Optical coherence tomography and hyperspectral imaging of vascular recovery in a model of peripheral arterial disease

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ABSTRACT

Peripheral arterial disease (PAD) leads to an increased risk of myocardial infarction and stroke, increased mortality, and reduced quality of life. The mouse hind limb ischemia (HLI) model is the most commonly used system for studying the mechanisms of collateral vessel formation and for testing new PAD therapies, but there is a lack of techniques for acquiring physiologically-relevant, quantitative data intravitaly in this model. In this work, non-invasive, quantitative optical imaging techniques were applied to the mouse HLI model over a time course. Optical coherence tomography (OCT) imaged changes in blood flow (Doppler OCT) and microvessel morphology (speckle variance OCT) through the skin of haired mice with high resolution. Hyperspectral imaging was also used to quantify blood oxygenation. In ischemic limbs, blood oxygenation in the footpad was substantially reduced after induction of ischemia followed by complete recovery by three weeks, consistent with standard measures. Three dimensional images of the vasculature distal to vessel occlusion acquired with speckle variance OCT revealed changes in OCT flow signal and vessel morphology. Taken together, OCT and hyperspectral imaging enable intravital acquisition of both functional and morphological data which fill critical gaps in understanding structure-function relationships that contribute to recovery in the mouse HLI model. Therefore, these optical imaging methods hold promise as tools for studying the mechanisms of vascular recovery and evaluating novel therapeutic treatments in preclinical studies.

Keywords: intravital imaging, peripheral arterial disease, optical coherence tomography, hyperspectral imaging

1. INTRODUCTION

Peripheral arterial disease (PAD) affects millions of individuals and leads to high rates of myocardial infarction and stroke, increased mortality, and reduced quality of life\(^1\). The mouse hind limb ischemia model is used extensively for studying the mechanisms of collateral vessel formation and for testing new therapeutic treatment strategies for PAD\(^2\). Current techniques for characterizing angiogenesis, arteriogenic vessel remodeling and physiologic restoration of normoxia in the ischemic hind limb include histology, laser Doppler perfusion imaging (LDPI) and microcomputed tomography, but these techniques suffer from their respective limitations including 2D and user-subjective data, semi-quantitative data with limited imaging depth, and being post mortem procedures requiring contrast agents\(^3\) (Table 1).

Currently, a combination of these anatomical and functional measures is employed to gain insight into vascular structure and function in this model. This requires excessive use of mice and access to multiple instruments making thorough experimentation expensive and time-consuming. An intravital, quantitative technique for evaluating vascular response to hind limb ischemia would have potential for answering previously-inaccessible structure-function questions about vascular development and may facilitate testing of new treatments for PAD.

Spectral domain optical coherence tomography (OCT) can non-destructively visualize structural features in tissue at cellular-level resolution and imaging depths of several mm. Doppler OCT detects motion in the sample, enabling quantification of blood flow velocity in 3D as a function of the measured phase shift\(^4\). Additionally, speckle variance OCT allows for improved 3D visualization of microvessel morphology compared to Doppler OCT, but does not provide the potential for absolute velocity quantification\(^5\). Recently, feasibility of imaging microcirculation in skeletal muscle was demonstrated with ultrahigh sensitive optical microangiography\(^6\). Hyperspectral imaging has been used to measure the oxygen saturation of hemoglobin, an important measure of vascular oxygenation, in the mouse brain and in tumors \textit{in vivo}\(^7\). \(^8\). This quantitative technique can provide physiological information about hemoglobin saturation on the regional tissue level, which when combined with the microvessel morphology and blood flow endpoints obtained with OCT methods provides a complete picture of the structure-function relationships involved in the vascular response to hind limb ischemia. Table 1 summarizes the relative strengths and weaknesses of these optical techniques in comparison to...
those of the current standard techniques used to study recovery in this preclinical model. The structure-function information that can be obtained with OCT and hyperspectral imaging together enables a more thorough understanding of PAD, and the ability to quantitatively assess the effectiveness of potential therapies. In this work, we have applied OCT and hyperspectral imaging techniques to non-invasively (without the use of contrast agents) track the changes in vascular morphology, blood flow, and hemoglobin oxygen saturation intravitaly in a mouse model of PAD. We have also compared this approach to a standard imaging method (LDPI) commonly used in this model.

Table 1. Strengths and weaknesses of standard (top box) and proposed (lower box) techniques for vascular assessments in the hind limb ischemia model.

