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Case Report

In vivo microscopy: A non-invasive diagnostic tool

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ABSTRACT

In recent years, a new optical technology known as in vivo microscopy (IVM) has emerged as a promising and non-invasive diagnostic adjunct. IVM includes high-resolution, microscopic imaging of intact tissue for disease detection without the need of tissue removal. IVM offers the opportunity to carry out a 'real time' inspection at a microscopic level during the clinical examination of both soft and hard tissues of the oral cavity. It make use of the refracted light emitted after being hit by incident light at a specific wavelength. As it is a non-invasive method it could in bypass all the difficulties and problems related to a biopsy and the subsequent histological examination, which still represents the gold standard for definitive diagnosis.

1. Introduction

In-vivo microscopy (IVM) is a technology, that allow tissue to be viewed in living patients, at high resolution, in real time, at an approximate depths of 200–300 μm without tissue removal, fixation, freezing, or staining.[1] It can examine both soft and hard tissues of the oral cavity. It uses the emission of refracted light after being hit by incident light at a specific wavelength.[2] In vivo confocal images correlate well with histological sections and can therefore be considered 'optical biopsies'.[3] The ability to visualize cells in living tissue, without the need of biopsies and fixation, gives new and valuable information in health and disease.[3] The oral cavity, on the other hand, has been less investigated with in vivo microscopy. IVM produces images of microscopic tissue features by measuring tissue optical properties, such as reflectance, scattering, absorption, and fluorescence emission, which are frequently altered in disease states. IVM has been proposed for a range of clinical applications, including disease diagnosis, disease risk stratification, longitudinal monitoring of patients, and surgical margin delineation.[4]

2. Discussion

In Vivo Microscopy (IVM) is an exciting field where microscopic images are obtained in vivo, in real-time, during clinical procedures. IVM imaging technologies use light and rapidly produce 2D or 3D (tomographic) microscopic images. Each IVM technology measures the different tissue optical properties and offers different capabilities in parameters, such as imaging depth, resolution, field of view (FOV), and acquisition time. The choice to use a specific IVM technology is determined by optimally balancing trade-offs in these parameters to meet the minimum performance requirements of the desired clinical application.

Types of In-vivo microscopy:

1. Multimodal imaging.
2. Optical coherence tomography
3. Reflectance confocal microscopy

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4. Multiphoton microscopy

2.1. Multimodal imaging

The HRME is a low-cost fluorescence microscope that comes in a compact box with a flexible probe that measures 0.79 mm in diameter. The clinician applies proflavine, a vital fluorescent dye that stains cell nuclei, to the tissue of interest with a cotton-tipped applicator. The probe is then gently pressed against the proflavine-stained mucosa, allowing direct visualisation of the surface epithelial nuclei. The depth of the investigation is believed to be between 20 and 50μm. When the clinician has a decent image, he or she can depress a foot pedal to freeze the current frame, then save and analyse the image. HRME pictures of the normal mucosa and benign lesions show small, uniformly spaced nuclei, whereas HRME images of dysplastic or malignant lesions show big, crowded, and irregularly shaped nuclei. An automated computer programme recognises nuclei and calculates metrics associated with dysplasia and cancer, such as the nuclear/cytoplasm ratio and the density of aberrant nuclei, to offer an objective result.[4]

2.2. Optical coherence tomography

OCT has been effectively used in clinical settings for a variety of anatomic locations, including the coronary arteries and the oesophagus, and it is now being researched for a variety of additional applications. Oral cancer has been imaged using OCT by a few studies. OCT creates a 3-dimensional volumetric map by stitching together 2-dimensional tissue cross-sections of light scattering. When compared to most other IVM methods, OCT has micron level resolution with a high penetration depth (usually 2 mm) and FOV (up to 1 cm x 1 cm). The high penetration depth enables for evaluation of cellular invasion by allowing visibility of the whole epithelial thickness and superficial connective tissue.[5]

2.3. Reflectance confocal microscopy

A pinhole is employed in reflectance confocal microscopy (RCM) to reject out-of-focus light reflected from the sample. The pinhole increases resolution and permits pictures of various depths of tissue to be obtained, as well as possible distinction of different degrees of dysplasia. RCM systems can scan the basement membrane and superficial connective tissue, with the exception of hypertrophic lesions.[6]

2.4. Multiphoton microscopy

Multiphoton microscopy is a form of fluorescence imaging that can produce picture cross-sections of tissues at varying depths, up to 1 mm. Because of the considerable penetration depth, it may be possible to assess cellular infiltration beyond the basement membrane. The excitation light source in multiphoton microscopy employs light with twice the required fluorescence excitation wavelength, so fluorescence occurs only when two photons excite a fluorophore at the same time. At a certain place, this process occurs with a high frequency. The excitation light source may penetrate deeper into tissue since there is no out of focus fluorescence absorption, and the longer wavelengths utilised for excitation reduce scattering effects. Multiphoton microscopy based on autofluorescence measures the same signals as previous AFI instruments, but with the extra benefit of depth-sectioned viewing of microscopic details. Multiphoton microscopy equipment, on the other hand, can usually only image a few hundred microns in diameter and require significantly higher light intensity.[7]

