Real-time assessment of \textit{in vivo} renal ischemia using laser autofluorescence imaging

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Abstract. Potentially transplantable kidneys experience warm ischemia, and this injury is difficult to quantify. We investigate optical spectroscopic methods for evaluating, in real time, warm ischemic kidney injury and reperfusion. Vascular pedicles of rat kidneys are clamped unilaterally for 18 or 85 min, followed by 18 or 35 min of reperfusion, respectively. Contralateral, uninjured kidneys serve as controls. Autofluorescence and cross-polarized light scattering images are acquired every 15 s using 335-nm laser excitation (autofluorescence) and 650±20-nm linearly polarized illumination (light scattering). We analyze changes of injured-to-normal kidney autofluorescence intensity ratios during ischemia and reperfusion phases. The effect of excitation with 260 nm is also explored. Average injured-to-normal intensity ratios under 335-nm excitation decrease from 1.0 to 0.78 at 18 min of ischemia, with a return to baseline during 18 min of reperfusion. However, during 85 min of warm ischemia, average intensity ratios level off at 0.65 after 50 min, with no significant change during 35 min of reperfusion. 260-nm excitation results in no autofluorescence changes with ischemia. Cross-polarized light scattering images at 650 nm suggest that changes in hemoglobin absorption are not related to observed temporal behavior of the autofluorescence signal. Real-time detection of kidney tissue changes associated with warm ischemia and reperfusion using laser spectroscopy is feasible. Normalizing autofluorescence changes under 335 nm using the autofluorescence measured under 260-nm excitation may eliminate the need for a control kidney. © 2005 Society of Photo-Optical Instrumentation Engineers. [DOI: 10.1117/1.1993327]

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1 Introduction

Transplant surgeons often face the difficult question of whether organs from a marginal cadaveric donor are suitable for transplantation. It is currently difficult to quantify how much warm ischemic organ damage has occurred, especially if the donor has experienced significant hemodynamic instability. Organ procurement in brain-dead and nonheartbeating donors also causes a variable amount of additional warm ischemia. Transplanting organs that have sustained significant preimplantation warm ischemic damage may leave the recipient with unacceptably poor allograft function and adversely affect graft and patient survival. On the other hand, as transplant waiting lists continue to grow, it becomes increasingly important to maximize utilization of viable organs from marginal donors. A number of physiological tests have been devised to answer the question of how much warm ischemic organ damage has occurred,\textsuperscript{1}\textsuperscript{-3} but these tests are hindered by clinical practicality as well as hypothermic preservation. Post-transplant monitoring is currently the only reliable way of assessing ischemic organ damage; unfortunately, at that stage the organ is already in the recipient and may have to be removed if found to be nonviable.

Three fundamental questions regarding ischemia in a transplanted organ are apparent. First, how much ischemic damage has an organ sustained? Second, how does the organ respond to intervention aimed at halting or reversing ischemic damage? Third, how will the organ function after it is transplanted? The ideal method for analyzing organ ischemia and answering these questions should be employable at any stage of transplantation—while the organ is still in the donor, after it has been removed (during hypothermic preservation), while it is being implanted, and after reperfusion. The method should also be capable of yielding real-time, nearly instantaneous results so that clinical decisions can be made in a timely fashion.

We have previously demonstrated the efficacy of optical spectroscopy in quantifying prepreservation warm ischemia \textit{ex vivo} in hypothermically preserved rat kidneys.\textsuperscript{4} In this
study, our aim was to explore optical spectroscopic methods for in vivo real-time assessment of warm ischemia in rat kidneys. Rather than analyzing a small portion of the kidney surface, we utilized spectroscopic imaging methods to capture changes occurring over the entire surface of the kidney. Our specific goals were as follows: 1. to demonstrate that optical spectroscopy, utilizing laser autofluorescence imaging, is a feasible means of in vivo real-time monitoring of both ischemia and reperfusion in rat kidneys; 2. to investigate whether these methods might eventually be able to predict an inability to recover from extended ischemic injury; 3. to explore the effects of different laser excitation wavelengths, as well as hemoglobin absorption, on spectroscopic measurements; and 4. to determine whether changes in tissue fluorophore identity versus quantity are responsible for the changes observed during ischemia/reperfusion.

