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Research Article

A MAGIC BOX FOR THE DIAGNOSIS OF ORAL LESIONS WITH SPECIAL EMPHASIS AT THE GENETIC AND MOLECULAR LEVEL

Vikas Singla[1]., Neha Aggarwal[2]., Neha Singla[3] and Sonam Barwar[4]

[1]Dental officer, Army Hospital, Kurukshetra, Haryana
[2]Department of Oral Medicine and Radiology, Manav Rachna Dental College, Faridabad
[3]Practioner, Zirakpur, Punjab
[4]Department of Dental Surgery, VMMC & Safdarjung Hospital, New Delhi

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ABSTRACT

Oral mucosa is very much vulnerable to a large number of diseases. In the past decades, the adjunctive techniques have emerged with claims of enhancing oral mucosal examinations and facilitating the detection of premalignant and malignant lesions. The advancements in these fields have led to the development of diagnostic tools at both the clinical and molecular level for the early detection of oral mucosal lesions in the oral cavity which remains the best way to ensure patient survival and quality of life. As the era advances, now there is more impact on the genes and the molecules for the diagnosis of oral diseases. So, our emphasis is more concentrated on advanced techniques. Some of the recent adjunctive techniques which shows promising results for the detection of oral lesions are Vizilite, Autofluoroscence, VELscope, Oral CDx brush biopsy etc. This article highlights the importance of conventional as well as recent advanced techniques used in the diagnosis of oral lesions.

INTRODUCTION

In the past decades, adjunctive techniques have emerged with claims of enhancing oral mucosal examinations and facilitating the detection of and distinctions between oral benign, premalignant and malignant lesions (OPML). Clinicians, who use these tools may be unaware of the state of the evidence supporting their effectiveness. Techniques that are promoted or assessed to improve earlier detection and diagnosis of oral malignancy include toluidine blue (TB), ViziLite Plus with TBlue (Zila Pharmaceuticals, Phoenix), ViziLite (Zila Pharmaceuticals), Microlux DL (AdDent, Danbury, Conn.), Orascoptic DK (Middleton, Wis.), VELscope (LED Dental, Vancouver, British Columbia, Canada) and OralCDx (Oral CDx Laboratories, Suffern, N.Y.) brush biopsy.[1]

The principal methods for assessing mucosal changes include recognition of risky behavior and high-risk individuals which primarily begins with clinical methods. These lesions can be detected by visual inspection and are amenable for large-scale screening efforts by the use of toluidine blue stain. Toluidine blue stain is an effective means of picking up malignant changes in premalignant lesions. It has been valued by surgeons as a useful way of demarcating the extent of a lesion prior to excision. While not currently approved by the FDA for use as an oral cancer screening technique in the United States, toluidine blue has been championed in other parts of the world for several decades as a means of identifying clinically occult lesions in patients whose oral mucosa may otherwise be normal –i.e, as a screening test or adjunct.[2]

Adjuncts for detection of lesions and selection of biopsy sites include vital tissue staining (with Toluidine blue) and exfoliative cytology. Unfortunately, sensitivity of cytological diagnosis in a meta-analysis of 1306 cases from 14 studies showed an average of only 87.4% (ranging from 73.8 to 100%).3 Some of the adjunctive techniques which shows promising results for the detection of oral mucosal lesions are reviewed below:

- Oral CDx brush biopsy
- Chemiluminescence
- Fluorescence visualization
- Confocal Microscopy
- Optical coherence tomography
- Fluorescence spectroscopy
- Elastic Scattering spectroscopy
- Raman spectroscopy

Corresponding author:* **Vikas Singla
Dental officer, Army Hospital, Kurukshetra, Haryana

