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Clinical-Penile cancer

Optical biopsy of penile cancer with in vivo confocal laser endomicroscopy

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Abstract

Introduction: Surgical management of penile cancer depends on accurate margin assessment and staging. Advanced optical imaging technologies may improve penile biopsy and organ-sparing treatment. We evaluated the feasibility of confocal laser endomicroscopy for intraoperative assessment of benign and malignant penile tissue.

Patients and methods: With institutional review board approval, 11 patients were recruited, 9 with suspected penile cancer, and 2 healthy controls. Confocal laser endomicroscopy using a 2.6-mm fiber-optic probe was performed at 1 or 2 procedures on all subjects, for 13 imaging procedures. Fluorescein was administered intravenously approximately 3 minutes prior to imaging for contrast. Video sequences from in vivo ($n = 12$) and ex vivo ($n = 6$) imaging were obtained of normal glans, suspicious lesions, and surgical margins. Images were processed, annotated, characterized, and correlated with standard hematoxylin and eosin histopathology.

Results: No adverse events related to imaging were reported. Distinguishing features of benign and malignant penile tissue could be identified by confocal laser endomicroscopy. Normal skin had cells of uniform size and shape, with distinct cytoplasmic membranes consistent with squamous epithelium. Malignant lesions were characterized by disorganized, crowded cells of various size and shape, lack of distinct cytoplasmic membranes, and hazy, moth-eaten appearance. The transition from normal to abnormal squamous epithelium could be identified.

Conclusions: We report the initial feasibility of intraoperative confocal laser endomicroscopy for penile cancer optical biopsy. Pending further evaluation, confocal laser endomicroscopy could serve as an adjunct or replacement to conventional frozen section pathology for management of penile cancer.

Keywords: Penile neoplasms; Microscopy; Confocal; Surgery; Computer-assisted

Abbreviations: CLE, confocal laser endomicroscopy; H&E, hematoxylin and eosin; OCT, optical coherence tomography; PDD, photodynamic diagnosis; SCC, squamous cell carcinoma

1. Introduction

Penile cancer is rare, accounting for 0.4% of malignant diagnoses in the United States and Europe, and up to 6% of cancers in the developing world [1]. However, penile cancer is associated with significant disease and treatment related morbidity [2]. Management is focused on achieving thorough oncologic control while preserving function, cosmesis, and quality of life [1,3−5]. Squamous cell carcinoma (SCC) is the most common histological type and 80% of cases involve the foreskin or glans [3]. Well and moderately differentiated superficial or locally invasive disease has an excellent prognosis, while poorly differentiated tumors require more aggressive treatment.

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The current paradigm for penile cancer management includes biopsy for clinically indeterminate lesions and immediate resection for highly suspicious lesions [2]. Complete excisional biopsy is often performed for small lesions of the glans or circumcision for the foreskin, while incisional biopsy may be first used for larger lesions to obtain a pathological diagnosis. Incisional biopsies may be limited by under-sampling leading to inappropriate treatment [6]. Histologically confirmed carcinoma in situ (CIS) may be managed with topical therapy, laser treatment, or glans resurfacing [7]. Small, low-stage invasive tumors, and select higher stage tumors are amenable to penile-preserving surgery which may preserve the patient's ability to void upright, have sexual intercourse, as well as maintain self-esteem [3,8]. While a 2-cm surgical margin was traditionally recommended in penile-preserving surgery to reduce local recurrences, current evidence suggests a 5-mm margin is sufficient [7,9,10]. Intraoperative frozen section is recommended to assess margin status, but the utility of frozen section in reducing margin positivity is unclear [7,11]. Frozen sections are further limited by under-sampling, processing time, and the use of the tissue of interest [12]. The limitations associated with incisional biopsies and frozen sections underscore the need for improved intraoperative diagnostics.

Increasingly, optical imaging technologies are under investigation as an adjunct in surgical oncology to improve differentiation of cancerous from benign tissue and provide delineation of margin status [13]. Small reports have demonstrated the feasibility of optical coherence tomography (OCT) and photodynamic diagnosis (PDD) to distinguish benign from malignant penile lesions [14,15]. Confocal laser endomicroscopy (CLE) is an optical biopsy technique that employs a laser light source and fluorescein as a contrast agent to provide in vivo subsurface tissue characterization with excellent spatial resolution resembling histopathology [16]. CLE has been applied in the gastrointestinal tract to improve diagnosis and surveillance of esophageal, gastric, pancreatic, and colorectal cancers [17–19]. In head and neck, normal squamous epithelium and SCC have been characterized using CLE, with normal squamous cells as flat and uniform polygonal cells whereas SCC as disorganized, heterogeneous cells with dark epithelium [20].

