Intraoperative Optical Biopsy during Robotic Assisted Radical Prostatectomy Using Confocal Endomicroscopy

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Purpose: Intraoperative optical biopsy technologies may aid in the identification of important anatomical landmarks and improve surgical outcomes of robotic assisted radical prostatectomy. We evaluate the feasibility of confocal laser endomicroscopy during robotic assisted radical prostatectomy.

Materials and Methods: A total of 21 patients with biopsy proven prostate cancer scheduled for robotic assisted radical prostatectomy were recruited. After intravenous administration of fluorescein 15 patients underwent in vivo intraoperative confocal laser endomicroscopy of prostatic and periprostatic structures using a 2.6 or 0.85 mm imaging probe. Standard robotic instruments were used to grasp and maneuver the confocal laser endomicroscopy probes for image acquisition. Confocal laser endomicroscopy imaging was performed ex vivo on fresh prostate specimens from 20 patients. Confocal video sequences acquired in vivo and ex vivo were reviewed and analyzed, with additional image processing using a mosaicing algorithm. Processed confocal images were compared with standard hematoxylin and eosin analysis of imaged regions.

Results: Confocal laser endomicroscopy was successfully integrated with robotic surgery, including co-registration of confocal video sequences with white light and probe handling with standard robotic instrumentation. Intraoperative confocal laser endomicroscopy imaging of the neurovascular bundle before and after nerve sparing dissection revealed characteristic features including dynamic vascular flow and intact axon fibers. Ex vivo confocal imaging of the prostatic parenchyma demonstrated normal prostate glands, stroma and prostatic carcinoma.

Conclusions: We report the initial feasibility of optical biopsy of prostatic and periprostatic tissue during robotic assisted radical prostatectomy. Image guidance and tissue interrogation using confocal laser endomicroscopy offer a new intraoperative imaging method that has the potential to improve the functional and oncologic outcomes of prostate cancer surgery.

Key Words: prostatic neoplasms; prostatectomy; microscopy, confocal; erectile dysfunction; surgery, computer-assisted

CANCER control and recovery of urinary and sexual function after radical prostatectomy are related to surgical quality.1 Since Walsh's initial description of anatomical radical prostatectomy there have been efforts to better...
understand pelvic anatomy to refine surgical technique. Robotic assisted radical prostatectomy is currently the most common surgical treatment for localized prostate cancer in the United States. Technological advances of the robotic platform include a magnified field of view, tremor filtration and improved surgeon ergonomics. Despite advances in understanding pelvic anatomy and surgical technologies, significant variation remains in the surgical outcomes of radical prostatectomy, including positive surgical margins (range 6.5% to 32%) and erectile dysfunction (range 7% to 80%).

Image guided surgery may improve intraoperative navigation and surgical outcomes. Optical imaging technologies offer excellent spatial and temporal resolution, are easily integrated into the operating room and can be manipulated with instruments commonly used in minimally invasive surgery. For radical prostatectomy in vivo and ex vivo feasibility studies have been reported using near infrared fluorescence imaging, OCT and MPM. Similar to OCT and MPM, confocal laser endomicroscopy is an optical biopsy technology that aims to provide on demand, high resolution imaging reminiscent of standard histopathology. CLE is approved for endoscopic applications in gastroenterology, pulmonology and urology. CLE is based on a 488 nm laser in conjunction with fluorescein, a Food and Drug Administration approved fluorophore with a demonstrated safety record.

We have demonstrated the cystoscopic application of CLE for the optical diagnosis and grading of bladder cancer, as well as in vivo visualization of glandular structures in the prostatic urethra. We assessed the feasibility of intraoperative CLE during RARP and evaluated potential clinical applications. We developed an intraoperative confocal imaging protocol, characterized in vivo microscopic features of prostatic and periprostatic anatomy, and compared ex vivo imaging of fresh surgical prostate specimens to histopathology.

MATERIALS AND METHODS

Instrumentation

Confocal endomicroscopy was performed with Cellvizio®, and 2.6 or 0.85 mm outer diameter fiberoptic probes were used for image acquisition (fig. 1, A). The 2.6 mm probe has a spatial resolution of 1 μm, a tissue penetration depth of 60 μm and FOV of 240 μm. The 0.85 mm probe has a spatial resolution of 3.5 μm, a penetration depth of 50 μm and a FOV of 320 μm. Probes were sterilized before use with the Sterrad® system.