- Lab on chips
- PET Scan

Oral Brush Biopsy CDX System

Oral brush biopsy is a recent development that is been heavily marketed to dentists and was introduced in 1999. Oral brush cytology uses a special brush to collect the epithelial cells. Oral brush cytology may be a good tool for monitoring patients with chronic mucosal changes, such as leukoplakia, lichen planus, post-irradiation and patients with a history of oral cancer who require long term surveillance of their ongoing mucosal changes. The technique uses a round stiff bristle brush to collect cells from the surface and subsurface layers of a lesion by vigorous abrasion. The brush is placed in contact with oral epithelium and rotated with firm pressure 5 to 10 times. If properly performed, the brush collects cells from all three layers of the epithelium: basal layer, intermediate layer and superficial layer (Fig 1). The cellular material collected on the brush is transferred to a glass slide and flooded with fixative (alcohol or propylene glycol). After the slide is dry, it is sent to a special laboratory where the specimen is evaluated by computer system as well as pathologists to determine whether the biopsy has penetrated the basement membrane. If the sample is adequate, a computer that is specifically programmed to detect oral epithelial dysplastic and malignant cells scan each brush cytology specimen and displayed on a high resolution color video monitor. The pathologist classifies the oral brush cytology specimen into one of three categories: [4]

1. Negative-No epithelial abnormality is detected. Oral lesions with negative results require the same clinical follow-up as negative histologically sampled lesions.
2. Positive - Definitive cellular evidence of epithelial dysplasia or carcinoma is present. If a result is positive, the patient should always be referred for scalpel biopsy and histology to grade and stage the lesion.
3. Atypical - Abnormal epithelial changes are present. These abnormal cells originate most often from a precancerous or cancerous lesion, although they may also develop in a benign inflammatory lesion such as lichen planus. Therefore, all atypical results require referral for scalpel biopsy and histology.[4]

The advantages are as follows: It is a chair side test that does not require any topical or local anaesthesia, results in minimal discomfort or bleeding, takes seconds to perform and it may be used with more frequency than one might perform or refer for standard incisional or excisional biopsy. The disadvantages are: the tissue specimen may be disintegrated, the architectural information necessary to stage and grade the lesion is absent. Oral cytology serves as a trigger and not a substitute for traditional scalpel biopsy and histology. It needs the pathologists who are specialized in the working of computers.[4]

Chemiluminescence

The term "chemiluminescence" was first coined by Eilhardt Weidemann in 1888. It refers to the emission of light from a chemical reaction. ViziLite Plus is based on the principle of chemiluminescence comprises of a chemiluminescent light source to improve the identification of lesions and a dye to mark those lesions identified by ViziLite.[5] Vizilite passes over oral tissue that has been treated with the rinse solution, normal healthy tissue will absorb the light and appear dark, abnormal tissue will appear white. (Fig 2) Vizilite kit consists of a Vizilite 1% acetic acid solution, a capsule or a lightstick. Vizilite 1% acetic acid solution contains purified water, acetic acid, sodium benzoate, raspberry flavor and base of propylene glycol and alcohol. The vizilite capsule or chemiluminescent light stick comprises an outer flexible plastic capsule containing Aspirin or acetyl salicylic acid and an inner fragile glass vial containing hydrogen peroxide. Activation of the capsule is achieved by flexing it wherein, the inner fragile glass vial ruptures releasing the hydrogen peroxide. The chemicals react to release the energy which excite the electrons in the fluorescent dye and converts this energy into the light of the white-blue color with a wavelength ranging from 430-580nm. The light lasts for approximately 10 minutes & is long enough to do a thorough visual oral examination. The advantages are as follows: easy to operate, safe procedure, non-invasive technique which is capable of detecting early asymptomatic precancerous and cancerous lesions in the oral cavity, it is capable of delineating the sharp borders between normal and abnormal oral mucosa. The disadvantages are that it is expensive, can be used only once for each patient, fails to identify biopsy sites at times, false positive results indicating that vizilite is non-specific and results in many unnecessary biopsies.[5]

