Optical methods for the assessment of microvascular perfusion and oxygenation
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SUMMARY AND CONCLUSIONS
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Technological advancements have always been the driving force behind clinical and experimental research. Prior to their employment in research, however, new techniques often require optimization, evaluation, and validation. After Van Leeuwenhoek’s introduction of microcirculatory microscopy [Van Leeuwenhoek, 1688, 1689], for instance, its application was long limited due to the unavailability of sophisticated illumination techniques that enable imaging of the microcirculation in intact human organ surfaces. It took over 300 years before dark field illumination [Sherman et al., 1971] and orthogonal polarization imaging [Slaaf et al., 1987] were developed and incorporated into a clinically applicable device [Groner et al., 1999]. It was only since then that microcirculatory microscopy could be applied in numerous studies in various clinical scenarios and, as a result, became an important technique in clinical research [e.g., De Backer et al., 2002, 2004, 2007; Boerma et al., 2005, 2007, 2010].

Today, our group focuses on the ‘translation’ of clinical scenarios into (patho)physiological concepts and on the development of therapeutic strategies based on insights obtained in the clinic and in the lab. As the microcirculation has a key role both in health and disease, the main emphasis of our studies is on this important physiological compartment and to support our research lines, the purpose of the work described in this thesis was to develop and validate new and available technologies for the assessment of microvascular perfusion (Part I) and oxygenation (Part II).

Part I – Microvascular perfusion assessment

As discussed in the General introduction, orthogonal polarization spectral (OPS) imaging imaging has had a major impact on clinical research and greatly contributed to the field of intravital microcirculatory microscopy. However, several shortcomings were still present, which include suboptimal imaging of the capillaries due to motion-induced image blurring by movement of the OPS device, the tissue, and/or flowing red blood cells. This led to difficulties in measuring blood flow velocities in these vessels. Driven by the success of OPS imaging and the drawbacks it has, we developed a novel imaging modality for the microcirculation, which we have termed sidestream dark field (SDF) imaging. In Chapter 1, we showed that OPS and SDF imaging provided similar values for capillary diameters and red blood cell velocities in the human nailfold microcirculation, validating the use of SDF imaging for clinical measurement of the microcirculation. SDF imaging, moreover, provided significantly higher image quality with more detail and higher capillary and venular contrast and enabled imaging of individual red and white blood cells and measurement of the endothelial glycocalyx thickness.

Since SDF imaging technology is incorporated into a hand-held microscope some operational issues arise in terms of axial and lateral instability of the
microscope probe, potentially causing pressure artifacts and image drifting, respectively. Therefore, in Chapter 2, we developed, evaluated, and validated an image acquisition stabilizer (IAS) for the SDF device. The IAS was based on creating adherence of the SDF probe to the sublingual tissue by applying negative pressure to the periphery of the microscopic field of view. We found that the IAS did not affect microcirculatory perfusion in the SDF imaging field of view and prevented pressure artifacts up to a significantly greater force applied by the SDF probe onto the tissue. Furthermore, we showed that the duration of maintaining a stable image sequence was significantly increased with the IAS.

For evaluation of the effects of interventions and (drug) therapy, SDF images are analyzed to assess (alterations in) microvascular density and perfusion. However, the offline analysis of the SDF images is a time consuming venture (>30 min) requiring a significant amount of user interaction. In Chapter 3 we therefore developed a rapid and fully automatic method for the assessment of microvascular density and perfusion in SDF images. To this end, we improved the algorithms for microvascular density assessment previously developed by our group and introduced a new method for microvascular perfusion assessment that was inspired by laser speckle imaging: temporal SDF image contrast analysis (tSICA). For the validation of the new algorithm for microvascular density assessment, we reanalyzed 325 SDF video clips of a study in intensive care patients and compared the results to (semi-)manually found microvascular densities. We showed that the new method was very rapid (<30 seconds) and adequately recovered microvascular density. The performance of the tSICA method for microvascular perfusion assessment was tested in several video simulations and in one high quality SDF video clip where the microcirculation was imaged (on one spot) before and during circulatory arrest in a cardiac surgery patient. With the video simulations we showed that the detection of flow using the tSICA method is limited by high cell densities and velocities at normal imaging rate, which severely impedes the applicability of this method in real SDF images. However, in high quality SDF video clips, the tSICA method is able to discriminate between perfused and non-perfused microvasculature.

Although sublingual SDF imaging is gaining a more prominent role in clinical research, it can only image the microcirculation in a small tissue volume and requires physical contact with the tissue surface. Furthermore, measuring red blood cell velocities using SDF imaging is only possible for a limited range as shown in the previous chapter. Hence, in clinical and experimental settings, a macroscopic, non-contact technique with high spatial and temporal resolution would be preferable. A technique potentially providing such features is laser speckle imaging (LSI). However, whether LSI is indeed sensitive to changes in capillary perfusion has not been properly validated. As SDF imaging is a validated intravital microscopic technique for measuring red blood cell velocities in individual capillaries (albeit for a limited range), in Chapter 4 we used it for validating LSI
responses to alterations in capillary perfusion. We found that changes in perfusion as measured using LSI correlated well with changes in capillary red blood cell velocities as measured using SDF imaging. We have furthermore shown that LSI is capable of measuring tissue perfusion in high temporal and spatial resolution and that this technology can be used to assess microvascular reactivity to (patho)physiological challenges.