Intraoperative CLE during Robotic Assisted Surgery

The study was conducted with Stanford University institutional review board and VAPAHCS (Veterans Affairs Palo Alto Health Care System) Research and Development Committee approval. Patients with clinically localized prostate cancer scheduled for RARP were recruited. Two surgeons (JCL and JTL) performed the operations.
and image acquisition. Standard 5-port placement consisting of a 12 mm camera port, 3, 8 mm robotic ports and a 12 mm assistant port was applied. The decision for nerve sparing was based on clinical staging, technical feasibility and surgeon discretion. The majority of CLE imaging was performed with a 2.6 mm probe introduced through the 12 mm assistant port (fig. 1, B). The robotic needle driver was used to grasp the distal metal tip for imaging (fig. 1, C). For the 0.85 mm probe 3 strategies were compared for intracorporeal maneuvering, including 1) insertion via a standard laparoscopic cholangiogram catheter holder operated by bedside assistant, 2) insertion via a 5Fr angiocatheter through assistant port and grasping using the robotic needle driver, and 3) insertion via a 19-gauge angiocatheter introduced suprapubically as a needlescopic port and grasping using the robotic needle driver (fig. 1, D).

Approximately 5 minutes before dissection of the neurovascular bundle, 2.5 ml 10% sodium fluorescein (Akorn, Lake Forest, Illinois) were administered intravenously. For imaging the probe tip was positioned perpendicular to the tissue for en face contact and rinsed with irrigation as needed to remove blood or debris. Images were acquired as video sequences at 12 frames per second. TilePro™ was used to simultaneously view the white light stereoscopic view of the operative field and confocal imaging (fig. 1, C and D). Prostatic and periprostatic structures, including levator fascia, NVB before and after the nerve sparing procedure, prostatic capsule, bladder neck, urethral stump and pelvic floor were imaged in situ, reviewed in real time and recorded for additional off-line analysis.

Ex Vivo CLE of Prostate Specimens
Ex vivo CLE was performed within 1 hour of specimen retrieval. To optimize prostate parenchymal staining an additional 2.5 ml 10% fluorescein were administered intravenously before the division of the prostatic pedicles. CLE image acquisition was performed by manual manipulation of the 2.6 mm probe. Imaged regions on the surface of the prostate included the prostatic capsule, posterolateral surface corresponding to the location of the NVB and apical margins. To characterize parenchymal structures the prostate was sectioned transversely with the assistance of a surgical pathologist (RVR). Each 5 mm thick prostate slice was divided into quadrants and systematically imaged in a defined pattern. Additional fluorescein was applied topically (2 minute incubation then 7 minute saline wash to remove excess fluorescein) to enhance visualization. After imaging the tissues were fixed in formalin and sent for hematoxylin and eosin staining and histopathological analysis. Immunohistochemistry against S100 proteins was performed on select sections (Histo-Tec, Hayward, California) to identify nerves.

Data Analysis
In vivo and ex vivo confocal video sequences were reviewed, edited and analyzed off-line using Cellvizio Viewer v1.6 software. A built-in mosaicing algorithm was used to compile consecutive images into a single larger composite image. Processed confocal images were compared with corresponding hematoxylin and eosin stains and reviewed with a surgical pathologist (RVR).

RESULTS
Between December 2012 and March 2015, 21 patients (mean age 62 years, range 49 to 69) scheduled for RARP at VAPAHCS were recruited. Patients underwent bilateral (16) or unilateral (5) nerve sparing RARP. In vivo CLE imaging was performed in 15 patients and ex vivo imaging was performed on 20 prostates. Patient characteristics and imaging details are described in the supplementary table (http://jurology.com/).

Overall 105 in vivo confocal video sequences from 15 patients were collected. The average image acquisition time was 10 minutes (range 3 to 18) per participant. An average of 7 video sequences (range 4 to 12) was obtained per case. The average duration of imaging at each area was 91 seconds (range 6 to 303). In 1 patient the metal tip of the 2.6 mm probe broke off during handling by the robotic instrument and was removed with a laparoscopic grasper without complication. There were no adverse events related to fluorescein administration.

