENT as a random effect. Univariate analyses of variance (ANOVAs) were conducted with post hoc Bonferroni correction to compare groups. The times of extrema (SO_2: minimum, BF: maximum) were calculated via linear regression via the following equation, which is based on the linear regression formula $Y = aX + b$ where a is the regression slope and b is the intercept with the Y-axis:

$$\text{Day of extremum} = (b \text{ before} - b \text{ after})/(a \text{ after} - a \text{ before}) \quad (1)$$

Owing to the lack of comparable human data in the literature, the authors could not run an a priori sample size calculation. The patient sample size was therefore determined by the number of patients available in the 18-month test period.

3. Results

Thirty-five patients were included in the study: 20 with prospective longitudinal measurements and 15 nonunion patients with a single measurement. In addition, 28 healthy control participants received a single measurement, serving as the control group. In this healthy control group, age was normally distributed, and there were no age effects on the outcome parameters. The patient and participant characteristics of the separate groups are shown in Table 1.

3.1. Longitudinal findings

The SO_2 did not drop to the lowest level immediately but later decreased to the minimum value, which was followed by an increase (Fig. 2). BF increased to a maximum, followed by a decrease. Fig. 2 shows the longitudinal trajectories of the parameters SO_2, rHb and BF at 10- and 16-mm depths in relation to those of the healthy control and nonunion groups. Table 2 shows the days of the calculated minimum (SO_2) and maximum (BF) values. A greater than twofold difference was observed in the time until the maximum between 10 and 16 mm in the BF; the maximum was reached later when it was measured deeper in the fracture. In the fracture group, the average values were significantly greater in the 10 mm fracture measurement than in the 16 mm fracture measurement (SO_2 $p < 0.001$, rHb $p = 0.003$, BF $p < 0.001$; Fig. 2). Similarly, in the healthy control group, significantly greater absolute values at the 10-mm depth than at the 16-mm depth were found for SO_2 and rHb but not for BF, and this difference was not significant (SO_2 $p = 0.027$, rHb $p = 0.002$).

3.2. Nonunion

As the nonunion types could not be clearly determined for several of the cases, all nonunion cases were pooled for analysis. Fig. 2 shows the findings of the nonunion group compared with those of the longitudinal fracture and control groups. The nonunion patients and healthy controls differed in SO_2 (10 mm, $p < 0.001$; 16 mm, $p = 0.038$) but not in rHb or BF (Table 1). The average SO_2 values of the nonunion group did not differ from the minimum values of the longitudinal fracture group. As shown in Fig. 2, the variability in values among nonunion patients was large, which indicates possible differences among the separate nonunion types. There were no differences between the measurement depths in the nonunion group.

Table 1
Patient and participant characteristics and average perfusion parameter values.

Parameter	Healthy control group	Nonunion	Fracture (SO_2 min, rHb average, BF max)	P value control vs. nonunion	P value control vs. fracture	P value nonunion vs. fracture
Total n	28	15	20			
Sex (m, f)	12, 16	10, 5	7, 13			
Age (mean ± SD)	58.61 ± 19.62	54.00 ± 15.81	60.80 ± 18.24	1.000	1.000	0.847
SO_2 10 mm (mean ± SD)	48.10 ± 11.56	27.42 ± 23.95	27.93 ± 12.61	**< 0.001***	**< 0.001***	1.000
rHB 10 mm (mean ± SD)	42.43 ± 9.31	51.60 ± 13.73	53.63 ± 14.97	0.056	**0.005***	1.000
BF 10 mm (mean ± SD)	88.42 ± 37.13	115.84 ± 35.40	176.22 ± 48.04	0.116	**< 0.001***	**< 0.001***
SO_2 16 mm (mean ± SD)	39.82 ± 15.38	27.11 ± 20.62	17.73 ± 10.31	**0.038***	**< 0.001***	0.243
rHB 16 mm (mean ± SD)	49.30. ± 6.39	55.47 ± 15.03	46.23 ± 19.18	0.252	0.809	**0.035***
BF 16 mm (mean ± SD)	95.02 ± 36.27	114.93 ± 39.82	132.20 ± 30.57	0.253	**0.020***	0.478

Values are shown for the healthy control, nonunion, and fracture groups. For the fracture group, as the data are longitudinal, the following values were used: SO_2: minimum, rHb: average, BF: maximum. P values are shown in bold and with an asterisk when significant.