<table>
<thead>
<tr>
<th>Method</th>
<th>3D</th>
<th>Resolution</th>
<th>Functional</th>
<th>Intravital / Longitudinal</th>
<th>Endpoint(s)</th>
<th>Weakness</th>
</tr>
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<tr>
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<td></td>
<td></td>
<td>Vessel density</td>
<td>Sampling error</td>
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<tr>
<td>LDPI</td>
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<td>Low</td>
<td></td>
<td></td>
<td>Semi-quantitative ratio</td>
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<tr>
<td>Micro-CT</td>
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<td>High</td>
<td></td>
<td></td>
<td>Morphology</td>
<td>Contrast agent</td>
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<td>OCT</td>
<td></td>
<td>High</td>
<td></td>
<td></td>
<td>Absolute velocity and shear rate, morphology</td>
<td>2 mm depth</td>
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<td></td>
<td>Low</td>
<td></td>
<td></td>
<td>Hemoglobin Saturation</td>
<td>Superficial</td>
</tr>
</tbody>
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2. METHODS

2.1 Hind limb ischemia model

Hind limb ischemia was induced in 8-10 week old male A/J mice (Jackson Labs) according to the well-established model of femoral artery ligation and transection. Surgery was carried out under 1.5-2.5% isoflurane anesthesia and at normal body temperature. After administration of pre-operative analgesia (10 mg/kg ketoprofen) and removal of hair, the surgical site was cleansed with alternating iodine and chlorhexidine scrubs. A 5-mm unilateral incision was then made over the right medial thigh of the mouse. The femoral artery and vein were dissected away from the femoral nerve and were ligated with 6-0 silk sutures at two locations: immediately distal to the origins of the epigastric artery and deep branch of the femoral artery, and approximately 2 mm distal to the first ligation proximal to the origin of the nearest distal branch (Fig. 1). The artery and vein were transected between the two ligations leaving a gap of 2-3 mm. The wound was irrigated with sterile saline and the incision was closed with interrupted 5-0 nylon sutures. The contralateral limb served as the control. Analgesia was administered subcutaneously every 18-24 hours post-operatively until animals exhibited normal appearance and behavior. Mice were fed a standard chow diet ad libitum and had free access to water. All protocols were approved by the Institutional Animal Care and Use Committee of Vanderbilt University and done in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Figure 1. Schematic of mouse hind limb and locations of femoral artery and vein ligations (X’s) for the surgical induction of hind limb ischemia.
2.2 Laser Doppler perfusion imaging

Perfusion in the footpads was measured by LDPI (Perimed) at 0, 3, 7, 14 and 21 days post-surgery under isoflurane anesthesia and normal body temperature. For analysis of perfusion data sets, ROIs in each image were defined by outlining the footpads. Perfusion values were obtained for each footpad by averaging the values for all pixels contained within the ROI, and the ischemic footpad value was normalized to that of the control.

2.3 Hyperspectral imaging

Two-dimensional diffuse reflectance images were collected with a 200 W halogen lamp coupled into a 10-mm core diameter liquid light guide for epi-illumination. The collection head consisted of a Varispec VIS-20 liquid-crystal tunable filter (LCTF) for bandlimited optical filtering (CRI, Inc.), a variable focal length (f = 28-80 mm) camera lens (Nikon), and a 512 x 512 pixel PhotonMax CCD camera (Princeton Instruments), resulting in a sampling density of 58 μm. Following surgery (day 0) and at 3, 7, 14 and 21 days post-surgery, control and ischemic footpads were imaged non-invasively through the skin (500 to 620 nm in 10 nm increments) under isoflurane anesthesia and normal body temperature. Hemoglobin saturation images of the footpads were calculated from hyperspectral data using an extension of the Beer-Lambert law and linear least-squares regression\(^8\),\(^11\).

2.4 Optical coherence tomography

A spectral domain optical coherence tomography (OCT) system (Bioptigen) with 25 μm lateral resolution and an 860 nm center wavelength, 51 nm bandwidth superluminescent diode (SLD) source (6.4 μm axial resolution in air) was used (Fig. 2). The SLD light is fiber coupled and split between a reference mirror and the sample arm using a 50/50 fiber coupler, while X-Y galvos in the sample arm perform lateral scanning. At each time point following induction of ischemia, Doppler B-scans (4 mm length) were acquired in a region containing the distal femoral artery and vein with 80 μs integration time per A-line, 800 A-lines/B-scan, and a Doppler number of 8. For 3 mm by 3 mm speckle variance OCT volume scans acquired in the same region, an 80 μs integration time per A-line, 200 A-lines/B-scan, 200 B-scans/C-scan, and a Doppler number of 6 was used. Doppler OCT B-scans were processed to reduce speckle noise and bulk motion artifact using a noise threshold from the corresponding structural image and a histogram-based correction method on the Doppler phase shift data\(^12\), respectively. To visualize vessel morphology at this distal location on the limb, volume data sets were processed with a speckle variance technique\(^5\) that computes the variance in each pixel over repeated A-lines at each lateral position. The resulting speckle variance images represent OCT flow signal for perfused vessels within the imaged volume. Noise due to signal fall-off was excluded by thresholding the corresponding structural volume to create a mask, and an average signal projection over the depth (approximately 1 mm) of the speckle variance OCT volume was used to visualize all vessels present in the volume.