Comparison of Imaging Probes
We compared the intraoperative handling and image quality of the 2.6 and 0.85 mm probes, testing them in 11 and 4 participants, respectively. The 2.6 mm probe was previously validated for bladder cancer imaging. The 0.85 mm probe was described for CLE of pancreatic cysts through a 19-gauge biopsy needle and upper urinary tract through standard ureteroscopes. The smaller 0.85 mm probe has the potential for greater flexibility for intraoperative probe deployment. While inserted through the 12 mm assistant port, both probes were compatible with in-parallel insertion of additional instruments without significant loss of pneumoperitoneum. To minimize trauma to the optical fibers, the 2.6 mm probe was handled by grasping the distal metal tip (fig. 1, C), whereas the 0.85 mm probe was inserted via a laparoscopic cholangiogram instrument (1), 5Fr catheter (2) or a 19-gauge angiocatheter for maneuvering with the robotic needle holder (fig. 1, D).

Overall the flexibility of the fiberoptic probes enabled efficient access to various pelvic anatomical landmarks for imaging. Given its higher spatial resolution, the image quality from the 2.6 mm probe was significantly better than that from the 0.85 mm probe and, thus, the 2.6 mm probe was used exclusively after the fourth case (see supplementary table, http://jurology.com/). The image quality of the probes did not decrease noticeably with repeated sterilization.

NVB Imaging
Prior ex vivo studies indicate that NVBs are located posterolateral of the prostate and enclosed in
lateral pelvic fascia. Intraoperative CLE of regions corresponding to the NVB was performed before and after nerve sparing dissection. Characteristic confocal features of the NVB include parallel thin dark lines corresponding to axonal fibers, bordered by dark cells consistent with adipocytes, and interspersed with vessels with flowing erythrocytes (fig. 2 and supplementary video, http://jurology.com/). Generally, confocal features of NVBs were not visualized until the lateral pelvic fascia was incised. NVBs were visualized with the 0.85 mm (fig. 2, A) and 2.6 mm imaging probes (fig. 2, B-E). The mosaicing algorithm was applied off-line to generate a wide field view of the NVB (fig. 2, G). In vivo CLE identified the NVB in 11 of 15 patients. In 1 case residual nerve tissues were observed on the prostatic capsule after initial dissection, prompting additional dissection and CLE confirmation of NVB separation from the prostate.

Identification of Prostatic and Periprostatic Structures

Representative in vivo confocal images of prostatic and periprostatic structures are shown in figure 3. The prostatic capsule, bladder neck margin, urethral stump, levator ani and obturator nerve were imaged. Imaging of the prostatic capsule demonstrated striated fibrous tissue with occasional small caliber vasculature. Given the relatively small FOV of confocal laser endomicroscopy, the prostatic capsule was not comprehensively imaged in vivo. No discernible prostatic parenchymal features such as glandular structures were observed in vivo. Confocal imaging of the bladder neck mucosa showed normal urothelium with umbrella and intermediate cells as well as the underlying vasculature of the lamina propria, consistent with previous bladder imaging.

Ex Vivo Confocal Imaging of Fresh Prostate Tissue

To further characterize the confocal imaging features with hematoxylin and eosin correlation, fresh prostate specimens were imaged ex vivo. A total of 259 imaging sequences were collected from 20 subjects. The prostate was imaged intact to visualize the capsular features (fig. 4, A), followed by imaging of prostate sections to visualize the parenchymal structures (fig. 4, B). In patients who underwent a nonnerve sparing procedure residual NVBs were observed on the prostate specimen, and confirmed by hematoxylin and eosin and immunohistochemistry staining of myelin specific antigen S100 (fig. 2, E and supplementary figure, http://jurology.com/).

While most prostate cancer arises from the peripheral zone, given the 60 μm penetration depth of CLE, we did not expect to visualize stromal and glandular structures through an intact capsule unless there were positive surgical margins or extracapsular extension. In specimen 7 with pT3B disease CLE imaging along the lateral prostatic capsule showed glandular structures distinct from the surrounding fibrous capsule (fig. 4, C and supplementary table, http://jurology.com/). This patient was confirmed to have multifocal ECE on pathological examination. On prostate sections benign prostatic glands were characterized by lobular structures with a rim of increased surrounding fluorescence (fig. 4, E). Benign features such as corpora amylacea were easily identified as round circumscribed structures within glands (fig. 4, G). Prostatic glands in tissues found to contain

Figure 2. CLE images of NVB. Nerve axons visualized with 0.85 mm probe (A) and 2.6 mm probe (B-G). Nerves were visualized before (B) and after (C, D) NVB dissection. Residual nerve structures were present on prostatic capsule after neurovascular dissection (E). Intact NVB seen ex vivo on nonsparing prostate specimen (F). Panoramic image of NVB generated with mosaicing algorithm from images obtained during in vivo CLE, with erythrocytes within blood vessels on left and nerve fibers on right (G).
DISCUSSION