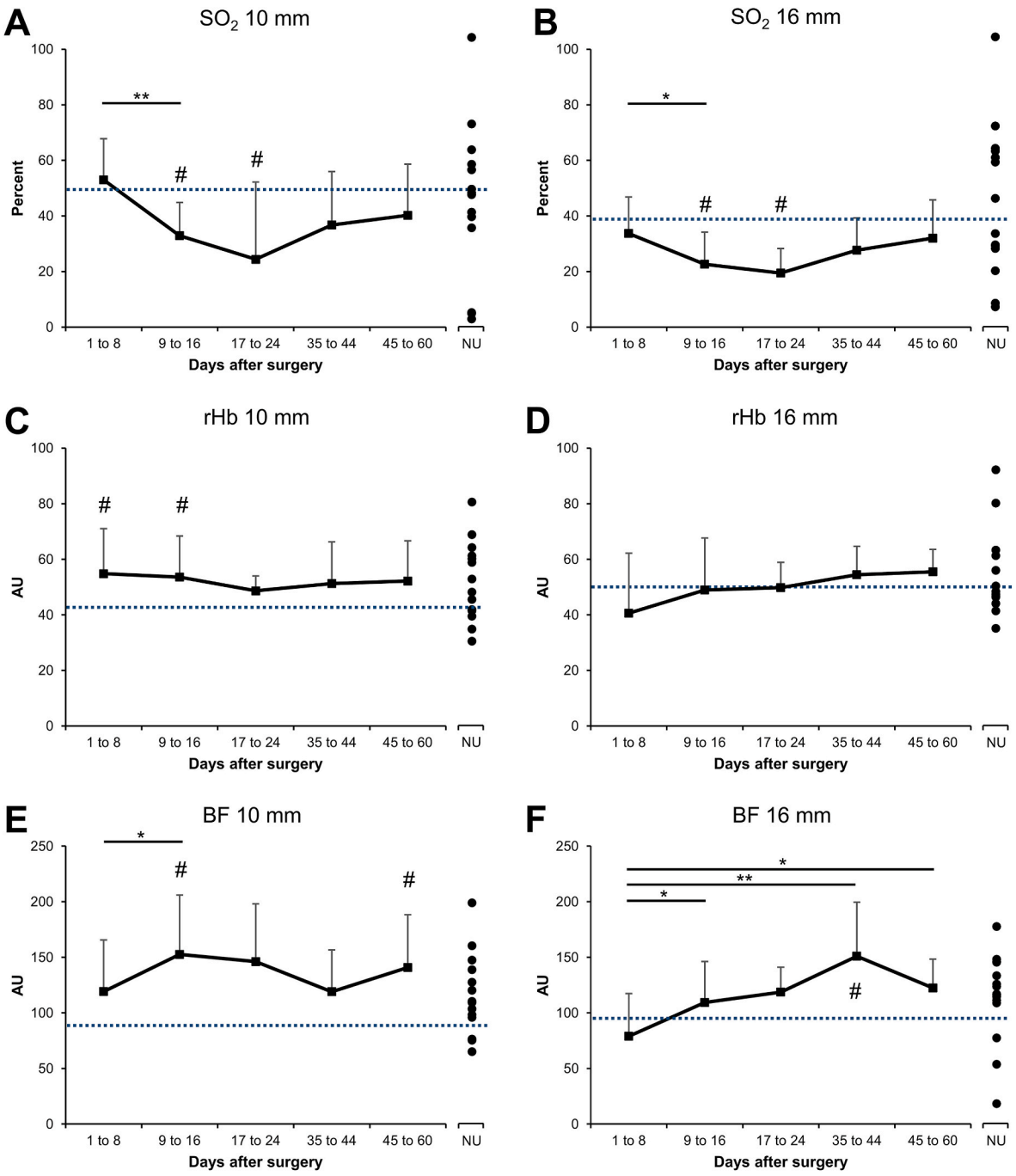

Fig. 2. Measurement findings. Changes in SO_2 (A, B), rHb (C, D) and BF (E, F) for 10 mm (A, C, E) and 16 mm (B, D, F) over time. Error bars show the standard deviation. Dotted blue line: average of the healthy control group. Individual data points of nonunion patients (NUs) are shown to the right of each graph. #: Significant difference from healthy controls (univariate ANOVA); Differences between time intervals (linear mixed effects model): *p < 0.05, **p < 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

