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Optical imaging with a high-resolution microendoscope to identify sinonasal pathology☆

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ABSTRACT

Objectives: High-resolution microendoscopy (HRME) is an optical imaging modality that allows real time imaging of epithelial tissue and structural changes within. We hypothesize that HRME, using proflavine, a contrast agent that preferentially stains cell nuclei and allows detection of cellular morphologic changes, can distinguish sinonasal pathology from uninvolved mucosa, potentially enabling real-time surgical margin differentiation.
Study design: Ex vivo imaging of histopathologically confirmed samples of sinonasal pathology and uninvolved, normal sinus epithelium.
Setting: Single tertiary-level institution.
Subjects and methods: Five inverted papillomas, one oncocytic papilloma, two uninvolved sinus epithelia specimens, and three inflammatory polyps were imaged ex vivo with HRME after surface staining with proflavine. Following imaging, the specimens were submitted for hematoxylin and eosin staining to allow histopathological correlation.
Results: Results show that sinonasal pathology and normal sinus epithelia have distinct HRME imaging characteristics. Schneiderian papilloma specimens show increased nuclear-to-cytoplasmic ratio, nuclear crowding, and small internuclear separation, whereas normal sinus epithelia specimens show small, bright nuclei with dark cytoplasm and relatively large internuclear separation. Inflammatory polyps, however, have varying imaging characteristics, that resemble both Schneiderian papilloma and normal sinus epithelia.
Conclusions: This study demonstrates the feasibility of HRME imaging to discriminate sinonasal pathology from normal sinus epithelia. While the system performed well in the absence of inflammation, discrimination of inflamed tissue was inconsistent, creating a significant limitation for this application. Novel imaging systems such as HRME with alternative contrast agents may assist with real-time surgical margin differentiation, enabling complete surgical resection of inverted papilloma and reducing recurrence rates.

1. Introduction

Schneiderian papilloma is a benign neoplasm of the sinonasal tract characterized by a high rate of recurrence and the potential for malignant transformation [1]. Patients typically present with symptoms of nasal obstruction, rhinorrhea, or unilateral epistaxis. On exam, Schneiderian papilloma often appears indistinguishable from inflammatory polyp, which makes diagnosis without histopathological analysis difficult.

These benign tumors arise from the respiratory mucosa of the sinonasal tract, predominately along the lateral nasal wall [1]. The incidence of these tumors is between 0.5% and 4.0% of all nasal tumors [2]. Schneiderian papillomas can be divided into three different morphological types: inverted, oncocytic (cylindric or columnar cell), and exophytic (fungigorm, septal) papillomas. Literature reports exophytic papillomas as the most common type, but in practicality, inverted papillomas are known as the most common type and oncocytic papillomas as the least common [3]. While their etiology is unclear, there is some evidence of association with human papilloma virus (HPV) [4]. Krouse developed a staging system for inverted papilloma, which divides stage

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(I–IV) exclusively by anatomical location [5]. The newest staging system for inverted papilloma carries prognostic weight and categorizes clinical stage by anatomical location and recurrence rate [6]. Although benign, Schneiderian papillomas are locally destructive tumors of the sinonasal tract associated with squamous cell carcinoma in approximately 5–15% of patients [7,8]. Squamous cell carcinoma may be present at the time of diagnosis or occur metachronously after malignant transformation [9]. Similar to squamous cell carcinomas, the mainstay of treatment for Schneiderian papilloma is complete surgical resection with clear margin discrimination [10]. Surgical resection may involve excision by an open, endoscopic, or combined approach. The endoscopic approach is now the mainstay of treatment, limiting surgical morbidity and improving visualization of diseased mucosa [11].