We and others have previously demonstrated the feasibility of CLE for numerous urologic applications including evaluation of bladder and upper tract urothelial carcinoma, characterization of small renal masses, and delineation of anatomic landmarks at the time of robotic radical prostatectomy [21–25]. In this study, we evaluate the feasibility of using CLE for optical biopsy of penile cancer.

2. Patients and methods

2.1. Patient recruitment

The study was conducted with Stanford University institutional review board and Veterans Affairs Palo Alto Health Care System (VAPAHCS) approval. We recruited patients scheduled to undergo biopsy, circumcision, or penectomy at VAPAHCS for suspected penile cancer for the study. Two patients undergoing CLE for nonpenile cancer urologic procedures were recruited as healthy controls.

2.2. Instrumentation

We performed CLE using the Cellvizio clinical system (Fig. 1, Mauna Kea Technologies, Paris, France) consisting of a 488-nm laser scanning unit, an image processor, and a 2.6-mm outer diameter fiberoptic probe (CystoFlex UHD) with a 240-μm field of view and 60-μm depth of penetration. CLE videos were acquired at 12 frames per second. Probes were sterilized before each use with the STERRAD system (Advanced Sterilization Products, Irvine, CA).

2.3. Intraoperative confocal laser endomicroscopy

Following initial visual inspection of the penile lesion, 2.5 ml of 10% sodium fluorescein (Akorn Inc., Lake Forest, IL) was administered intravenously (IV) 2 to 3 minutes prior to CLE imaging. The CLE probe was hand held and positioned in direct en face contact with the area of interest (Fig. 1B). At least 2 video sequences of 20 seconds were obtained for each region of interest. To facilitate correlation of CLE imaging with histopathology, the CLE imaging procedure was video-recorded and the regions of interest imaged with CLE were marked with ink and/or suture prior to sending to pathology. In some cases, additional ex vivo CLE was performed on the fresh surgical specimens in the operating room after excision. Surgical procedures

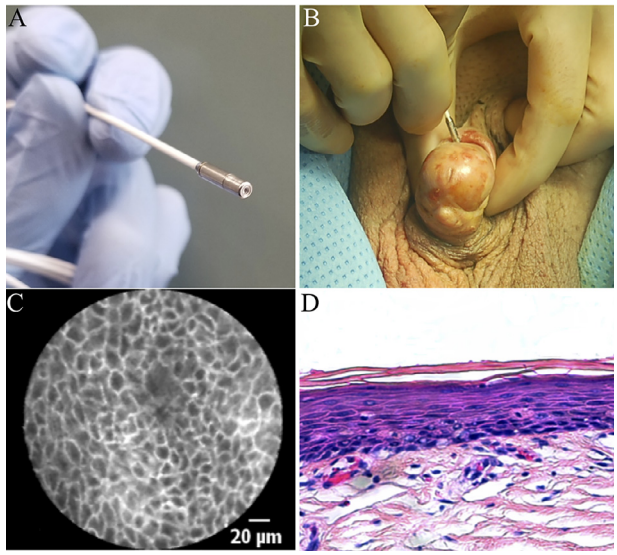

Fig. 1. Confocal laser endomicroscopy of the penis. (A) The 2.6-mm fiberoptic probe used for CLE. (B) Image acquisition with the probe in direct en face contact with a lesion at the glans. (C) CLE imaging of normal portion of glans showing squamous epithelium with corresponding H&E (D), 200×.

(i.e., biopsy, radical circumcision, partial penectomy, or radical penectomy) were performed based upon clinical parameters and independent of CLE findings. The specimens were then sent for standard histopathological assessment with hematoxylin and eosin (H&E) staining.

2.4. Data analysis

Descriptive statistics of patient characteristics were compiled and analyzed. CLE video sequences acquired both in vivo and ex vivo were processed, reviewed, and analyzed using the Cellvizio Viewer software, version 1.6.2. Videos obtained at the time of surgery were reviewed and CLE images annotated for localization to corresponding histopathology slides. Composite images consisting of 2 or more frames were compiled using a built-in mosaicing algorithm to enable a larger field-of-view [26]. Individual confocal frames, composite mosaics, and selected clips and their corresponding H&E sections were reviewed with a genitourinary pathologist (CK).