Subsequently, in Chapter 5, we evaluated the use of LSI for assessing microvascular perfusion heterogeneities in the rat renal cortex. For this purpose, LSI was employed during complete renal I/R (1, 10, and 45 min of renal artery occlusion) and during heterogeneous renal I/R (air embolisms). We have used two types of LSI image analysis yielding either a single LSI perfusion value for the entire renal cortex or an LSI perfusion histogram, describing the perfusion heterogeneity in the renal cortex. We found that LSI is able to detect differences in reperfusion dynamics following different durations of complete ischemia and that renal microvascular perfusion heterogeneities can be quantitatively assessed by recovering LSI perfusion histograms.

Part II – Microvascular oxygenation assessment

The primary site of oxygen delivery from blood to cells is the microcirculation and therefore, microcirculatory oxygenation is considered a parameter of key (patho)physiological importance. During endotoxemia, however, the balance between renal microcirculatory oxygen delivery and cellular oxygen consumption is significantly disturbed. Although oxygen diffusion, which relies on radial oxygen gradients between the microvasculature and tissue, represents a crucial step in cellular oxygen delivery, no studies have simultaneously assessed renal microvascular and interstitial oxygen tensions under endotoxemic conditions. Therefore, in Chapter 6, we aimed to study the complex changes associated with endotoxemia that occur in different compartments of the kidney (cortex and medulla) and at different levels of cellular oxygen delivery (microvasculature and interstitium) in relation to global and renal hemodynamics. To this end, we simultaneously assessed renal artery blood flow and microvascular and interstitial oxygen tensions in the renal cortex and medulla using ultrasonic flowmetry, dual wavelength phosphorimetry, and tissue oxygen tension monitoring, respectively, in an endotoxemic rat model. We found distinctive temporal changes in the different regions. While the gradient between microvascular and interstitial oxygenation was higher than that reported in metabolically less active organs, this did not change during a short-term endotoxemic insult.

While the previous chapter provides integrative insight of the complex changes associated with endotoxemia that occur in different compartments of the kidney (cortex and medulla) and at different levels of cellular oxygen delivery (microvasculature and interstitium), this study did not account for the underlying oxygenation distributions. Under the (patho)physiological conditions associated
with endotoxemia, however, it has been shown that hypoxic tissue could co-exist with normoxic tissue. These hypoxic tissue fractions could potentially lead to organ dysfunction and therefore their detection is essential. Instead of mono-exponential curve fitting of the phosphorescence decay trace, providing a single microvascular oxygen tension value for the phosphorimetric measurement volume, multi-exponential curve fitting could be used for the analysis of the decay traces, which provides a distribution (or histogram) of microvascular oxygen tensions. Although it is known that oxygen-quenched phosphorescence decay traces can be analyzed using multi-exponential curve fitting, its application until now has been limited to a few (patho)physiological studies, probably because the reliability of the recovered oxygen tension histograms has never been extensively evaluated and lacks documentation. The aim of Chapter 7 was to extensively evaluate the ability of multi-exponential fitting analysis to adequately determine oxygen tension histograms from simulated and in vivo-obtained phosphorescence decay traces. We characterized how noise affects the recovery of oxygen tension histograms and elaborately validated the use of phosphorimetry in combination with multi-exponential curve fitting analysis for recovering low, high, and bimodal oxygen tension distributions.

In Chapters 5 and 7 we have established that LSI and phosphorimetry can be used to reliably assess renal microvascular perfusion and oxygenation histograms, respectively. The purpose of Chapter 8 was to combine these modalities to investigate the role of renal hypoperfusion in the development of renal microcirculatory dysfunction in endotoxemic rats. We hypothesized that prevention of endotoxemia-induced hypotension by immediate fluid resuscitation would prevent the development of renal microcirculatory dysfunction. We have shown, however, that prevention of renal macrovascular hypoperfusion by immediate fluid resuscitation could not fully protect renal microcirculatory dysfunction. Furthermore, we demonstrated that prevention of hypotension by immediate fluid resuscitation reduced renal inflammatory activation. This indicates that in clinical scenarios, the endotoxemia-induced hypotension prior to the start of fluid resuscitation leads to an ischemia-reperfusion insult that potentially leads to the activation of renal inflammation. Hence, this study shows that during endotoxemia, renal microcirculatory dysfunction can exist under conditions of maintained macrovascular perfusion that this could be associated with renal failure.
Conclusions

This thesis describes the development, evaluation, and validation of key optical methods for the in vivo assessment of microvascular perfusion (sidestream dark field imaging and laser speckle imaging) and oxygenation (phosphorimetry). It thereby supports the research in our group on the ‘translation’ of clinical scenarios into (patho)physiological concepts and on the development of therapeutic strategies based on insights obtained in the clinic and in the lab. Furthermore, this thesis might serve as a frame of reference for investigations focused on perfusion and oxygen (re)distribution during disease and therapy and encourage researchers to employ the described techniques possibly revealing new insights into complex disease states and treatment strategies.