We report the initial feasibility of in vivo CLE during RARP. We demonstrated the ease of integrating CLE with robotic surgery, including co-registration of confocal video sequences with white light imaging, probe handling with standard robotic instrumentation and tremor-free image acquisition. We characterized in vivo imaging features of clinically relevant prostatic and periprostatic anatomical landmarks, particularly the NVB. Intraoperative CLE was performed successfully with 2.6 and 0.85 mm probes, with the 2.6 mm probe offering superior image quality. Dynamic imaging of intact NVBs demonstrated parallel axonal fibers lined by adipocytes and small caliber vessels. In vivo microscopy features of the NVB were confirmed with ex vivo CLE and standard hematoxylin and eosin in prostate samples where nerve sparing was not performed.

Erectile dysfunction is a complication of radical prostatectomy that can be minimized by preservation of the NVB. Since components of the NVB have variable distribution and location and cannot be visualized directly during surgery, nerve sparing techniques are based on gross inspection and minimizing thermal energy use near the presumed NVB location. Intraoperative visualization of microscopic features may better guide nerve sparing surgery and provide real-time feedback for adequate dissection. Our results suggest that CLE may be used to map NVB location. Dynamic characterization of the intact NVB after dissection may serve as a marker of successful preservation of the NVB.

Positive surgical margin status is an adverse oncologic outcome of radical prostatectomy that might be improved by image guided identification of ECE at surgical margins. Ex vivo CLE of prostatic carcinoma were characterized by smaller, less regular lobular structures without a surrounding rim of fluorescence (fig. 4, I).

Figure 3. In vivo CLE of prostatic and periprostatic structures with corresponding stereoscopic views from robotic prostatectomy as insets. Confocal characteristics of fibrous prostatic capsule (A), urothelium of bladder neck margin (B), levator ani muscle fibers (C) and axons of obturator nerve (D).
sections revealed benign and cancerous glandular structures. While most of the patients in this series had organ confined disease, in the patients with pT3b disease we were able to detect apparent ECE of carcinoma. CLE could be used in conjunction with preoperative magnetic resonance imaging for targeted intraoperative imaging of areas concerning for ECE. Identification of any glandular structures at surgical margins would prompt the surgeon to redirect the plane of dissection.

CLE differs from other optical biopsy technologies. Compared to clinical OCT systems, CLE offers a higher spatial resolution but a lower penetration depth. MPM offers spatial resolution similar to CLE and improved depth of penetration. However, current studies using MPM are limited to ex vivo human specimens and in vivo animal studies. While OCT and MPM do not require the administration of exogenous dye, fluorescein is inexpensive, has a proven safety profile and may be coupled with targeting agents for molecular imaging using CLE to further improve optical diagnostics.

The small sample size and pilot nature of this study precluded diagnostic accuracy assessment of CLE imaging of the prostate. Furthermore, the impact that CLE may have on long-term functional outcomes is unknown as CLE was not used to direct surgical guidance. A larger sample size and defined
clinical end points will be necessary to assess the clinical benefits of CLE during RARP. Visualization of intact nerves does not equate to functional nerves. Future integration of CLE with molecular imaging agents or nerve stimulator may provide physiological feedback of nerve function. Other technical limitations include the small FOV of CLE, which makes intraoperative surveying of large surface areas impractical. This may not negatively impact the usefulness of CLE for nerve sparing procedures as it was possible to scan the length of the NVB within 90 seconds. However, it may limit the use of CLE for the detection of incidental ECE. While CLE imaging is optimal within 20 minutes of fluorescence administration, we demonstrated the feasibility of fluorescein re-dosing. Future investigation of topical contrast administration with an endoscopic spray catheter may offer alternative strategies for intraoperative CLE without the requirement of intravenous fluorescein.

CLE is a promising technology for microscopic imaging during RARP. CLE optical biopsy of live tissue may provide a new method for the intraoperative identification of the NVB with spatial and temporal resolutions not previously described. Additional experience is required to assess the usefulness of CLE in detecting surgical margin status, and to evaluate if this promising imaging technique will translate to improved oncologic and functional outcomes.

CONCLUSIONS

CLE of the prostate and NVB is feasible during RARP. Nerve fibers can be visualized and differentiated from vessels and connective tissue. Ex vivo CLE can be used to identify prostatic glandular structures and ECE. Additional prospective analysis is required to assess the clinical benefits of CLE guided nerve sparing RARP.

REFERENCES