3. Results

3.1. Patient characteristics

Between April 2013 and June 2018, 11 patients were enrolled to undergo penile CLE. Patient characteristics, including stage, are shown in Table 1 [27]. Nine patients were scheduled to undergo biopsy or surgery for suspected or confirmed penile cancer and 2 patients received IV fluorescein for other CLE indications (mean age 72 years, range 66–82). Patients underwent radical circumcision ($n = 1$), partial penectomy ($n = 5$), or radical penectomy ($n = 3$). Two patients had CLE performed during both initial diagnostic biopsy and subsequent definitive surgery. CLE imaging was performed in vivo during 12 procedures and ex vivo on 6 specimens. On final histopathology, 8 patients had penile SCC and 1 patient who underwent re-excision for a prior positive surgical margin had benign inflammatory changes. The most common lesion location was the glans.

3.2. CLE image acquisition

A total of 83 video sequences (48 in vivo and 35 ex vivo) were collected for this study. The average image acquisition time for each case was 5 minutes, 22 seconds (range: 23 seconds to 12 minutes, 41 seconds). The average duration of imaging of each area was 51 seconds (range: 23–90 seconds). There were no adverse events related to image acquisition or IV fluorescein administration.

3.3. CLE findings

CLE imaging of normal, inflammatory, and cancerous tissue was performed. Normal squamous epithelium was characterized by homogenous cells with uniform size and shape with distinct cytoplasmic membranes (Fig. 2A and D). Distinct circular aggregations of cells corresponding to dermal papillae containing vasculature were seen in select images of normal epithelium (Fig. 2B and E). These imaging features of normal squamous epithelium were seen in both healthy controls and normal-appearing skin adjacent to cancerous regions in all penile cancer patients. An example of benign reactive inflammation under CLE and corresponding H&E is shown in Fig. 2C and F. This was a case of a patient (Table 1, case 13) who underwent incisional biopsy at an outside institution that showed T1 SCC at the biopsy margin and was referred to our institution for partial penectomy. CLE of the biopsy bed at the time of the partial penectomy showed diffuse, dark, and small cells with indistinct borders (Fig. 2C) and the corresponding histology showed benign reactive changes (Fig. 2F). The final pathology of the partial penectomy did not show residual cancer.

Table 1
Patient characteristics and diagnoses.

Case	Age	Procedure	Lesion location	CLE imaging	Stage	Grade
1	71	(Control)	–	In vivo	N/a	N/a
2	71	(Control)	–	In vivo	N/a	N/a
3	69	Radical penectomy	Shaft	Ex vivo	T2	Moderately differentiated
4	70	Partial penectomy	Glans	In vivo	Tis	N/a
5	66	Radical penectomy	Glans	In vivo	T1b	Poorly differentiated
6	82	Partial penectomy	Glans	Both	T1b	Poorly differentiated
7[a]	69	Biopsy	Glans	In vivo	T1b	Poorly differentiated
8[a]		Partial penectomy	Glans	Both		
9[b]	73	Biopsy	Glans	In vivo	Tis	Moderately differentiated
10[b]		Partial penectomy	Glans	Both		
11	77	Radical penectomy	Glans	Both	T1b	Poorly differentiated
12	78	Circumcision	Prepuce	In vivo	Tis	Poorly differentiate
13	72	Partial penectomy	Glans	Both	Inflammation	N/a

[a] Same patient.
[b] Same patient.

