Using Optical Coherence Tomography (OCT) to Evaluate Human Donor Kidneys Prior to and Following Transplantation

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Abstract

The most common insult to donor kidneys destined for transplantation is Acute Tubular Necrosis (ATN). The extent of ATN will affect post-transplant function and is a significant risk factor for long-term graft function and survival. Optical Coherence Tomography (OCT) is a rapidly emerging imaging modality that can function as a type of “optical biopsy”, providing non-invasive images of tissue morphology in situ and in real-time. In this paper, we review studies that support the use of OCT and Doppler based OCT (i.e., DOCT) to image the renal microstructure and blood flow of human donor kidneys. We conclude that OCT/DOCT imaging of donor kidneys prior to and following transplantation can provide transplant surgeons with a means for predicting ATN and post-transplant renal function.

Keywords: Optical Coherence Tomography (OCT); Doppler Optical Coherence Tomography (DOCT); Acute Tubular Necrosis (ATN); Delayed Graft Function (DGF); End-Stage Renal Disease (ESRD); Kidney transplantation; Uriniferous tubules; Renal blood flow

Imaging Donor Kidneys to Determine their Status

End-stage Renal Disease (ESRD) is associated with both high mortality rates and an enormous economic burden [1]. The preferred treatment option for ESRD that can extend patients’ lives and improves their quality of life is kidney transplantation. However, ischemic insult suffered by kidneys awaiting transplantation frequently causes acute tubular necrosis (ATN) that leads to varying degrees of delayed graft function (DGF) after transplantation. Also, ATN represents a significant risk for eventual graft and patient survival [2,3], and can be difficult to discern from rejection. In present clinical practice, there is no reliable real-time test to determine the viability of donor kidneys and whether or not donor kidneys might exhibit ATN. Therefore, there is a critical need for objective and reliable real-time tests to predict ATN to use these organs safely and utilize the donor pool optimally.

Previously it has been shown that the non-invasive imaging techniques (i.e., tandem scanning confocal microscopy-TSCM) could be used to determine the degree of ATN by analyzing the superficial nephrons of living kidneys in animal models and that these observations correlate with post-transplant renal function [4,5]. This is not surprising in that the status of superficial proximal convoluted tubules is indicative of the status of proximal convoluted throughout the entire kidney cortex. Non-invasive microscopic techniques are necessary for this determination because conventional microscopy results in artifacts that are difficult to distinguish from ATN [6]. Other investigators have also used near-infrared confocal microscopy [7] and multi-photon microscopy (MPM) [8-10] to demonstrate the ability to perform non-invasive imaging of kidney structure and function in animal models. However, the maximum penetration depth of those techniques for kidney imaging is very limited (≈100 µm for TSCM, and ≈200-300 µm for MPM), which makes it difficult to impossible to non-destructively image the human kidney, especially if an intact human renal capsule surrounds it. Indeed, in a previous clinical trial, we found that the limited penetrating ability of TSCM precluded us from imaging human donor kidneys even when an attempt was made to remove the renal capsule [unpublished observations]. Also, conventional bulky systems like TSCM and MPM are awkward and especially difficult when attempting to image the kidney in the clinical arena. Therefore, a non-invasive microscopic procedure that has enough penetrating ability to image the human kidney parenchyma and determine the extent of ATN would provide invaluable clinical information regarding kidney function.