These locally aggressive tumors have a high rate of recurrence, ranging from 5% to 75%, depending on the extent of surgical resection [7–9]. This high rate of recurrence, regardless of the type of procedure, can be attributed to multi-centricity of the tumors as well as incomplete excision [11]. Therefore, complete resection with negative margins is a key for limiting the risk of recurrence and potential morbidity of additional therapeutic interventions. Unfortunately, due to paranasal sinus mucosal inflammation, which is often present, clinical examination is inadequate to determine appropriate surgical margins. Therefore, surgeons currently rely on frozen section analysis to determine margin status intraoperatively. Histological examination of inverted papilloma reveals epithelial hyperplasia and multi-layering with fingerlike inversions into the underlying epithelium [12]. Oncocytic papillomas are characterized by multilayered epithelial proliferation of columnar cells with abundant eosinophilic and granular cytoplasm [3]. Neoplastic epithelium may be composed of a variable mix of columnar cells, mucocytes and squamous cells, with admixed intraepithelial inflammatory infiltrate [9].

Novel modalties are needed to provide real-time margin differentiation to limit recurrence rates and reduce operative time. Optical imaging technologies such as high resolution microendoscopy (HRME) enable non-invasive visualization of structural and morphological changes in tissue epithelium [13–15]. Prior studies, including those from our group, have demonstrated the utility of HRME in detecting neoplastic changes within the head and neck and upper gastrointestinal tract as well as cholesteatoma in the middle ear [16–19].

HRME has been previously described by Muldoon et al. [20] As pictured in Fig. 1, the system is composed of a fiberoptic probe, a blue LED light source, and a CCD camera linked to a laptop computer. By inserting a 1-mm fiber bundle image-based microscope into the nasal cavity, the HRME allows for real-time image capture. In addition, the portability of this system allows for ex-vivo imaging of tumor and margins after resection. The system uses proflavine, a fluorescent topical contrast agent to stain the nucleus of the cells to allow for visualization. Proflavine, a dye in the family of aminoacridines, is the major component of acriflavine, which has been used topically for in vivo imaging studies and is routinely used in Europe and Australia during endoscopy [21]. Proflavine preferentially stains cellular nuclei and avidly binds to DNA in a reversible and non-covalent manner [22,23]. This staining pattern is ideal for cancer imaging applications, allowing visualization of cellular architecture, nuclear-to-cytoplasmic ratios, and other features with minimal sample preparation or incubation time. Proflavine has been extensively studied in in vivo studies without any reported adverse events [21,24–26].

The ability to distinguish inverted papilloma from surrounding tissue with optical technology, and thus define the margins of the tumor in vivo, provides several potential therapeutic benefits. Here, we describe our ex-vivo investigation evaluating the utility of HRME to image sinonasal pathology. To our knowledge, this study is the first to evaluate the role of HRME in the sinonasal cavity. We hypothesized that HRME with the use of proflavine will highlight distinct morphologic and structural characteristics within pathological sinonasal mucosa when compared to normal sinus mucosa.

2. Methods

This study protocol was approved by the Icahn School of Medicine at Mount Sinai Institutional Review Board (GCO # 14-1644). Our study utilized anonymous tissue specimens stored within the Mount Sinai Biorepository Cooperative. Specimens included in the study were any tumor, mass, or lesion other pathological specimen obtained from the nasal cavity or sinuses. All specimens were labeled by a randomly assigned identification number. Proflavine was purchased in powder form from Sigma-Aldrich Chemicals (St. Louis, MO). A 0.01% solution of proflavine was applied topically to the specimen with a cotton-tipped applicator. The specimen was then rinsed with saline to remove any unbound dye and imaged with the fiberoptic probe. Images were recorded in video format (.avi). Each specimen was imaged at multiple sites and two to three HRME videos were acquired. After HRME imaging, the diagnosis of each specimen was confirmed with a histopathological analysis by a head and neck pathologist (EGD).

3. Results

We reviewed five specimens of inverted papilloma, one specimen of oncocytic papilloma, two specimens of normal sinus mucosa, and three specimens of inflammatory polyp. Schneiderian papilloma, including both inverted papilloma and oncocytic papilloma, displayed distinct imaging characteristics from normal sinus mucosa, often enabling discrimination between the two with HRME. Table 1 describes the imaging characteristics of Schneiderian papilloma (including inverted papilloma and oncocytic papilloma), inflammatory polyp, and normal sinus mucosa. Figs. 2–5, display representative images of inverted papilloma, oncocytic papilloma, normal sinus mucosa, inflammatory polyp, respectively, as seen on HRME imaging and corresponding histopathological H&E examination. Oncocytic papilloma, compared to inverted papilloma, has higher nuclear to cytoplasm ratio, smaller internuclear separation, and more sparse cytoplasm. Inflammatory polyps, however, have varying imaging characteristics, oftentimes within the same gross specimen, as displayed in Fig. 6. Features range from small, dimly lit nuclei with large internuclear separation (Images B and C) to large, bright nuclei with small internuclear separation (Images A and D).