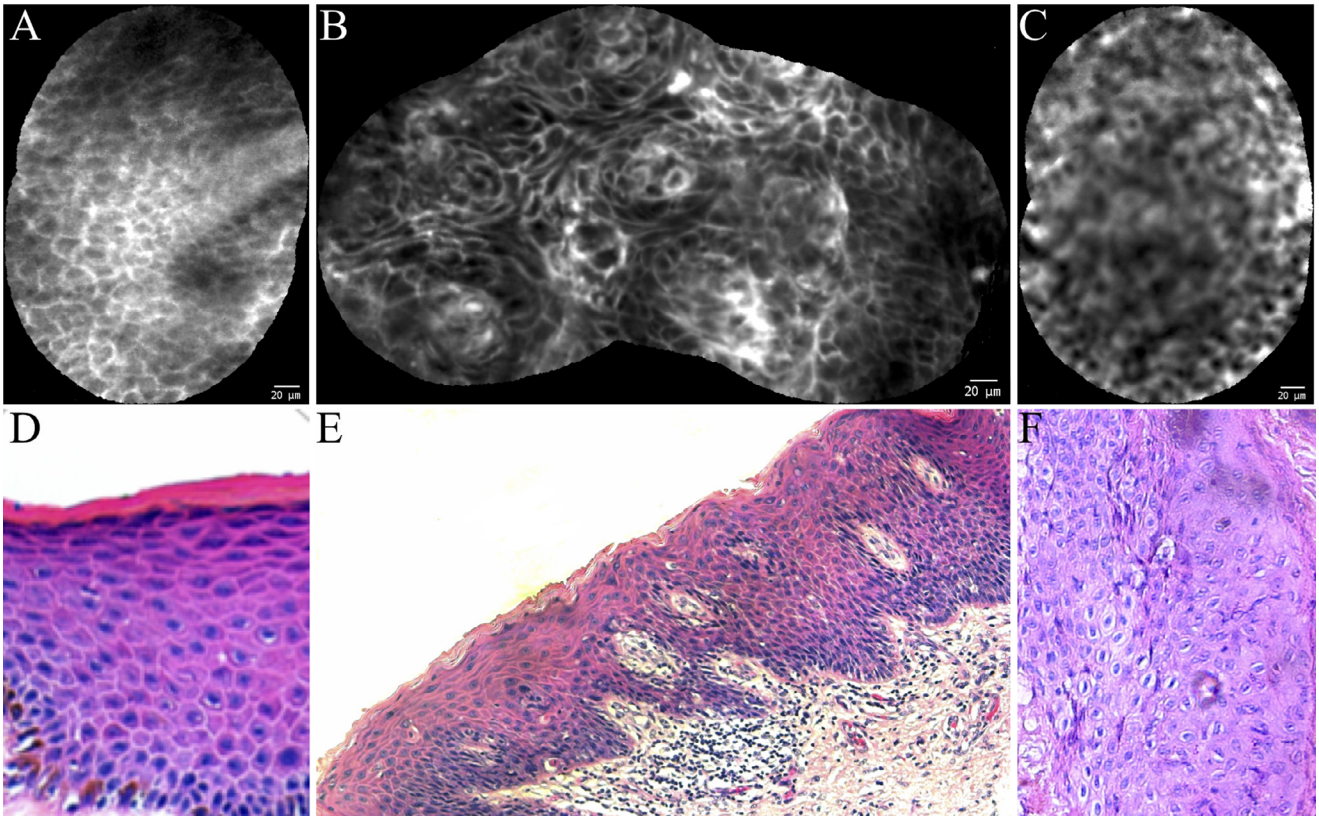

Fig. 2. In vivo confocal laser endomicroscopy features of normal and inflamed penile epithelium. CLE image mosaics (top panel) compared with corresponding H&Es (bottom panel). (A) Normal squamous epithelium showing homogenous cells with uniform size, shape, and with distinct cytoplasmic membranes; (B) Subsurface imaging of the dermal papillae with a "rose petal" pattern. Image processing with mosaicing algorithm enabled a field of view of over 500 μm. (C) CLE imaging of prior biopsy site showing diffuse small cells and indistinct cell borders. Corresponding H&Es (D) 200×, (E) 100×, and (F) 200×.

CLE imaging characteristics of penile SCC include disorganized microarchitecture, crowded cells with variation in size and shape, lack of distinct cytoplasmic membranes, and a hazy, moth-eaten appearance (Fig. 3). Imaging features of cancerous epithelium were identified in all patients with pathological-confirmed SCC ranging from CIS (Fig. 3A) to