This study is the first to present longitudinal perfusion data obtained by laser-Doppler and white-light spectroscopy from the tibial fracture gap of human patients. The main findings are as follows: 1. The SO_2 concentration did not drop to the lowest level immediately, as hypothesized, but rather decreased to a later minimum two to three weeks postsurgically, followed by an increase. In addition, the BF increased to a maximum, followed by a decrease. 2. The SO_2 values of the nonunion group did not differ from the minimum values of the longitudinal fracture group.

The presented findings are new with respect to the initial decrease in SO_2, which was followed by a marked minimum and then an increase. Previously, a more immediate onset of hypoxia was assumed. In sheep with osteotomies, Epari et al. (2008) reported a decrease in oxygen tension, mainly during the first day after fracture, when the measurement was conducted with an invasive probe. The slower SO_2 decline in this study could be explained by a continuation of some of the blood supply from the periphery via smaller cortical blood vessels and from blood flowing through the central artery in the bone, as well as by the steady increase in metabolism and oxygen demand associated with remodelling. The SO_2 value determined by O2C reflects the amount of

Table 2
Times of extrema (SO_2: minimum, BF: maximum).

Parameter	Interval of expected extremum (days) according to Fig. 2	Regression before and including interval with expected extremum	Regression after and including interval of expected extremum	Calculated day of extremum
SO_2 10 mm	17–24	−2.1116x + 60.857	0.5327x + 13.36	17.96
SO_2 16 mm	17–24	−1.1074x + 37.688	0.2849x + 16.10	15.50
BF 10 mm	0–16	3.2498x + 108.73	−0.4265x + 156.17	12.90
BF 16 mm	35–44	2.1793x + 74.39	−1.1617x + 186.34	33.51

haemoglobin oxygenation and thus is a measure of oxygen extraction. The SO_2 values decrease with increasing gas exchange. In the case of a high BF and low SO_2, haemoglobin experiences a large amount of oxygen extraction due to a high oxygen demand in the tissue, causing the loss of much of its oxygen despite the high flow. This decoupling of SO_2 and BF observed in our data may also be explained by the fact that the sprouting of new capillaries leads to a sufficient tissue oxygen supply and a reduction in O_2 removal from haemoglobin. In the normal healing process, cytokines and growth fractures induce vascularization of the fracture callus within 2–5 weeks (Menger et al., 2022a). The new blood vessels then provide cells, hormones, and nutrients to develop a callus from avascular cartilaginous tissue to mineralized woven bone (Menger et al., 2022b).

The finding that the local rHb concentration did not change over time is consistent with the fact that increased blood availability does not necessarily increase the local rHb concentration when a cavity such as a bone cavity or fracture gap is already filled with blood. In contrast, when rHb is measured in soft tissues, the concentration is observed to increase with vasodilation or venous blood pooling. In contrast with the present findings, in another investigation, the skin and subcutaneous tissue of the hip after surgery for trochanteric femur fractures showed increases in SO_2, BF and rHb content (Ganse et al., 2019).