OCT is a rapidly emerging imaging modality that can function as a type of “optical biopsy”, providing cross-sectional images of tissue morphology in situ and in real-time [11,12]. OCT is similar to ultrasound imaging, except that it uses the echo delay of light instead of sound to generate images. OCT is safer than X-ray technologies, much less expensive than MRI devices, and provides higher resolution images than ultrasound. By employing broadband optical light sources, OCT can achieve axial resolutions of 1-10 µm, much less of magnitude above that obtainable for clinical ultrasound. As a result, OCT can provide very high- resolution images of organs and tissues in a non-invasive manner. This potential has been demonstrated in a number of biomedical applications including ophthalmology [13-15], cardiology [16,17], gastroenterology [18-21], dermatology [22], dentistry [23], urology [24] and gynecology [25], among others. In contrast to other forms of non-invasive light microscopy, OCT can image with longer working distances, improved penetration depth and without the need for tissue contact. Not only can it image up to depths of ~1-2mm in most light- scattering tissues, OCT can also provide three-dimensional images in arbitrary planes. Finally, OCT can be performed using a thin flexible sterile endoscope or catheter [26,27] or even with a needle [28], enabling ease of use for minimally-invasive procedures and the possibility of imaging deep within a solid tissue or organ. Previously, we have demonstrated that OCT is able to provide clinically relevant information (morphology and blood flow) using a rat kidney model [29-31]. In addition, we showed that OCT has sufficient penetration depth and resolution to visualize human

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Received December 31, 2013; Accepted January 23, 2014; Published January 27, 2014

Citation: Andrews PM, Chen Y (2014) Using Optical Coherence Tomography (OCT) to Evaluate Human Donor Kidneys Prior to and Following Transplantation. J Nephrol Ther 4: 151. doi: 10.4172/2161-0959.1000151

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kidney microanatomy on intact human kidneys \textit{ex vivo} even when surrounded by an intact connective tissue renal capsule [32,33]. To make OCT more applicable to the clinical situation, we developed a handheld OCT unit that has proven highly effective in our preliminary clinical trials. OCT has proven especially valuable in studying the living kidney because not only are excisional biopsies invasive, damaging and can only sample a very small region of the kidney, they also produce severe artifacts that are difficult to distinguish from ischemia and other injuries [4]. In addition, OCT can detect blood flow \textit{in vivo} using the Doppler [34]. Doppler OCT (DOCT) combines the ability of OCT to capture high-resolution structural images with corresponding Doppler velocity maps that can be merged together to identify regions with moving reflectors, indicating blood flow. Thus, OCT/DOCT is a powerful tool that combines structural and functional imaging which could be used to evaluate kidney status \textit{in vivo} and in real-time during and following surgical procedures.

In this paper, we document the ability of OCT/DOCT to diagnose ATN in human donor kidneys both prior to and following their transplantation. We conclude that OCT imaging of donor kidneys can provide transplant surgeons with a means for predicting renal transplant outcomes.

**OCT System Setup and Design**

A custom-built OCT system with a fiber-optic, hand-held probe was used in this study, enabling real-time, intra-operative OCT imaging during kidney transplant procedures (Figures 1 and 2A,B). Briefly, the details of the OCT system used in our studies consisted of a Fourier-domain OCT system with swept-source laser operating at 1310 nm center wavelength and 100 nm bandwidth. The OCT system’s axial resolution was ~12 µm, and using a 2.0x objective in the sample arm, a transverse resolution of ~15 µm was achieved as determined by resolution chart. Wavelength sweeping frequency was 16 kHz enabling real-time 2D imaging. OCT image pixel dimensions were as follows: 1024 [X] by 512 [Z], and the associated 2D image scan dimensions were 3.75 mm [X] by 2.0 mm [Z]. The system’s sensitivity was with ~90 dB [31].

For operating room (OR) imaging, the hand-held OCT imaging device was assembled on a portable cart that can be easily wheeled into and out of the operating room. Figure 2A shows the portable imaging system being used in the OR. The imaging system was equipped with two output monitors facing opposing directions. One monitor enabled the operator to visualize and record the data while the second monitor let the physicians visualize the imaging in real-time.