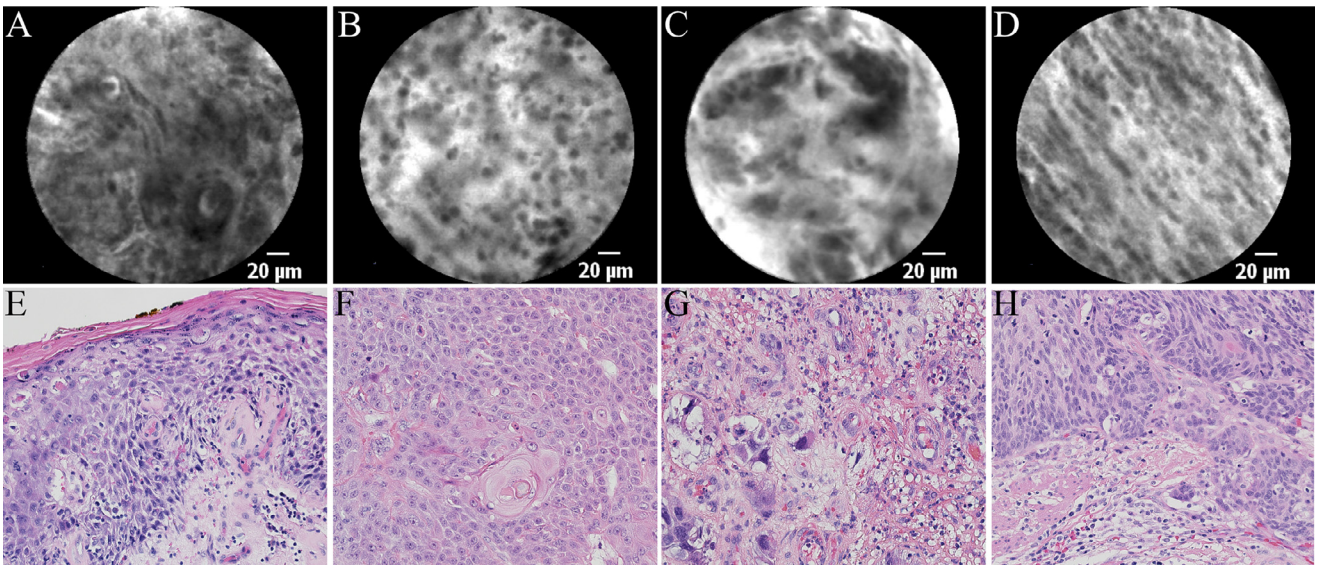

Fig. 3. Endomicroscopy features of squamous cell carcinoma of the penis. Characteristic features include disorganized microarchitecture, cells with variable size and shape, lack of distinct cytoplasmic membranes, and hazy, moth-eaten appearance (top panel A–D). Corresponding H&E sections at 200× magnification (bottom panel E–H). Images were derived from 4 separate subjects with pathological confirmation of penile cancer: (A & E) CIS; (B & F) T1a; (C & G) T1b; (D & H) T2.

invasive SCC (Fig. 3B–D) and in neither of the healthy controls. We were unable to identify definitive distinguishing features between the confirmed SCC cases and the single case of reactive inflammation (Fig. 2C).

To assess whether transition between cancerous and noncancerous tissue can be observed in situ microscopically, the CLE probe was used to scan across gross margins. A representative example of the transition point between benign epithelium and cancer on CLE and correlated to H&E is demonstrated in Fig. 4. A CLE video sequence of transition from benign squamous epithelium to CIS is shown in the Supplementary Video.

4. Discussion

We report the feasibility of in vivo real-time optical biopsy of penile cancer using CLE. The 2.6-mm CLE probe provided sufficient spatial resolution and tissue contrast to distinguish cellular architecture, borders, and size for characterization of healthy and malignant tissue in both in vivo and ex vivo specimens. Imaging of healthy controls allowed for classification of benign squamous epithelium as having cells of uniform size and shape with distinct cytoplasmic membranes and dermal papillae. Features of healthy tissue were seen during in vivo CLE of