Fluorescence Visualization

The VELscope System patented technology platform was developed by the British Columbia Cancer Agency in collaboration with MD Anderson Cancer Center. It is based on the direct visualization of tissue fluorescence and the changes in fluorescence that occur when abnormalities are present. It emits a safe blue light which excites the tissue from the surface of the epithelium through to the basement membrane and into the stroma beneath, causing it to fluoresce and abnormal tissue typically appears as an irregular, dark area that stands out against the otherwise normal, green fluorescence pattern of surrounding healthy tissue. (Fig 3) Direct visualization examination (red zone) underestimates the full extent of histologic alterations as detected by intra-operative frozen section histology (yellow zone). Fluorescence visualization (green zone) is a simple and real-time method for visualizing optical changes associated with subclinical premalignant disease. The boundaries established by fluorescence visualization may even extend beyond the zone of histologic changes to more fully encompass the field of genetic alterations (blue zone).[6] (Fig 4) The uses include early detection of oral cancer and dysplasia, detecting mucosal abnormalities that may not be clearly apparent to the naked eyes and to identify the margin of a clinically apparent lesion. The advantages are as follows: painless, non-invasive procedure and takes only one or two minutes and is easy to incorporate, flexible positioning of the device, disposable cap helps in maintaining the asepsis and camera adapter allows for tracking of lesions. The limitations are: some benign conditions such as physiologic pigmentation, amalgam tattoos, trauma etc may also appear as dark regions similar to malignancy. Some areas as attached gingiva, anterior tonsillar pillars, the buccal mucosa, lateral surfaces of the tongue and hard palate may sometimes show darker areas due to inflammation. Images viewed by eye through the VELscope

indicate a characteristic loss of fluorescence associated with malignant progression.[7]

In Vivo Confocal Microscopy

Confocal microscopy is an imaging technique for various researches in cell biology with an advantage of optical sectioning and high resolution imaging. In vivo confocal images from the oral cavity show the characteristic features such as nuclear irregularity which is used to differentiate OSCC (oral squamous cell carcinoma) from normal oral mucosa. This technology uses low intensity laser to image below the surface of the skin.[8]

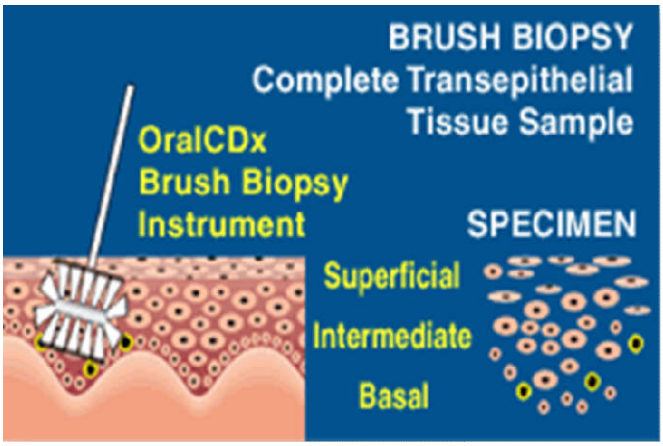
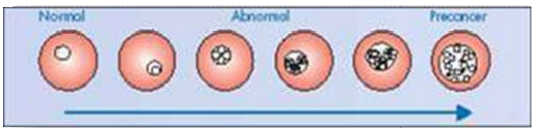