2 Methods

2.1 Renal Ischemia

All animal procedures were approved by the University of California, Davis Animal Use and Care Committee. Adult male Wistar rats weighing 300 to 500 g were anesthetized using 100-mg/kg sodium pentobarbital (50 mg/mL) injected intraperitoneally. Laparotomy was performed through a midline abdominal incision. Both kidneys and their respective vascular pedicles were then dissected free of surrounding fascia and fatty tissue, and mobilized anteriorly into the laparotomy wound so they could be imaged by an external camera. Care was taken to avoid twisting the vascular pedicle and causing premature ischemia in either kidney.

Unilateral warm renal ischemia was induced by left renovenous pedicle clamping and then studied by analyzing tissue autofluorescence under excitation with laser light of different wavelengths. Reperfusion was studied in vivo after unclamping of the renovenous pedicle. In all groups, the right kidneys were left uninjured and used as internal controls. At the conclusion of the imaging studies, all rats were sacrificed. Ischemia times in the experimental groups were chosen based on the previous demonstration by Calman and Bell that rat kidneys clamped for less than 50 min recovered, while those clamped for greater than 70 min did not.

Renal ischemia time in group 1 (n=4 rats) was 18 min, followed by unclamping and a reperfusion phase of 18 min. Images were acquired under a laser excitation wavelength of 335 nm. Renal ischemia in group 2 (n=4 rats) was 85 min, followed by unclamping and a 35-min reperfusion phase. Images were acquired under a laser excitation wavelength of 335 nm. Renal ischemia time in group 3 (n=3 rats) was 85 min, followed by unclamping and 35 min of reperfusion. Images were acquired under both 335- and 260-nm excitation during the warm ischemia and reperfusion phase. In group 4 (n=2 rats), we monitored the autofluorescence intensity under 260-nm excitation during 20 min of renal ischemia, followed by a 20-min reperfusion phase. In group 5 (n=4 rats), we performed cross-polarized light scattering imaging measurements at 650 nm to monitor the change of hemoglobin absorption during 20 min of ischemia time, followed by 30-min of reperfusion. In group 6 (n=2 rats), we studied kidney autofluorescence spectra using 335- and 260-nm excitation at the time of clamping (0 min) and at 75 min of warm ischemia.

2.2 Optical Spectroscopy Methods

In each experimental rat, both kidneys were exposed and a black, nonfluorescing cloth was used to cover the remainder of the rat’s body and viscera to avoid autofluorescence from other tissues. The anterior surfaces of both kidneys were simultaneously excited.

The kidneys in groups 1 and 2 were imaged using their native autofluorescence under exposure to 335-nm laser illumination. A set of lenses was used to enlarge the laser beam and its central portion was used to provide nearly uniform illumination of the kidneys. The laser illumination wavelength was obtained by second harmonic conversion of the output of an optical parametric oscillator (Opotek, Incorporated, Carlsbad, California) tuned at 670 nm. Excitation with 335 nm is known to penetrate tissue to a depth on the order of a few hundred μm, depending on tissue type. Resulting autofluorescent images from each pair of kidneys were captured by a liquid nitrogen cooled charge-coupled device (CCD) array detector (512×512 pixels, Roper Scientific, Trenton, New Jersey). A camera lens was used to project the images to the CCD detector through a 395-nm long wavelength pass filter placed in front of the camera. This combination of filter and detector captures images using the autofluorescence from the kidneys in the ~400- to 1000-nm spectral region. This range was selected to optimize signal-to-noise ratio and enable short image acquisition times. Both excitation and emission spectral bands for image acquisition were chosen based on our previous experience using 335-nm excitation with multiple narrow band and long pass filters. Starting from the time of left renal vascular pedicle clamping, images were taken every 15 s for the duration of the ischemia and reperfusion phases. The intensity of the captured images was normalized to the intensity map of the photoexcitation beam, which was recorded by acquiring the fluorescence image of the beam imprint on a fluorescent paper. Normalization was performed by pixel-by-pixel division of autofluorescence images by the images of the beam profile on the fluorescent paper. Images were analyzed using commercially available computer software (Roper Scientific) to calculate the ratio of autofluorescence intensity of the ischemic to control kidneys. The software calculates the average pixel intensity over a rectangular area of the image. We used this feature to obtain the average intensity of the kidneys by covering as many pixels as possible of the digitized image of each kidney.