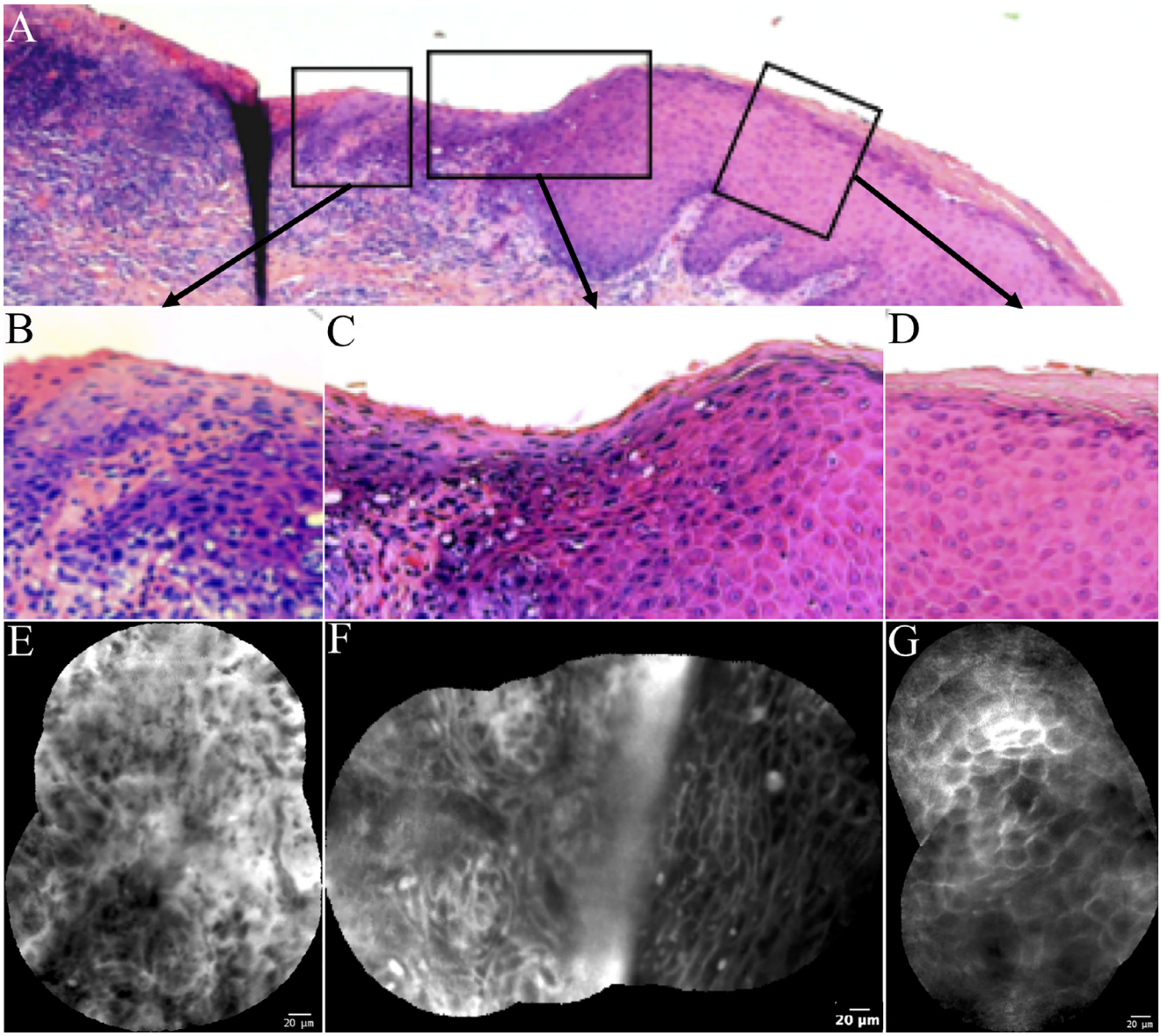

Fig. 4. In vivo evaluation of cancer margin using CLE. (A) Low-magnification (25×) H&E image of margin between normal squamous epithelium (right) and CIS (left). (B) H&E image (200×) of CIS. (C) H&E image (200×) of transition zone between CIS and normal epithelium. (D) H&E image (200×) of normal squamous epithelium. (E) CLE mosaic image of CIS with characteristic variable cellular morphology, density, and lack of cytoplasmic membranes. (F) CLE mosaic image of transition from CIS (left) to benign epithelium (right) and (G) CLE mosaic image of normal epithelium with uniform cellular morphology and distinct cytoplasmic membranes.

benign appearing tissue in patients with penile cancer. However, in the penile cancer patients, a distinct transition between healthy appearing and suspicious lesions was observed with CLE suggesting that CLE could be used to more precisely evaluate the cancer margin. CLE optical biopsy of SCC in vivo demonstrated a haphazard arrangement of highly variable cellular morphology, lack of cytoplasmic membranes, and a hazy, moth-eaten appearance. Ex vivo and in vivo CLE images were similar and CLE findings correlated with standard H&E.

