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Hyperspectral imaging for monitoring of perfusion failure upon microvascular anastomosis in the rat hind limb

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ABSTRACT

Background/purpose: Objective, reliable and easy monitoring of microvascular tissue perfusion is a goal that was achieved for many years with limited success. Therefore, a new non-invasive hyperspectral camera system (TIVITA™) was tested for this purpose in an in vivo animal model.
Methods: Evaluation of tissue oxygenation during ischemia and upon reperfusion was performed in left hind limb in a rat model (n = 20). Ischemia was induced by clamping and dissection of the superficial femoral artery. Reperfusion of the limb was achieved by microsurgical anastomosis of the dissected artery. Oxygenation parameters of the hind limb were assessed via TIVITA™ before and immediately after clamping and dissection of the artery, 3 and 30 min after reperfusion as well as on postoperative days 1 and 2. Thereby, the non-operated hind limb served as control. As clinical parameters, the refill of the anastomosis as well as the progress of the affected leg were assessed.
Results: In 12 from 20 cases, TIVITA™ recorded a sufficient reperfusion with oxygenation parameters comparable to baseline or control condition. However, in 8 from 20 cases oxygenation was found impaired after reperfusion causing a re-assessment of the microvascular anastomosis. Thereby, technical problems like stenosis or local thrombosis were found in all cases and were surgically treated leading to an increased tissue oxygenation.
Conclusions: The TIVITA™ camera system is a valid non-invasive tool to assess tissue perfusion after microvascular anastomosis. As it safely shows problems in oxygenation, it allows the clinician a determined revision of the site in time in order to prevent prolonged ischemia.

1. Introduction

One major problem in reconstructive surgery is necrosis of free flaps (Losken et al., 2008). To prevent tissue necrosis, an adequate perfusion of the flap is essential, including sufficient blood flow from arteries through capillaries to draining venules (Barker et al., 1989). Therefore, both - an intact macro- and microcirculation - are crucial to assure the flaps' viability.

When it comes to deficiencies in tissue perfusion, a timely assessment and initiation of the respective therapy is crucial to avoid ischemia-induced damages. Monitoring of tissue perfusion is challenging due to the lack of established objective and accurate diagnostic methods that are feasible in clinical routine. In addition to clinical monitoring, including capillary reperfusion and optical aspects of the target tissue, as the actual standard for evaluation of tissue perfusion, different techniques have been tested for postoperative perfusion monitoring including non-invasive methods like tissue oximetry, autofluorescence spectroscopy or laser Doppler velocimetry and invasive methods like indocyanine green angiography or implantation of Doppler probes near an anastomosis (Moyer and Losken, 2012; Losken et al., 2008; Rao et al., 2009; Lim et al., 2014; Chae et al., 2015). Beside these there is a variety of other methods for non-invasive perfusion monitoring, i.e. for assessment of wound healing, with experimental or clinical evidence level (Paul et al., 2015). However, none of these techniques were found superior to clinical examination.

Non-invasive hyperspectral imaging (HSI) in the visible and near infrared spectral range has been proved to gain a high amount of relevant information about physiological parameters in different medical

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application areas like diabetic foot and skin ulcer (Yudovsky et al., 2010; Chin et al., 2012), tissue perfusion measurements and wound analysis (Zuzak et al., 2002; Calin et al., 2015). Benefits of this method are the contact-free spectroscopic measurement over a larger area without the need for contrast agents or other invasive procedures.

It was the aim of this study to test the new HSI system TIVITA™ (Diaspective Vision, Pepelow, Germany) for assessment of tissue oxygenation in a rat model of surgically induced ischemia and subsequent reperfusion after microvascular anastomosis.

2. Materials and methods

All in vivo experiments (LALLF M-V/TST/7221.3-1-063/15) were conducted in accordance with the German legislation on protection of animals and the NIH Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council) and were performed in accordance with the recommendations of Good Laboratory Practices. The animals were housed in a specific pathogen-free facility with a 12 h light-dark cycle and had access to standard laboratory chow and water ad libitum.

In brief, 20 male Sprague Dawley rats (age between 10 and 15 weeks and a body weight (bw) between 200 and 300 g) were anesthetized by isoflurane (isoflurane 1.5 vol% and O_2 0.8 l/min). Additional analgesia was assured by subcutaneous (sc) injection of carprofen (4 mg/kg bw Rimadyl®, Zoetis, Parsippany-Troy Hills Township, New Jersey, USA). The animals were placed on a pad with an integrated heating plate for maintaining the body temperature at 37 °C. Perioperative anticoagulation was performed by sc injection of 250 IE heparin on day 0 and 125 IE heparin on days 1 and 2 respectively. During the observation time of 2 days, animals were orally treated with metamizol (5 drops in 100 ml water from a 500 mg/ml stock solution) to assure analgesia.