In groups 3 and 4, the same imaging system described above for groups 1 and 2 was used. Group 3 kidneys were imaged with 260- and 335-nm excitation, at 0, 18, and 85 min of ischemia. The 260-nm laser illumination wavelength was obtained from the same optical parametric oscillator (OPO) laser system. The goal of this set of experiments was to compare the optical spectroscopic properties of ischemic kidneys when imaged under different excitation wavelengths. We used the same spectral band imaging filter (395-nm-long wavelength pass) for both excitation wavelengths to facilitate their near-simultaneous use during the execution of the experiment. Our previous experience with probing different excitation wavelengths demonstrated that
excitation of hypothermically preserved rat kidneys ex vivo
with a laser tuned at 260 nm resulted in no changes (within
experimental error) in intensity ratio between a normal kidney
and a kidney that had undergone 60 min of warm ischemia
prior to hypothermic preservation. In the current study, we
examined whether ischemic kidneys, during in vivo measure-
ments under 260-nm excitation, would display similar spec-
 troscopic properties. To examine the same effect at shorter
ischemia times, group 4 experiments utilized 260-nm excita-
tion to monitor the autofluorescence from normal and injured
kidneys during 20 min of renal ischemia time, followed by
20 min of reperfusion.

To monitor any changes in the absorption by hemoglobin
during 20 min of ischemia and 20 min of reperfusion, normal
and injured rat kidneys in group 5 were studied using the
same experimental setup, with the exception that images were
obtained with cross-polarized light scattering at 650 nm. The
illumination was delivered to the kidneys using a fiber bundle
that was coupled to a white light source. Both a linear polar-
ized and a 650±20-nm (40-nm bandwidth) narrow band in-
terference filter were positioned at the output of the illumina-
tion fiber to deliver polarized and nearly monochromatic
illumination. A second polarizer with its polarization orienta-
tion orthogonal to that of the illumination was positioned in
front of the imaging lens to capture cross-polarized images of
the kidneys. Images were captured every 5 s, starting from
the time of left renal vascular pedicle clamping, for the dura-
tion of the ischemia and reperfusion phases. Images were ana-
yzed using the same methods described earlier.

During ischemic injury, the absorption spectrum of hemo-
globin changes due to transition from oxy- to deoxyhemoglo-in. A reverse change is expected during reperfusion when
fresh, oxygenated blood recirculates through the injured kid-
ney. Cross-polarized imaging was used to: 1. eliminate the
specular reflection component (which remains mostly polar-
ized and bears no information from below the surface of the
imaged organ); and 2. obtain images of kidneys generated by
photons that have penetrated the tissue, thus offering optimal
information regarding changes in the absorption within the
substance of the kidney. We chose 650 nm for imaging be-
cause there is a large difference in the absorption coefficient
between oxy- and deoxyhemoglobin in this part of the spec-
trum, allowing for visualization of the effect in an imaging
mode. Increased deoxyhemoglobin concentration leads to
more absorption of the 650-nm light and, if occurring during
these experiments, would be expected to result in decreased
intensity of the kidney images.

The autofluorescence spectra of kidneys from group 6
were recorded at 0 min and after 75 min of warm ischemia
using a back-illuminated CCD detector (1340×400 pixels,
Roper Scientific, Trenton, New Jersey) after the emission was
spectrally analyzed using a single-gating spectrometer
(TRIAX 320, Jobin Yvon Incorporated, Edison, New Jersey).
The tunable output of the OPO laser was used for excitation
with the laser wavelength alternately tuned at 335 or 260 nm
to capture the spectra for various ischemia times at both these
excitations. The autofluorescence was collected and focused
into the slit of the spectrometer using reflective optical ele-
ments. To monitor spectral change independently of autoflu-
rescence intensity, spectral profiles were normalized by divid-
ing each by its peak intensity.