**Clinical Trials (Methods)**

Prior to engaging in this research, the protocol was approved by the Institutional Review Boards at both Georgetown University and the University of Maryland. Patients scheduled to receive kidney transplant at Georgetown University Medical Center (Georgetown, Washington D.C.) were enrolled in this study. Informed consent was obtained from all patients prior to imaging. A total of 28 patients were enrolled in this study and for each patient we imaged the kidney prior to transplant (i.e., \textit{ex vivo}) and following transplantation and reperfusion (i.e., \textit{in vivo}).

Following procurement from the donor, the kidney was transferred to a sterile ice bath solution prior to its transplantation. During this time, the kidney was imaged using our hand-held OCT imaging probe (\textit{ex vivo} kidney). The entire hand-held probe with its six-foot length of cords was covered with a sterile camera sleeve. A 1.5 cm circular hole was cut in the end of the sleeve and covered with a commercially available, sterile, transparent “Tegaderm” film (3M Health Care, St. Paul MN). This set-up provided a sterile and moisture barrier without impeding the imaging laser beam. It took approximately 2-4 minutes to image the entire kidney \textit{ex vivo}. Following transplantation of the kidney into the patient and reestablishment of blood flow to the donor kidney, we imaged the transplanted kidney again (\textit{in vivo} kidney). For \textit{in vivo} kidney imaging, the interference fringe data (the complex OCT signal including both magnitude and phase information) was recorded to enable DOCT processing and analysis. The total time for surveying the transplanted kidney was approximately 2-4 minutes.

**Clinical Trials (Results)**

**Ex vivo Kidney Imaging**

While in a sterile ice bath prior to transplantation into the recipient, the entire donor kidney surface was surveyed by OCT (i.e., surveyed globally). Figure 3 shows representative OCT imaging of the \textit{ex vivo} kidneys prior to transplantation. The kidney parenchyma containing uriniferous tubules is visible beneath the intact kidney capsule. There were significant variations in the openness of lumens of the

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**Figure 1:** Schematics of the Fourier-domain OCT system used in kidney imaging studies. Inset shows the photo of OCT microscope.

**Figure 2:** A. Transplant surgeons using the sterilized hand-held OCT probe to image a transplanted human donor kidney in the OR. Both surgeons are looking at real-time images of the functioning kidney while they are imaging it. The OCT probe and associated wires are covered with a sterile camera sleeve. B. The OCT imaging probe covered with camera sleeve and transparent Tegaderm (arrow).
proximal convoluted tubules (Figures 3A and B). An analysis of post-transplant function (i.e., serum creatinine and BUN values) revealed that a decrease in the proximal convoluted tubule luminal diameters correlated closely with a poorer post-transplant renal function.

In vivo OCT Kidney Imaging

Following transplanting and reestablishing blood flow to the donor kidney, the kidney was imaged using OCT (Figures 4 and 5) and DOCT (Figure 6). Figure 4 depicts representative in vivo kidney OCT images from two patients (Figure 4A and 3B are from one, 4C and 4D are from another) showing cross-sectional profiles of superficial proximal tubules below the renal capsule. The openness of tubule lumens reflects a functioning post-transplanted kidney. The poorest post-transplant function was seen in one patient who had suffered an additional normothermic ischemic insult during reimplantation. This patient’s transplanted kidney did not show open tubules and suffered DGF.

The spatial distribution of opening tubule lumens exhibited variation from patient to patient. Example images of the variation of tubule morphology for both the size/shape and the density/uniformity are evident in Figure 5. Tubule size/shape was grouped to poor, moderate, and good. Tubule density/uniformity was also grouped to poor, moderate, and good categories. These images illustrate the visual appearance represented by the scoring values and can be used for standardizing the scoring system across multiple users for further analysis of tubule opening quantification.

OCT fringe data was also recorded during in vivo imaging to enable DOCT imaging for visualizing blood flow in real time as shown in Figure 6. OCT is displayed in gray scale and DOCT is overlaid with a color map. Blue-cyan represents blood flow in one direction while red-yellow represent blood flow in the opposite direction. In a previous publication, we have shown that DOCT will reveal glomerular blood flow and can be used to evaluate glomerular function/status [31].

