Oral mucosa optical biopsy by a novel handheld fluorescent confocal microscope specifically developed: technologic improvements and future prospects

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Objective. This pilot study evaluated the baseline effectiveness of a novel handheld fluorescent confocal microscope (FCM) specifically developed for oral mucosa imaging and compared the results with the literature.
Study Design. Four different oral sites (covering the mucosa of the lip and of the ventral tongue, the masticatory mucosa of the gingiva, and the specialized mucosa of the dorsal tongue) in 6 healthy nonsmokers were imaged by an FCM made up of a confocal fiberoptic probe ergonomically designed for in vivo oral examination, using light at the wavelength of 457 nm able to excite the fluorophore acriflavine hydrochloride, topically administered. In total, 24 mucosal areas were examined.
Results. The FCM was able to distinctly define epithelial cells, bacterial plaque, and inflammatory cells and to image submucosal structures by detecting their intrinsic fluorescence.
Conclusions. When compared with other devices, this FCM allowed the user to image each oral site at higher magnification, thus resulting in a clearer view. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:752-758)

To date, the diagnosis of suspicious or equivocal oral lesions is based on their conventional clinical examination and confirmed by the histopathologic report subsequent to a mandatory biopsy.[1] Diagnostic procedures can benefit from adjunctive tools. Among these, tissue autofluorescence visualization[2-5] and toluidine blue[6] and Lugol iodine vital staining[7] have been used to improve the ability to screen and clinically identify oral premalignant and malignant lesions in order to facilitate the diagnostic pathway[8] in a noninvasive, real-time way.

Biopsy providing definitive microscopic features remains the gold standard for the management of lesions with high-grade dysplasia or greater tissue change, which require treatment, and those with low-grade dysplasia that will usually be monitored over time with periodic comparative biopsies. As a surgical procedure, biopsy is invasive, and the selection of the biopsy site can be problematic. In a large lesion, multiple biopsies might be necessary for a more accurate histopathologic analysis; for areas with posttreatment mucosal change, repeated and excisional biopsies can cause more problems. It is acceptable to resect a relatively large area (approximately 1 to 2 cm) of normal-appearing mucosa around the visibly abnormal lesion to compensate for the limitation of the surgeon's ability to exactly determine the margins of carcinoma or dysplasia[9]; this approach produces better likelihood of complete excision but increases postoperative discomfort, thus resulting in low compliance among the patients, who may become reluctant to perform further follow-up biopsies. Noninvasive approaches that can help the clinicians to decide the timing and the best site for a diagnostic biopsy and to avoid unnecessary biopsies are needed.

Optical imaging technologies have shown promise in meeting that need. In vivo confocal microscopy, one such optical technology, has been widely used to investigate the tissue at microscopic resolution in a real-time fashion in clinical settings, such as ophthalmology,[10] dermatology,[11,12] gynecology,[13-16] and gastroenterology.[17-19] Due to its noninvasiveness and its time-saving nature, it could be advantageously performed at the point of care.

The application of confocal microscopy in the oral cavity is limited to some preliminary work previously reported.[20-23] Detailed descriptions of "confocal criteria" of healthy oral structures also appeared in

Statement of Clinical Relevance

The results represent a major technical advance in the development of this optical imaging modality for the in vivo oral mucosa examination, thus allowing examination of each site of the oral mucosa for cellular details during an otherwise routine examination.

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further works, adapting confocal microscopes designed for dermatologic use to be used in the oral cavity.[24,25]

The objectives of this pilot study were to image healthy oral mucosa to evaluate the baseline effectiveness of an easy-to-use, handheld fluorescent confocal microscope (FCM) specifically developed for in vivo oral evaluation and to criticize and compare results with previous works.

SUBJECTS AND METHODS

Subjects

Six healthy nonsmokers were enrolled at the Imaging Unit in the Department of Integrative Oncology of the British Columbia Cancer Agency of Vancouver, BC, Canada, after informed and written consent. The study was approved by the Institutional Research Board of the BC Cancer Agency/University of British Columbia (H11-00011). The series comprised 4 men and 2 women (mean age, 29.6 ± 4.6 years) without any oral mucosal conditions. They were subjected to FCM examination (see below for instrumentation) of different oral mucosal sites. In total, 24 mucosal areas were examined, as follows: 6 labial mucosae and 6 ventral surfaces of the tongue, 6 attached gingivae, and 6 dorsal surfaces of the tongue. The former 2 sites were used to represent nonkeratinized oral mucosa; the gingiva was used as a keratinized one; and the dorsal surface represented the specialized epithelium. Because the present work is a pilot study to define quality of images and details, comparing them with previous works, biopsies were not performed.