3 Results

3.1 Group 1: Imaging During 18 Min of Ischemia
and 18 Min of Reperfusion (335-nm Excitation)

Figure 1 shows a representative example of our experimental
observations in group 1, depicting the injured-to-normal au-
tofluorescence image intensity ratio over 18 min of warm is-
chemia, followed by an 18-min reperfusion phase. In this
figure, the intensity ratio of one out of every three images is
plotted over ischemia time. The intensity ratios rose slightly
from 1.0 to 1.04 during approximately the first minute of is-
chemia, and then declined steadily to 0.78 at the time of
unclamping. Intensity ratios for the other three 18-min injured
kidneys at the time of unclamping were 0.76, 0.78, and 0.78.
During the reperfusion phase, the intensity ratios rose with a
slightly lesser slope than that observed during their decline
with ischemia, finally reaching 0.98 after 18 min (Fig. 1).
Intensity ratios for the other three 18-min injured kidneys at
the end of the 18-min reperfusion phase were 0.97, 0.93, and
0.98.

3.2 Group 2: Imaging During 85 Min of Ischemia
and 35 Min of Reperfusion (335-nm Excitation)

Figure 2 illustrates a representative example of the injured-to-
normal autofluorescence image intensity ratio over 85 min of
warm ischemia, followed by a 35-min reperfusion phase. In
this figure, the intensity ratio of one out of every nine images
is plotted over ischemia time. The intensity ratios rose during
the first minute of ischemia, and then declined steadily. Between 50 and 60 min of ischemia time, the intensity ratios leveled off at 0.68, with no further decline observed for the remaining 25 to 35 min of ischemia. Intensity ratios for the other three 85-min injured kidneys at the time of unclamping were 0.62, 0.64, and 0.65. During the reperfusion phase, the intensity ratios did not change notably for any of the kidneys in group 2, remaining
between 0.62 and 0.70.
3.3 Group 3: Imaging During 85 Min of Ischemia and 35 Min of Reperfusion Using 335- and 260-nm Excitation

Figure 3 shows representative autofluorescence images of kidneys in group 3 that were subjected to ischemic injury for 18 min/h20851 Figs. 3/a and 3/b and 85 min (Figs. 3/c and 3/d), with their respective controls. Each pair of kidneys was imaged using both 335- excitation [Figs. 3/a and 3/c] and 260-nm excitation [Figs. 3/b and 3/d] through a 395-nm long pass filter. Under 335-nm excitation, we observed notable differences in autofluorescence intensity between the injured and control kidneys at 18 min of ischemia, and these differences were more pronounced at 85 min of ischemia. However, under 260-nm excitation, we observed no notable differences at 18 and 85 min of ischemia. Furthermore, no notable changes were observed during the reperfusion phase following 85 min of warm ischemia with 335- or 260-nm excitation (data not shown).

3.4 Group 4: Imaging of the Kidneys During 20 Min of Ischemia and 20 Min of Reperfusion Under 260-nm Excitation

The observation of almost no change in the autofluorescence intensity of kidneys undergoing ischemic injury and reperfusion under 260-nm excitation was further studied in the group 4 experiments. Autofluorescence images of the kidneys were recorded in real time under 260-nm excitation for 20 min of injury followed by 20 min of reperfusion. Figure 4 illustrates an example of the injured-to-normal autofluorescence image intensity ratio obtained during this process. Although there was a small decline during the injury phase followed by a recovery of the intensity during the reperfusion phase, the value of the ratio did not decline below 0.95.