First, the left groin region was shaved. The experimental setting started with a baseline analysis at day 0 to assess the unimpaired oxygenation of both hind limbs using the TIVITA™ Tissue camera system that was developed together with Diaspective Vision, Pepelow, Germany. Analysis included indexes for near-infrared perfusion (NIR), tissue oxygenation (StO_2) and tissue water (TWI). Assessment of these parameters was performed using the camera-specific software package TIVITA™ Suite that allows definition of different regions for perfusion analysis within the photographed tissue. The parameters are calculated in the circular areas by the software package. The position of the circulated area and its diameter can be chosen freely. To standardize the quantification of the parameters the circulated area was positioned always in the center of the midfoot of the respective hind limb and the diameter was chosen so that the edge of the circle reached from the medial to the lateral edge of the midfoot.

First the respective hind limb was positioned under the TIVITA™ (Fig. 1A). After baseline assessment, the left superficial femoral artery (SFA) was prepared and clamped with 2 microsurgical clamps (Fig. 1B). After 5 min of clamping the artery, oxygenation parameters were recorded from the left hind limb again, followed by surgical sectioning of the artery. This was followed by microsurgical anastomosis of the SFA using 10/0 Ethilon suture (Ethicon Inc., Somerville, New Jersey, USA) in single stich technique. Microvascular anastomoses were performed by two surgeons (EG & PWK). When anastomosis was completed *ante* and *retrograde* refills of the anastomosis were assessed. Therefore the artery was clamped respectively proximal or distal from the anastomosis with a micro forceps and scratched out with another micro forceps on the respective other side of the anastomosis forwards the anastomosis. A positive refill was defined as a complete filling of both directly proximal and distal parts of the artery referring to the anastomosis. Then, oxygenation of the left hind limb was assessed by means of the TIVITA™ camera system at 3 and 30 min postoperatively. In case of an impaired oxygenation of the hind limb reported by TIVITA™ or when refill of the anastomosis was prolonged or insufficient, the anastomosis was opened and revised for local complications like thrombosis or stenosis. Thereby an impaired oxygenation was defined as a reduction in StO_2 level of $>$ 30% compared to baseline condition.

After another 24 h, tissue oxygenation of both hind limbs was recorded again. On day 2, animals were anesthetized by isoflurane and oxygenation analysis was performed as before on both limbs. Then, the microvascular anastomosis on the left SFA was examined macroscopically and *ante* and *retrograde* refill were assessed as discussed above. After examination of the anastomosis and documentation of the perfusion of limb by means of TIVITA™, rats were sacrificed by an intravenous overdose of ketamine and xylazine (90/25 mg/kg bw) followed by exsanguination.

2.1. Statistics

After evaluating the variables for normal distribution employing the Kolmogorov-Smirnov test, differences in StO_2, NIR and TWI indexes before and after clamping the left SFA were analyzed by Student's *t*-test or Mann-Whitney *U* test by ranks in dependence of normal distribution. For analysis of the course of StO_2, NIR and TWI indexes over the time repeated measurement ANOVA was performed. All p-values resulted from two-sided statistical tests and values of $p < 0.05$ were considered to be statistically significant. Analysis was performed using the software package SigmaStat (Jandel, San Rafael, CA).

3. Results

In all animals, TIVITA™ reliably allowed assessment of tissue oxygenation and tissue water indexes over the course of time. Clamping time of the SFA averaged about 60 min. Each hyperspectral analysis took 5 s to gain the image of the respective hind limb and about another 20 s to gain the particular data.

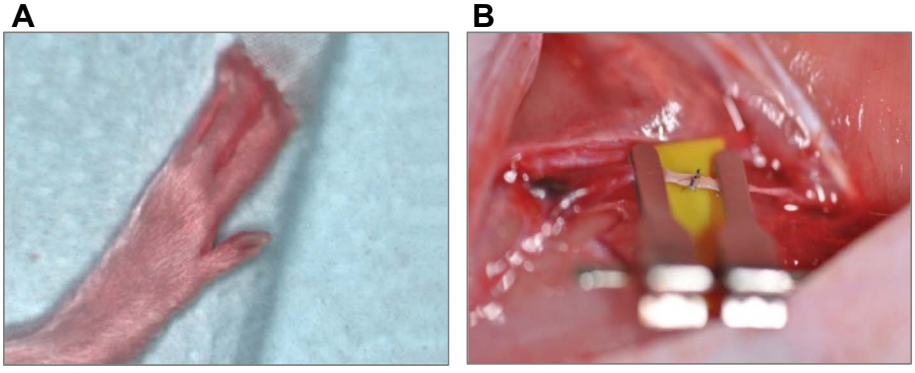

Fig. 1. Color image during positioning of the right hind limb showing the spatial resolution of the TIVITA™ (A). Intraoperative image after anastomosis of the SFA of the left hind limb (B). The artery was clamped by a microsurgical clamp. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)