4. Discussion

This study describes the first attempt to utilize optical imaging to identify sinonasal pathology. Our ex vivo study demonstrates that HRME has the ability to distinguish Schneiderian papilloma from normal sinus mucosa. HRME imaging of Schneiderian papilloma displays nuclear crowding with large prominent nuclei, sparse cytoplasm, and small internuclear separation. Normal sinus mucosa, on the other hand, displays small, bright nuclei with abundant cytoplasm and large internuclear separation. These distinct imaging characteristics raise the possibility of in-vivo use of HRME to discriminate between Schneiderian papilloma and uninvolved sinonasal mucosa, enabling real time surgical margin differentiation. In a recently published study, we reported high inter-rater reliability for identification of inverted papilloma using HRME imaging [27]. We demonstrate that otolaryngologists can be trained to distinguish between inverted papilloma and normal sinonasal mucosa using distinct qualitative imaging characteristics [28].

Our previous investigations have demonstrated the effectiveness and feasibility of HRME in identifying head and neck neoplasms. However, the accuracy of HRME and proflavine in identifying squamous cell carcinoma was limited due to the affinity of proflavine for keratin, which resulted in substantial background artifact and limited visualization of nuclei. In addition, the minimal depth of penetration prevents detection of submucosal disease [18]. HRME represents a potential tool to provide real-time, intraoperative identification of Schneiderian papilloma, enabling identification of tumor extent and the

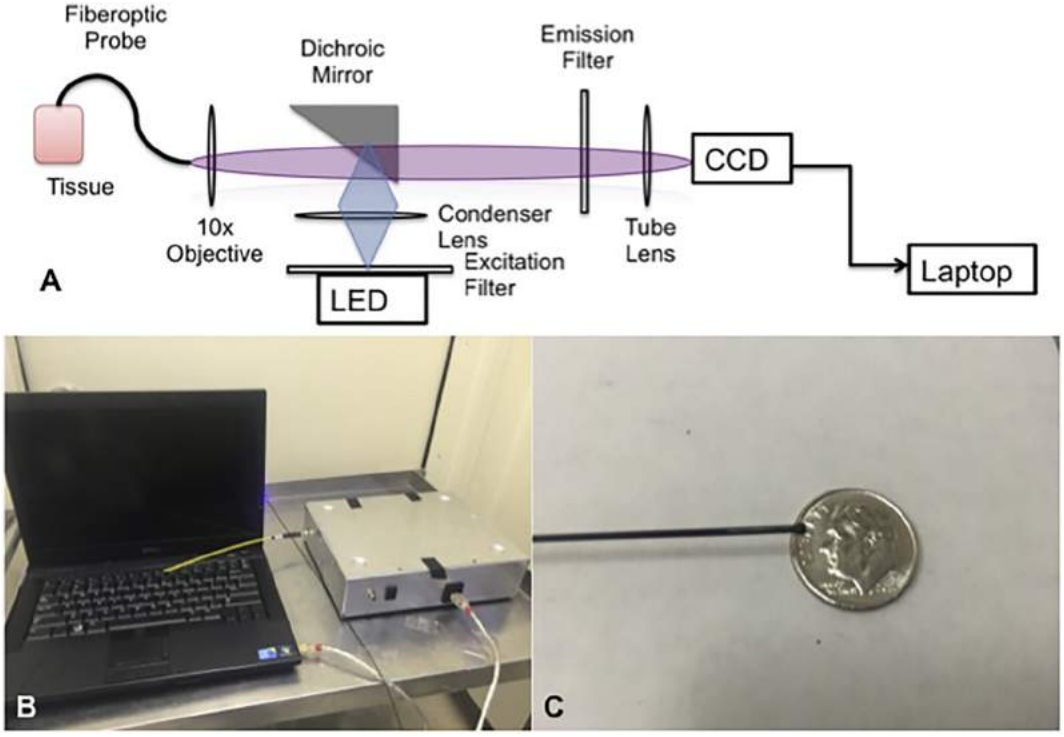

Fig. 1. High-resolution microendoscope (HRME). (A) Schematic of fiberoptic bundle microscope system (B) Photo of the HRME imaging system (C) HRME fiberoptic probe. CCD: Charge-coupled device. LED: light-emitting diode.