Figure 3: OCT image of a donor kidney showing open tubules (black arrows) prior to transplantation (A) and a donor kidney showing no open tubules prior to transplantation (B). The donor kidney seen at A exhibited a rapid return to normal function (two days), while that at "B" did not recover normal values until nearly two weeks. The brackets indicate the kidney capsule while the white arrows indicate the Tegaderm. Scale bar=500 µm.

Figure 4: OCT imaging of human kidneys following transplantation showing open uriniferous tubules below the renal capsule in two different patients (A and B are for one patient and C and D are for another). Tubules appear to be open with some degree of homogeneity throughout the images. Scale bar = 500 µm. (Reprinted with permission from JIOHS, 7 (4), 130064, 2014).

Figure 5: Examples of variation of in vivo OCT images of human kidneys for tubule size/shape and density/uniformity. Tubule size/shape: (B) poor (D) moderate (F) good. Tubule density/uniformity: (A) poor (C) moderate (E) good. Scale bar=500 µm. (Reprinted with permission from JIOHS, 7 (4), 130064, 2014).

Figure 6: In vivo human kidney showing open tubules and cortical blood flow. Open tubules appear round and relatively uniform. A larger blood vessel is seen in (B) against some smaller vessels observed in (A, C, and D). The smaller blue and red dots represent peritubular vessels. Data are from a single patient. Scale bar=500 µm. (Reprinted with permission from JIOHS, 7 (4), 130064, 2014).
Future studies

The preliminary studies described in this paper demonstrate the feasibility of using OCT to assess donor kidney status prior to and immediately following transplantation by examining tubular morphology and renal blood flow. With ~12 µm axial and ~15 µm transverse resolution and 16 kHz axial scan rate, OCT revealed renal tubules and cortical blood vessels in real-time during transplantation procedures. 3D imaging will be important in future OCT/DOCT imaging as it provides not only comprehensive volumetric information, but also enables integration of DOCT signals over en face plane to quantify flow information correctly [32-34]. Due to significant motion from the hand-held scanner, 3D OCT imaging could not be performed in clinical settings with our current image acquisition speed. Higher speed imaging systems, however, are now commercially available. For example, Thorlabs Inc. recently developed a VCSEL-based swept source laser running at 100 kHz axial scan rate at 1.3 µm with 100 nm tuning range [35]. Alternatively, a Fourier-domain mode-locked (FDML) laser [36] enables ultrahigh-speed OCT imaging up to MHz A-scan rates [37,38] and can be used for wide-field kidney imaging. These novel light sources will bring new clinical tools capable of volumetric imaging of tissue pathology.

Motion artifacts might be present during image acquisition especially during in vivo imaging. One can stabilize the kidney against the abdominal wall using standard instruments to reduce motion artifacts while imaging. The OCT hand-held probe (using the "cage" as a spacer) can also be gently placed on the kidney surface to further minimize motion. Also, the OCT probe can be attached to an articulating arm to reduce the tremor of the surgeons' hand. Finally, cross-correlation algorithms can be applied to correct the motion artifacts [39].

Conclusions

Recent studies have shown that OCT/DOCT is a powerful emerging medical imaging technology that can be used to evaluate the human kidneys prior to and following their transplantation. Preliminary result demonstrate that OCT/DOCT is a safe, non-invasive procedure that can assess donor kidney status in a timely fashion in the OR and provide important information that can be used to predict post-transplant kidney function.

Acknowledgements

We acknowledge the following individuals, all of whom contributed to the studies reviewed in this manuscript: Hsien-Wen Wang, Jeremiah Wierwille, Wei Gong, Jennifer Verbesey, Matthew Cooper, Seyed Ghasemian, Daniel Joh, Peter Stinson, et al. We also thank Dr. James Jiang and Alex Cable from Thorlabs Inc. for technical support.

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