Blood vessels have also been identified in nonunion tissue biopsies (Reed et al., 2002); however, the actual oxygenation within the callus tissue of nonunions, as measured in mice by photoacoustic imaging, is impaired (Menger et al., 2022a). This is in line with the findings of this study, which revealed lower oxygenation in nonunion patients than in healthy participants. In a study of tibial osteotomies in dogs, Laurnen and Kelly (1969) reported an increase in BF, which peaked after two weeks with sixfold flow values and returned to normal values after approximately 12 weeks. In a fracture with delayed union, the BF remained increased at thirteen weeks. In the present study, the BF was increased only at a depth of 10 mm but not at a depth of 16 mm in nonunion patients compared with healthy control participants. The nonunion group, however, was assessed at approximately 6 months after surgery, which was later than the 13-week period used in the study by Laurnen and Kelly (1969). The pathophysiology of nonunion formation in ischaemia was studied in a mouse model by Lu et al. (2007). Ischaemia significantly decreased the callus size, matrix production of bone and cartilage, and cell proliferation. Moreover, the presence of ischaemia increased the occurrence of early apoptosis and the amount of fibrous and adipose tissue adjacent to the fracture site during the third and fourth weeks after injury. In the mouse model, these alterations led to massive delays in union. There also seem to be structural differences in the fracture haematoma, where delayed healing is associated with thinner fibres and denser clot structures (Wang et al., 2016). Fischer et al. (2020) showed differences in perfusion among 34 patients with union, aseptic nonunion and infected nonunion via contrast-enhanced ultrasound. The wash-in rate of a contrast agent decreased with aseptic nonunion and increased with infected nonunion. The present study did not distinguish between nonunion types, but this should be done in future studies with a larger cohort of nonunion patients. Longitudinal perfusion parameter trajectories of patients who develop nonunion may be beneficial for determining whether these parameters serve to predict nonunion earlier. To determine if this is possible, it is necessary to conduct prospective longitudinal studies that are large enough to include a sufficient number of patients who develop nonunion.

The greater values at the 10 mm depth than at the 16 mm depth that were found in this study may be explained by the signal absorption of the laser and white light by the calcified bone tissue, which may also explain the similar findings in healthy control participants without fracture (Hanne et al., 2019; Howden et al., 2017; Nötzli et al., 1989). However, the differences between the 10 mm and 16 mm depths were not observed in nonunion cases, which might have been related to their delay in calcification.

Given the need for new methods to monitor fracture healing and for additional sensor options for smart implants, the present findings indicate that combined laser-Doppler and white-light spectroscopy may be a great addition to radiography and clinical impressions in daily clinical practice, as well as a suitable method for *in vivo* studies on fracture healing in human patients. The combination of both methods may allow to distinguish the separate nonunion types earlier on than only using one method alone. For example, nonunion caused by inflammation may have a greater effect on the BF than the SO_2 than nonunion caused by excess mechanical movement. In the future, larger studies with a great number of nonunion cases are needed to clarify this assumption. Fig. 3 shows parameters that may be assessed on the basis of the determined trajectories and that could serve as outcome parameters. Measurements of fracture perfusion could be valuable when studying the effects of fracture-stimulating devices and active implants (Ganse, 2024; Ganse et al., 2022). Measurements of the effects of different stimulation modes (i.e., frequencies, intensities, and time spans) on fracture SO_2 could help to determine the optimal stimulation regimen when developing such interventions. The effects of ultrasound or magnetic field stimulation, shock wave therapy and other interventions to treat and prevent nonunion could be assessed by monitoring changes in SO_2 in fractures *in vivo*. Continuous measurements of fracture SO_2 in patients could allow more individualized treatment, especially when risk factors for nonunion are present. In addition, the measurements presented here could also be of value to monitor healing of osteotomies or bone defects.

The first studies on instrumented or smart implants with embedded sensors for live monitoring of fracture healing have shown benefits for clinical practice (Ganse et al., 2022; Jeyaraman et al., 2023). Among the sensor options explored to date are force sensors (Ledet et al., 2022), strain gauges (Seide et al., 2012) and electrical impedance spectroscopy