Table 1
HRME imaging characteristics of inverted papilloma and normal sinus mucosa.

Cellular feature	Normal sinus	Schneiderian papilloma
Nucleus:cytoplasm	≤1	> 1
Internuclear separation	Large	Small
Nucleus morphology	Small, bright	Large, prominent
Cytoplasm	Abundant	Sparse

presence of multi-focal disease. Although the system performed well when discriminating Schneiderian papilloma from normal mucosa, HRME of inflammatory polyps resulted in inconsistent imaging characteristics, likely attributable to the variable cellular architecture of inflammatory polyps [28]. Our examination of inflammatory polyps resulted in imaging characteristics that were often indistinguishable from Schneiderian papilloma. As a result, HMRE with proflavine contrast cannot consistently distinguish inflammatory polyp from inverted papilloma. This issue is a significant limitation of this technology for this application, particularly because sinonasal pathology is often surrounded by inflamed tissue or difficult to distinguish from inflammatory polyps. Therefore alternative contrast agents or imaging modalities may offer improved accuracy to distinguish inverted papilloma from inflamed sinus mucosa. Optical technologies that allow for interrogation of tissue architecture, such as optical coherence tomography may offer improved discrimination over our current system. We are currently exploring several optical systems and alternative contrast agents to improve and enhance the accuracy for inverted papilloma.

Another potential application of HRME imaging technology is tumor surveillance. Schneiderian papilloma requires long-term follow up due to the propensity for recurrence and the potential for malignant transformation [29–31]. While most recurrences occur within the first 2 years of surgery, 17% recur after 5 years and 6% after 10 years [32]. Moreover, in their pooled review of 2047 cases, Mirza et al. described a 3.6% rate of metachronous carcinoma development with an average time interval of 52 months (range 6–180 months) [33]. Currently,

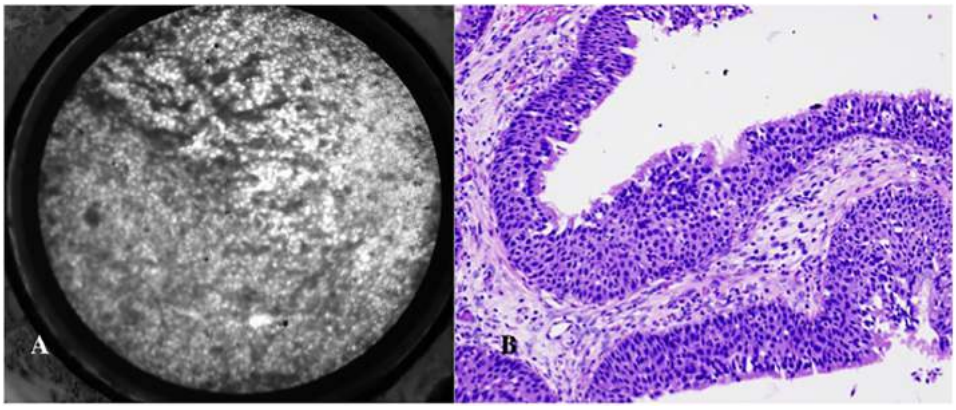

Fig. 2. HRME images (A) of inverted papilloma with corresponding H&E appearance, taken at 200× magnification (B).