An accurate histopathological diagnosis of penile cancer is paramount in guiding clinical management. CLE images have similar resolution to traditional H&E, and thus could serve as a valuable adjunct technology by potentially minimizing the need for intraoperative frozen section analysis. Pathological assessment of frozen sections and biopsy specimens does not typically involve the surgeon; however, CLE image interpretation can be performed by a trained urologist for real-time distinction of normal from abnormal tissue. Just as ultrasound is used to guide resection of renal cell carcinoma during partial nephrectomy, the ability to sample the entirety of a tumor and surrounding tissue at the time of initial biopsy may reduce under-sampling and help guide extent of excisional biopsy. Dynamic real-time microscopy for margin identification and determination of resection boundaries could thereby maximize penile preservation. Additional advantages of this approach over the use of frozen sections include the ability to review lesions repeatedly, improved tissue orientation when performed in vivo, and preservation of tissue for permanent processing. CLE may also be used in the clinical setting for operative planning, patient counseling and postoperative surveillance for local recurrence.

Feasibility studies of other imaging modalities as diagnostic adjuncts to histopathological analysis have been conducted. OCT creates cross-sectional images of squamous epithelium using back-scattered light [14]. Intensity measurements of back-scattered light and echo time delay are measured at several transverse positions to construct a 2-dimensional cross-sectional image of tissue. Cellular morphology can be inferred with a limited degree of certainty via measurements of epidermal thickness. Spatial resolution of OCT, however, is insufficient for visualization at the cellular level. Distinct cellular features observed using CLE are not visible on OCT. PDD utilizes blue-light excitation to reveal areas of increased fluorescence, which could indicate inflammation or malignancy [15]. PDD is limited as it is unable to discern microscopic features and margins. A direct comparison of CLE with these and other imaging modalities would be of significant interest in the future, but the relative rarity of the disease pose challenges in trial design.

Our study is limited by the small sample size which reflects the rarity of penile cancer in our patient population. As such, we were not powered to assess the diagnostic accuracy of CLE for penile cancer optical biopsy, nor were we able to prospectively compare the effectiveness of CLE and frozen section pathology to establish margin status. However, the CLE characteristics of SCC in our study are similar to those described in other cancers, where sensitivity and specificity of CLE for neoplasia are well over 90% [20,28]. The CLE imaging and image interpretation has a moderate learning curve and may be complicated by interobserver variability [29]. Improved diagnostic criteria and automated image interpretation software will facilitate CLE utility and may allow forsubclassification of penile lesions [30]. A collaborative relationship with surgical pathologists interested in in vivo microscopy will also be beneficial for applications in the clinical setting.

To characterize the morphological features of benign squamous epithelium and penile SCC with CLE, we used IV fluorescein as a contrast agent. This allowed for sufficient resolution of in vivo cellular architecture within a few minutes of fluorescein administration. While ex vivo images had slightly worse resolution, likely due to lack of circulating contrast agent making interpretation more difficult, we anticipate the main clinical utility of CLE to be for in vivo use. In this study, CLE was not used to direct clinical management so the long-term oncologic and functional impact of using CLE is unknown. In 1 patient with a positive margin from previous resection of SCC within an ulcerated lesion, only benign reactive changes and inflammation were identified by H&E upon further resection. CLE imaging at the prior resection site was distinct from healthy tissue but not clearly distinguishable from cancer. Additional CLE imaging of benign penile lesions will be necessary to refine diagnostic criteria for use in cases of re-resection for prior positive margins to determine if distinguishing characteristics can be identified. Further investigation is also needed to characterize CLE features of benign inflammatory conditions such as balanoposthitis.

Despite the limitations of the current study, CLE is a promising technology for optical biopsy of penile cancer. A prospective, multicenter study is needed to determine the accuracy of CLE in identifying penile cancer. CLE may play a role as a real-time microscopy technology that aids urologists in the selection of suspicious lesions to biopsy and in determining margin status for optimal outcomes. In addition to the management of the primary penile tumor, the utility of CLE to evaluate lymph node status in these patients should be explored.

5. Conclusions

CLE is a promising imaging technology that may aid intraoperative decision-making at the time of penile biopsy or penectomy. CLE can be used to generate real-time, in vivo microscopic examination of the penis for evaluation and demarcation of cancer. Using CLE, benign squamous

epithelium can be identified and distinguished from SCC. Additional prospective analysis is needed to assess the benefits of CLE in the diagnosis and management of patients with penile cancer.

Conflict of interest

Authors declare that they have no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2019.08.018.

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