![Schematic of spectral domain OCT system containing a superluminescent diode source.](http://proceedings.spiedigitallibrary.org/)
3. RESULTS

LDPI measurements of perfusion revealed a substantial decrease in perfusion in the ischemic footpad on day 0 after surgery (Fig. 3A). The ischemic footpad measurements for all animals were normalized to the control footpad and complete recovery of perfusion was achieved by the end of the time course (Fig. 3B).

Figure 3. Representative LDPI images of the ischemic and control footpads on day 0 post-surgery (A) and on day 21 post-surgery (B).

Immediately after surgery, hemoglobin oxygen saturation calculated from hyperspectral images (Fig. 4A) was reduced in the footpad of the ischemic limb relative to the control footpad (Fig. 4B). Analysis of hemoglobin saturation images over the time course of 21 days revealed increases in oxygenation of the ischemic footpad that correspond with the time-dependent improvements in perfusion observed with LDPI.

Figure 4. Representative diffuse reflectance image (550 nm) of the ischemic and control footpads on day 0 post-surgery (A). Representative hemoglobin oxygen saturation map of control and ischemic footpads at day 0 post-surgery (B).

Over the 21 day time course, OCT imaging through the skin of the mouse hind limb indicates that blood flow is restored in the femoral artery and vein along with smaller, nearby vessels distal to the ligations. Doppler B-scans acquired in the distal femoral artery and vein region showed that blood flow in these vessels was completely absent after surgery. By day 21 post-surgery, perfusion is recovered in the ischemic limb as shown by representative control (Fig. 5B) and ischemic limb (Fig. 5D) Doppler OCT images. Speckle variance OCT volumes of hind limb vessel morphology are shown as an average intensity projection over ~1 mm in depth in Fig. 6. On day 0 following surgery, there is no OCT flow signal present in speckle variance OCT volumes of the ischemic limb in comparison to a control limb volume (Fig. 6A). Within the first two weeks following surgery, vessels in the distal ischemic limb remodel and flow signal is restored (Fig. 6B). Additionally, vessels with the corkscrew morphology associated with arteriogenesis in the mouse hind limb ischemia model are present. Finally, the femoral artery and vein are perfused and OCT flow signal reveals the morphology of these vessels by day 21 of recovery (Fig. 6C).
Figure 5. Representative structural (A) and corresponding Doppler shift (B) OCT B-scan images of the distal femoral artery and vein region imaged through the skin in a control limb. Representative structural (C) and corresponding Doppler shift (D) OCT B-scan images of the distal femoral artery and vein region in an ischemic limb at day 21 post-surgery.

Figure 6. Representative average intensity projections of OCT flow signal in the distal femoral artery and vein region in a control limb (A), an ischemic limb at day 14 (B), and an ischemic limb at day 21 (C). Scale bars = 500 μm.

4. DISCUSSION

Here, we have established hyperspectral imaging and optical coherence tomography techniques for acquiring time course measurements of vascular structure and function intravitally in the mouse model of PAD without the use of contrast agents or invasive procedures. This combination of measurements allows for non-invasive time-course studies of the angiogenesis and arteriogenesis processes and provides unprecedented insight into the relationship between vessel structure, hemoglobin oxygenation, and blood flow in this setting. The ability to perform these measurements intravitally is critical to determining whether morphological changes correspond to improvements in function. Previously, post-mortem techniques and multiple cohorts of mice were required for time course analyses of vascular changes. This optical imaging approach overcomes limitations associated with standard methods such as LDPI, micro-CT and histology and
could ultimately lead to a more detailed understanding of vascular growth and remodeling in ischemic tissues, providing a valuable tool for development of new pro-angiogenic therapies. Doppler OCT in particular holds potential for quantification of the changes in absolute velocity and shear rate in the hind limb vessels over time which is particularly relevant to the flow-driven process of arteriogenesis. Furthermore, the combination of non-invasive OCT and hyperspectral imaging techniques enables longitudinal studies with physiologically-relevant readouts which reduce the variation inherently present between animals. This will accelerate throughput, increase statistical power, and decrease costs of basic studies in comparison to existing techniques for assessing pro-angiogenic therapies in the mouse hind limb ischemia model.

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REFERENCES