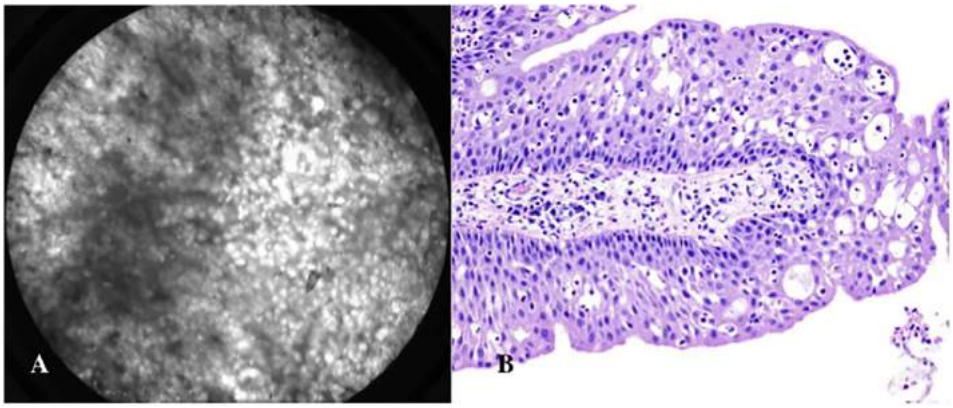

Fig. 3. HRME images (A) of oncocytic papilloma with corresponding H&E appearance, taken at 200× magnification (B).

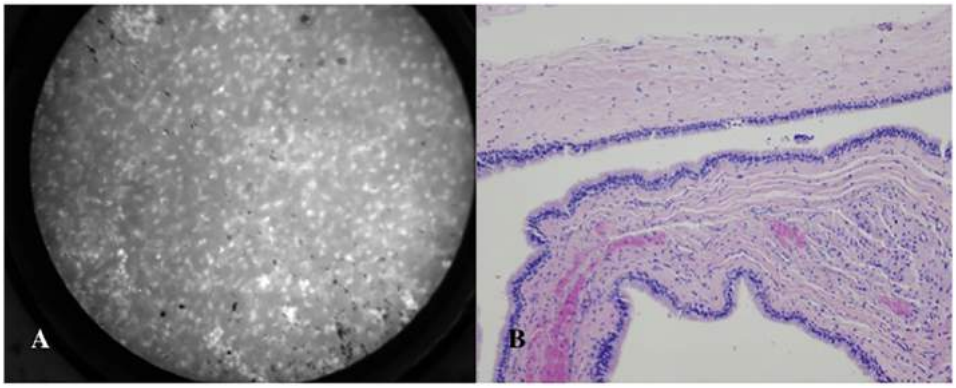

Fig. 4. HRME images (A) of normal sinus mucosa with corresponding H&E appearance, taken at 200× magnification (B).

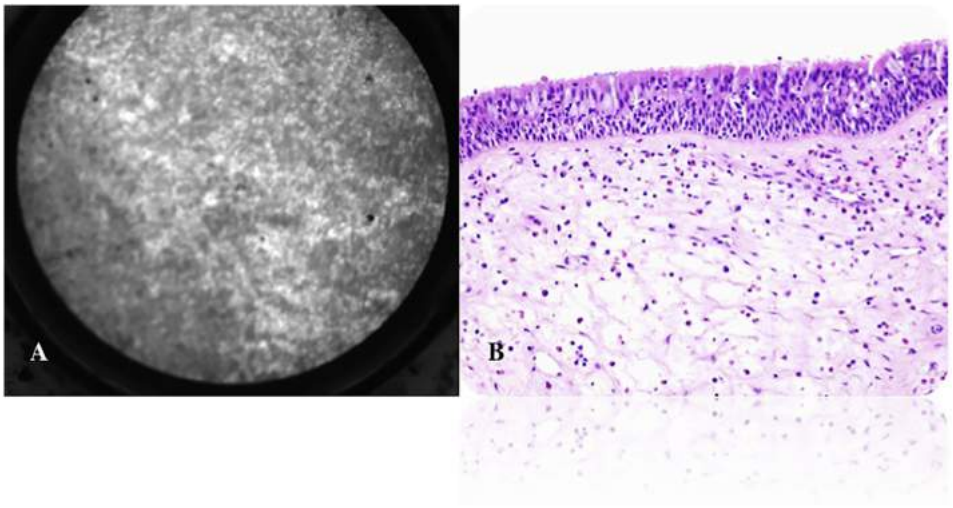

Fig. 5. HRME images (A) of an inflammatory polyp with corresponding H&E appearance, taken at 200× magnification (B).

routine follow-up after surgical resection relies on visual inspection via endoscopic surveillance, imaging studies, and biopsy to monitor for recurrence. However, the use of HRME during clinical follow-up may allow for early detection of recurrence, and direct biopsies after evaluating areas of interest optically to reduce imaging procedures and improve the accuracy of surveillance for this disease.