3.5 Group 5: Imaging with Cross-Polarized Light Scattering at 650-nm During Ischemia and Reperfusion

Figure 5 demonstrates an example of our experimental results using cross-polarized light scattering with 650-nm imaging during 20 min of ischemia and 20 min of reperfusion. The ratio of the intensity of the injured over that of the normal kidney declined to ≈0.75 within the first 5 min of injury. The decline continued during the following 15 min of injury at a much slower pace to reach a minimum value of 0.67. Following reperfusion, the intensity ratio increased very rapidly and its value exceeded 1.0 to reach a maximum value of 1.1. This behavior was in sharp contrast to the measured change of the autofluorescence intensity ratio under 335-nm excitation (see Fig. 1). A similar behavior was observed in the intensity ratios for the kidneys of the other three rats of group 5—a fast decline of the ratio during the first 5 min of injury followed by a slower decline during the remaining 15 min of injury. The minimum values of the ratios for these three cases were...
0.74, 0.76, and 0.83. Following unclamping, as fresh blood circulated through the kidneys, the recovery of the intensity ratios toward their initial values (1.0) was rapid and almost identical to the case shown in Fig. 5. In one case (the kidney that had minimum value of 0.74), the repertusion phase led to an overshoot above 1 to a maximum value of 1.3. In the remaining two cases, the ratio reached a maximum value of 1.03 and 1.05.

3.6 Group 6: Emission Spectral Profiles at 335- and 260-nm Excitation

Figure 4 shows emission spectra, normalized to peak autofluorescence intensity, of injured kidneys in group 6 excited with 335- and 260-nm laser light. The spectra represent an average from all animals using spectra from five different sites in each kidney. At 0 min of ischemia, kidneys excited with 260 nm demonstrated a peak in autofluorescence at around 330 nm, whereas those excited with 335 nm had peak autofluorescence at around 470 nm. Although image intensity changed with increasing ischemia time for kidneys excited with 335 nm (see results of groups 1, 2, and 3), the spectral profiles did not change with increasing ischemia time for either excitation wavelength.

4 Discussion

Growing waiting lists for solid organ transplantation necessitate the development of methods to expand the donor pool. All too frequently, organs from donors having experienced significant hemodynamic instability or prolonged cardiac arrest are discarded because of concerns over irreversible ischemic damage. To date, no reliable and practical means of assessing and quantifying ischemic damage in potentially transplantable organs exists. We previously investigated the ability of laser autofluorescence and light scattering spectroscopy imaging techniques to assess the degree of warm ischemia in hypothermically preserved rat kidneys. In this series of experiments, we explored optical spectroscopic imaging as a means of quantifying in vivo renal injury, thus eliminating the need for a control kidney.

The advantage of using spectral imaging, rather than a single point spectroscopic measurement, as has previously been done, is that the intensity from the entire kidney can be analyzed as a single measurement. Thus, meaningful results can be obtained despite clinically frequently observed macroscopic heterogeneity in tissue structure, and differences in the degree of ischemia from one point to another. Because of the heterogeneity of ischemia across the kidney, data taken from one small point may not represent the whole surface; as a result, multiple measurements must be taken and averaged.

We found that laser autofluorescence imaging of kidneys in live rats was technically feasible, and that such imaging could be accomplished at short enough intervals (i.e., seconds) so that the acquired data could be processed and made available for interpretation in real time. The autofluorescence intensity of the injured kidneys decreased with increasing warm ischemia time. However, isolated, absolute measurements could not be relied on to give accurate, reproducible information, mainly due to variations in the observed absolute autofluorescence intensity between different animals. Therefore, we used each animal’s contralateral, uninjured control kidney to normalize measurements made of the ischemic kidney. The optical spectroscopic measurement of ischemia (and reperfusion) was thus quantified by the injured-to-normal autofluorescence intensity ratio.

Clamping the renal vascular pedicle and inducing warm ischemia resulted in an initial slight rise in the injured-to-normal intensity ratio. After about 1 min of ischemia, the intensity ratio began a steady decline. The slope of this decline was very consistent between the experimental animals. In our experiments, all injured kidneys that were unclamped after 18 min and reperfused, experienced a steady increase of the intensity ratio toward the baseline ratio. Kidneys that continued to experience warm ischemia out to 85 min reached an intensity nadir at about 50 min, after which almost no change was observed. On unclamping and reperfusion, the intensity ratio of these kidneys did not change either. Previous experiments suggest that rat kidneys subjected to less than 50 min of ischemia can usually recover, while those subjected to more than 70 min do not. Variable recovery can be expected between 50 and 70 min. Our results suggest, therefore, that the rebound of intensity ratios observed in the 18-min ischemic kidneys after reperfusion may be indicative of physiological recovery, while the lack of such a rebound in the kidneys with 85 min of ischemia may reflect the fact that these kidneys did not recover and may have undergone irreversible, significant injury. To confirm our spectroscopic method’s ability to predict physiological recovery, however, it would be necessary to follow the injured kidney’s physiological performance for some time after ischemia. Moreover, it would be important to test our method’s predictive power in
kidneys injured between 50 and 70 min, where recovery may be variable.