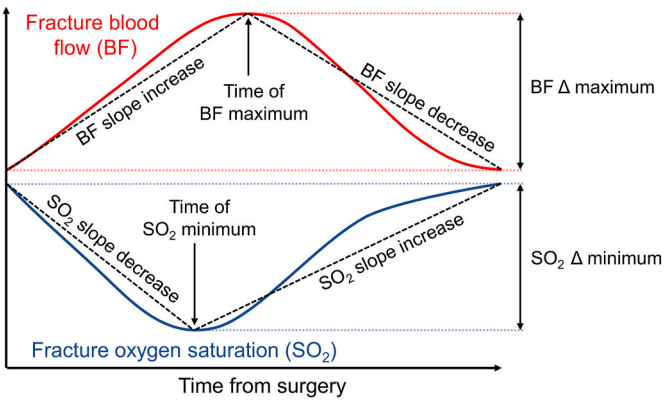

Fig. 3. Suggested parameters that could be used in future studies to compare intervention groups or for a prediction model. These are based on the findings of this study for monitoring fracture healing via laser-Doppler- and white-light-spectroscopy-based perfusion measurements of SO_2 and BF.

(EIS) (Lin et al., 2019). On-board perfusion measurements have to our knowledge not previously been discussed as a sensor option for smart fracture implants or wearable devices. The present study shows that the characteristic trajectories in BF and SO_2 may be a great addition to mechanical measurements. Future studies should conduct combined mechanical and perfusion measurements in the same animals or patients to determine how changes in stiffness and perfusion correlate. This correlation is of particular interest as it may be the key to allow to distinguish between the separate nonunion types. While mechanical measurement data do not allow to determine the underlying reason of delays in fracture healing, perfusion measurement may be suitable to distinguish whether the healing delay is caused by an infection (likely increased perfusion), by excessive movement in the fracture gap (likely unchanged perfusion), or by reduced blood supply (likely decreased perfusion). The authors therefore suggest to conduct studies that combine several sensor modalities in an even larger patient collective in a longitudinal clinical study. In addition, animal experiments with a purposely induced decrease in blood supply, an osteosynthesis with excessive play, and an induced bacterial infection could be a model to clarify the effects of these conditions on the data obtained by the separate sensor types.

Limitations of this study include the low number of follow-up measurements beyond three weeks and the low number of nonunion patients who could not be subclassified. It would be desirable to conduct a study with more frequent or, if possible, even daily measurements over the entire time span of fracture healing. Ideally, the follow-up time should be expanded to determine at what time the SO_2 and BF return to the values of the healthy controls. In addition, potential sources of variability could be taken into account, such as individual patient factors (e.g., age, vascularization, comorbidities) and methodological aspects like sensor placement consistency and a standardized time of measurements during the day.

5. Conclusions

This study presents a new approach to a highly relevant clinical problem by showing that laser-Doppler and white-light spectroscopy measurements allow monitoring of bone fracture healing via characteristic trajectories in SO_2 and BF. Given the need for new methods to monitor fracture healing and predict nonunion, the present findings indicate that combined laser-Doppler and white-light spectroscopy may be a great addition to radiography and clinical impressions in daily medical practice, as well as a possible method for *in vivo* studies on fracture healing in human patients. Using this technology, it may be possible to distinguish between the separate nonunion types early on. In addition, this new sensor option may be a great addition to smart fracture implants or wearable devices. Apart from fracture healing, this method may also be suitable to monitor healing of osteotomies and other bone defects.

CRediT authorship contribution statement

Oana Scholz: Writing – review & editing, Data curation. **Cedric Nowicki:** Writing – review & editing, Data curation. **Elke Warmerdam:** Writing – review & editing, Supervision. **Sandra Rother:** Formal analysis, Writing – review & editing. **Bergita Ganse:** Writing – original draft, Visualization, Supervision, Project administration, Methodology, Formal analysis, Conceptualization.

Ethics approval and consent to participate

Ethical approval was obtained from the institutional review committee of the Saarland Medical Board (Ärztekammer des Saarlandes, Germany, application number 127/22). Written informed consent was obtained according to the Declaration of Helsinki prior to the start of the experiments.

Funding source

This work was funded by the Werner Siemens Foundation, project Smart Implants.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bergita Ganse reports financial support was provided by Werner Siemens Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The data were collected by Oana Scholz and Cedric Nowicki for their doctoral thesis projects and pooled for this paper. The authors thank Christina Höft for her support.

Data availability

The data set analysed during the present study is available from the corresponding author on reasonable request. Access may be granted based on a collaboration agreement. The requesting institution needs to fall within the eligibility criteria of German data protection law.

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