In vivo FCM

Fluorescence confocal microscopy is an imaging technique based on the detection of fluorescent light emitted by an endogenous marker or an exogenous substance applied to the living tissue when illuminated by a specific wavelength. This work examines a prototype of a handheld fluorescent confocal microscope, specifically developed for oral examinations (BC Cancer Agency, Imaging Unit, Integrative Oncology, Vancouver, BC, Canada). The system was based on previously reported laser-scanning designs.[22,26] The handheld wand employed a custom (7-element) 3×/1.0 numerical aperture objective lens with a 240-μm field of view. Blue excitation light was provided by a 457-nm laser diode (Melles Griot, Carlsbad, CA, USA). Reflected excitation was blocked by a 475-nm long-pass filter (Chroma Technology, Bellows Falls, VT, USA), thus allowing detection of the fluorescence emitted by acriflavine hydrochloride (AH) as a contrast agent topically applied to the mucosal surface.[22] AH and its derivatives have been previously used for fluorescence imaging in the European, Asian, and Australian gastrointestinal literature without any adverse effects noted,[18] and El Hallani et al.[27] found AH to be the best contrast agent when compared with other types, thus supporting our choice.

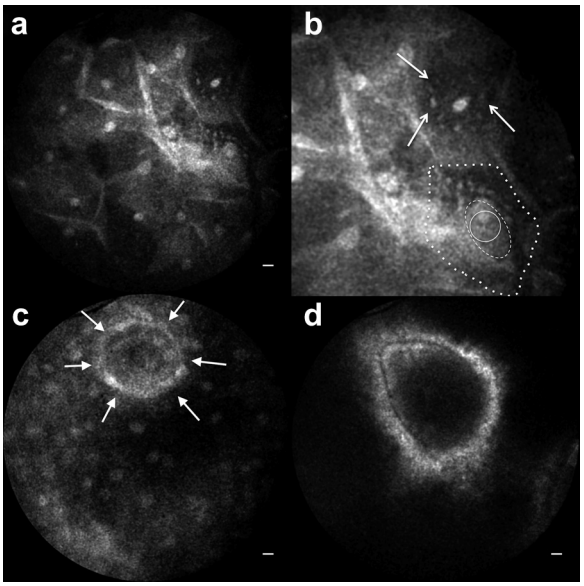

Fig. 1. Lip imaging with a fluorescent confocal microscope. (**a**) Keratinocytes appear as plump, roundish cells, defined by bright boundaries, gray cytoplasm, and a central strongly bright nucleolus surrounded by a dark nuclear halo. (**b**) Detail of the squared selection in (**a**), showing 2 keratinocytes with perinuclear bright granules (*white arrows*) and whose contours, perinuclear granules, and nucleolus are marked by *dotted lines* from outer to inner. (**c**) At the lowest epithelial layers, some roundish structures (*white arrows*), made up by cells organized in a bright ring, resemble a minor salivary gland duct. (**d**) The dark ring surrounded by a bright halo corresponds to a connective tissue papilla limited by epithelium. Scale bars, 10 μm.

FCM acquisition method

After the application of 0.05% AH on the mucosal surface for 5 minutes, the volunteer washed out the excess using water; then FCM examination took place. En face, single, 240 × 240-μm FCM images and videos were collected from each mucosal layer of the different mucosal subtypes (covering, masticatory, and specialized mucosa), starting from the most superficial visible layer of the tissue and progressing to the deepest visible layer.

Because the FCM probe was still being modified at the time of this study, the imaging depth could not be accurately and quantitatively determined. For these reasons, based on the knowledge gained in reflectance confocal microscopy imaging,[24] the imaged layers were conventionally classified on the basis of their appearance as follows: superficial layer, related to the first layers of keratinocytes; stratum spinosum, corresponding to the homonymous histologic layer; lower layer, corresponding