Emission spectra at 335-nm excitation demonstrated a dominant peak at 470 nm. While increasing ischemia time correlated with changes in autofluorescence intensity, the spectral profile did not change notably. These observations suggest that the quantity of tissue fluorophore excited under these parameters changed with ischemia, but that the identity of the fluorophores involved did not change. The intrinsic mitochondrial fluorophore NADH exhibits a peak in its emission spectrum at 470 nm when excited at 335 nm, and is therefore likely the molecule responsible for the changing spectroscopic properties associated with ischemia in our study. During cellular respiration, NAD is reduced to NADH to serve as an electron transporter in the oxygen-dependent process that regenerates the cellular energy unit ATP. Without flow of oxygenated blood, NADH accumulates in tissues and causes an increase in autofluorescence when excited with the appropriate wavelength. We observed an initial increase in autofluorescence intensity over approximately the first minute after the onset of ischemia, possibly corresponding to an increase in NADH levels. This short-term, self-limited increase in NADH has also been observed by others, and may be due to the finite amount of NAD available for conversion to NADH. However, after this initial relatively small rise, we observed a steady decrease in intensity with increasing ischemia time, coupled with a rise in intensity after reperfusion. These findings suggest that with increasing ischemia, one or more nonfluorescent byproducts of cellular ischemia (e.g., pyruvate, lactate, potassium) may have accumulated steadily and may have interacted with NADH, resulting in a decrease of its fluorescence intensity.

The light scattering imaging results under 650-nm illumination suggest strongly that the changes in the autofluorescence intensity do not arise from, or correlate with, changes in the absorption of the residual blood within the kidney. The pattern of the change in hemoglobin absorption, as illustrated in Fig. 5, is clearly different from the pattern observed with the autofluorescence intensity under 335-nm excitation (Fig. 1). Within the first five minutes after clamping the vascular pedicles, the rapid change in hemoglobin absorption contrasts with the initial increase, followed by a small decrease, in autofluorescence. Additionally, the hemoglobin absorption returns to and even exceeds its original value within 2 min of unclamping, while the autofluorescence increases at a much slower pace during this reperfusion phase. Furthermore, the emission spectra (Fig. 6) did not show any significant changes with increasing ischemia time that would be expected if changes were due to enhanced absorption by blood.

In the setting of clinical transplantation, an uninjured control kidney for normalizing autofluorescence intensity measurements is not available. Figures 3 and 4 demonstrate that virtually no change in autofluorescence intensity is observed with ischemic injury under 260-nm excitation. The dominant emission peak at 330 nm with this excitation wavelength suggests tryptophan as the active fluorophore. Unlike NADH, tryptophan is not directly involved in aerobic or anaerobic cellular respiration; the spectroscopic properties of tryptophan would therefore be expected to remain “inert” during ischemia. Our results suggest that autofluorescence intensity at 335 nm, which changes with ischemia, can therefore be normalized to autofluorescence intensity measured at 260-nm excitation in the same tissue, eliminating the need for a control kidney. This step is crucial in the evolution toward clinical practicality and will be further explored in future experiments.

5 Conclusion

Optical spectroscopy utilizing laser autofluorescence is a useful means of monitoring renal ischemia in real time. Spectroscopic measurements during the reperfusion phase following reperfusion appear to correlate with the degree of warm ischemic damage. Therefore, this novel optical spectroscopic technique may be capable of predicting the ability of renal tissue to recover from an ischemic insult. Spectral analysis of injured kidneys suggests that NADH may be the fluorophore responsible for the spectroscopic changes with ischemia. Finally, the “inert” excitation wavelength of 260 nm may eliminate the need for an uninjured control kidney as a means of normalizing image intensity.

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References