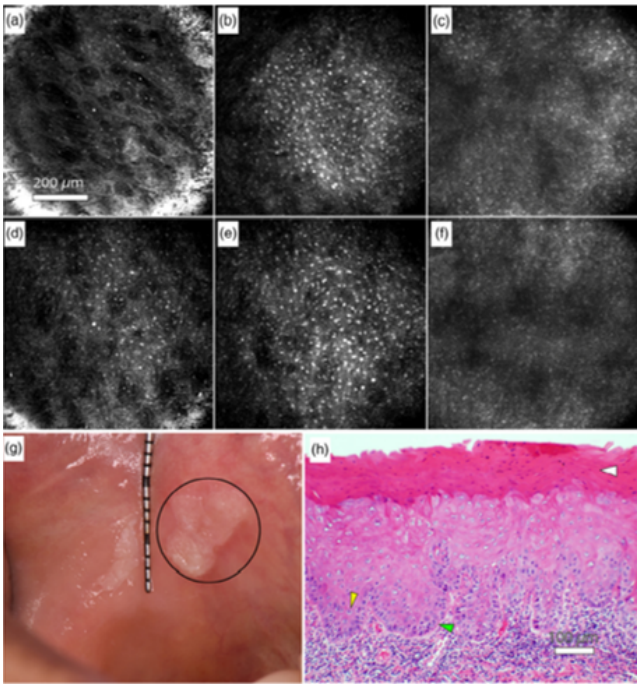

Fig. 1: In-vivo microscopyof normal buccal mucosa; **(a):** 10 μm (superficial epithelium); **(b):** 70 μm (spinous layer); **(c):** 120 μm (approaching basement membrane); **(d):** 10 μm (superficial epithelium); **(e):** 70 μm (spinous layer), and **(f):** 120 μm (approaching basement membrane); **(g):** Photograph of the leukoplakia, including the imaged lesion (circle) (h)Hematoxylin and eosin (H&E) slide of imaged leukoplakia diagnosed as mild to focally moderate dysplasia. Yellow arrow: Increased nuclear to cytoplasmic ratio. Green arrow: Loss of polarity of basal cells. White arrow: Hyperkeratosis.

2.5. Indications of in-vivo microscopy

1. Gastrointestinal tract endoscopic imaging, excellent for making differential diagnoses and identifying areas for biopsy to improve diagnostic yield, decrease sampling errors, decrease morbidity, and trim health

care costs.
2. Organ screening such as the esophagus for Barrett esophagus, can identify small foci of disease, follow up on previously treated areas, and monitor therapies.
3. Imaging of coronary vessels during interventional cardiology procedures.
4. Ophthalmology exams.
5. Various oral premalignant and malignant lesions.
6. Reflectance confocal microscopy for skin Reflectance confocal microscopy (RCM) is an excellent aid to histology to diagnose pigmented skin lesions.

2.6. Benefits of in vivo microscopy

1. In the case of lung nodules or breast cancer, guide biopsy site acquisition in real time to get targeted and diagnostically relevant biopsies.
2. Conduct a comprehensive test for occult microscopic disease, such as Barrett Esophagus capsule screening.
3. Obtain microscopic diagnoses when tissues cannot be easily or safely excised, as in coronary arteries, retinal screening or pulmonary fibrosis.
4. Guide and assess the efficacy chemotherapy, laser ablation, guided surgical ablation, focused ultrasound, cryotherapy, radiofrequency, and brachytherapy

3. Limitations of Biopsy[4]

Though biopsy is a gold standard for diagnosis it has various limitations as compared to in-vivo microscopy.

1. Bias in sampling due to site selection.
2. To perform a proper biopsy, a trained doctor is necessary.
3. For diagnosis, a trained pathologist and processing facilities are necessary.
4. Long delay (days) between diagnosis and treatment.
5. Variation between and within observers.
6. Patient pain and morbidity.

Thus in comparison to biopsy IVM is a technology which allows tissue to be viewed in high resolution ,in real time without the need for processing, fixation, freezing or staining thus is non-invasive, with no delay in diagnosis and is painless and comfortable to the patient.

3.1. Role of pathologist

As a pathologist, one should take advantage of their expertise in microscopy and histopathologic diagnoses to interpret IVM images. Many experts believe that IVM will become integral to the practice of pathology. These early IVM imaging systems are the precursors to subsequent imaging systems with better resolution. Pathologists can now play a key role in the development, validation, and clinical implementation of this technology as it matures, bringing their histopathologic expertise to the proper interpretation of IVM images and establishing a future role for pathologists in both clinical image analysis and potentially as interventional microscopists. Pathologists can employ these technologies in their own pathology practise as in vivo tools for intraoperative margin and sentinel lymph node evaluation, guided sampling of surgical tissues in the grossing room, tissue triaging for molecular and genomic research, and more.[8]

3.2. Future uses of IVM

1. Biopsy guidance in lung or breast lesions.
2. Diagnosis in organs that cannot be biopsied safely, such as brain lesions.
3. At-risk patients, such as those with fibrotic lung disease, who cannot safely undergo a biopsy procedure.

4. Conclusion

Though biopsy is the gold standard for diagnosing dysplasia and cancer, but it is limited by morbidity, time and resource requirements, and the risk of sampling bias. Thus in vivo microscopy is a promising technology for diagnosing and monitoring oral diseases, and it has the potential to give a non-invasive technique for cancer and pre-cancer detection. However, numerous technological constraints must be overcome before it can be extensively used in stomatology. Future research must provide statistical analyses, enhance the amount and quality of findings, and convert research into clinical practise.

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6. Conflict of Interest

The authors declare no potential conflicts of interest concerning the authorship and publication of this article.

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