5. Conclusion

HRME has been utilized with success to identify neoplastic processes in the head and neck and gastrointestinal system. This study represents the first study to assess the utility of HRME for pathology of the sinonasal cavity. HRME represents a novel optical imaging technique that does have potential to distinguish Schneiderian papilloma from normal sinus mucosa. Optical systems such as HRME may allow real-time intra-operative surgical margin differentiation, enabling complete surgical resection of Schneiderian papilloma and reduced recurrence rates, as well as enhanced post-operative surveillance. However, HRME with proflavine contrast is unable to distinguish inflammatory polyps from inverted papilloma. As a result, further investigations must be conducted to determine an optimal contrast agent or optical system that highlights distinctive cellular and sub-cellular

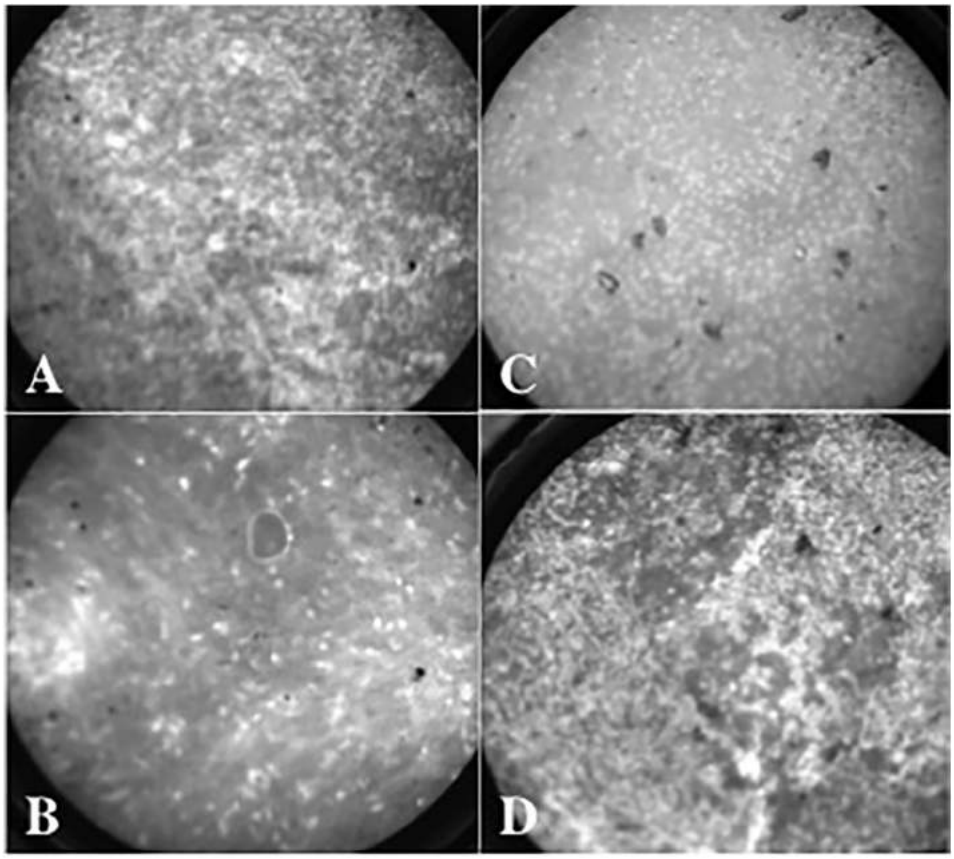

Fig. 6. HRME images from three different inflammatory polyps. (A) and (B) represent images from the same gross specimen. (C) and (D) are images from additional inflammatory polyps.

architecture, and can reliably differentiate between inverted papilloma, normal sinus mucosa, and inflammatory polyps.

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