Fig 1



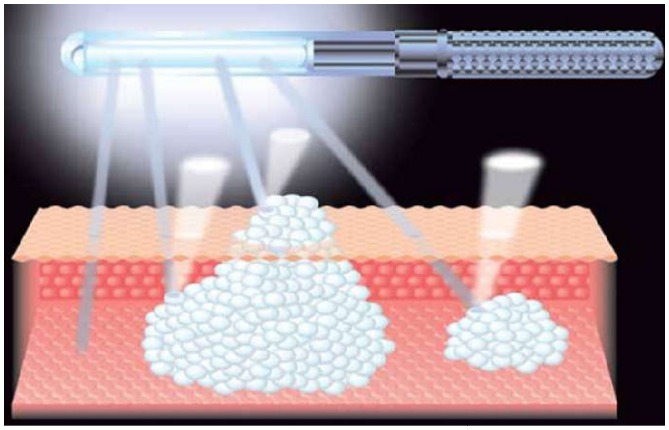


Fig 2

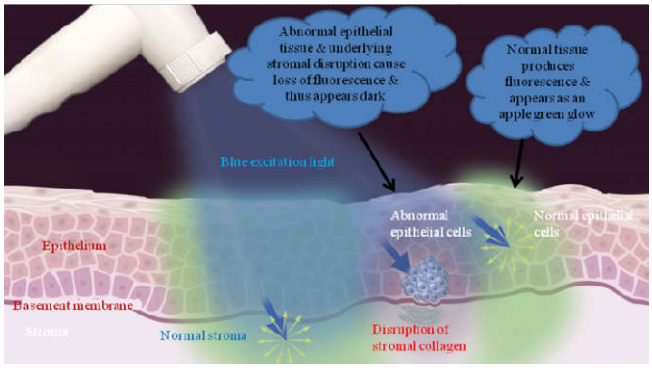


Fig 3

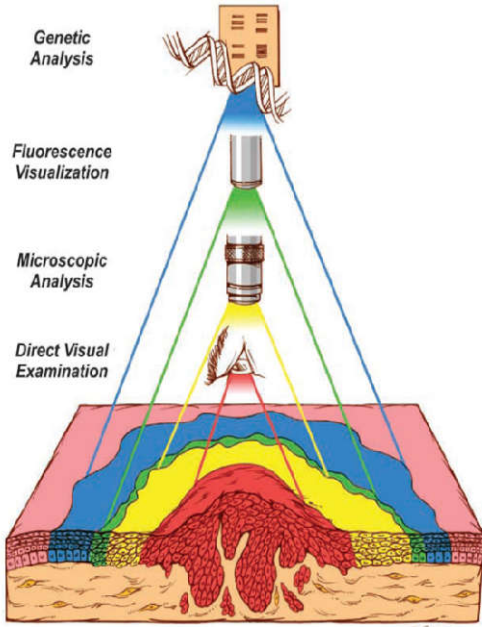


Fig 4

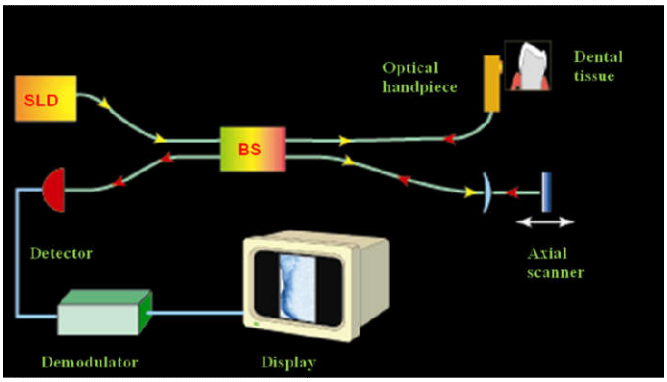


Fig 5

Optical Coherence Tomography

This was invented in 1990 at Massachusetts institute of technology. It was first reported by Huang & co workers in 1991.[9] It is a non invasive diagnostic technique that detects the tissue interfaces based on their differential reflection of light. It uses infrared radiation for imaging. It is based on a white light fiber optic Michelson interferometer. Output from a low coherence light source is split at the 2 x 2 fiber optic coupler and directed toward the sample and reference arms of the interferometer. Reflections from the mirror and backscattered light from the sample are recombined at the coupler and propagated to the detector and light source. An interferometric signal is detected when the distance to the reference and sample arm reflections is matched to within the source coherence length. A scanning retro-reflector varies the path length of the reference arm for each transverse location on the sample. Loss in signal intensity caused by birefringence effects in the optical fiber is corrected using polarization paddles. A cross sectional image is produced by transversely scanning the beam across the sample and collecting a reflectance versus depth profile at each transverse location. The reflectance intensities are recorded digitally on a grey-scale image as a function of transverse and axial distances. (Fig 5) The indications include soft tissue imaging and early diagnosis of oral mucosal lesions, earlier detection of incipient tooth lesions, viewing of tooth

restoration interface, measurement of periodontal pocket depth without physical probing and to determine the radiation induced mucosal damage. The advantages are as follows: uses non ionizing radiation, it does not require direct contact with the body, it is compact, easy to use and images can be read on the chair-side monitor, it generates an image with up to 25 times resolution as compared to ultrasound and 10 times as compared to standard radiograph.[10]

Fluorescence Spectroscopy

Auto fluorescence Spectroscopy Auto fluorescence spectroscopy has emerged as a promising tool for oral cancer detection. The system consists of a small optical fiber which produces various excitation wavelengths and a spectrograph which receives and records on a computer and analyzes it with the help of software, the spectra of reflected fluorescence from the tissue. However, the technique is controversial and often found with unclear results. Overall, it seems to be very accurate for distinguishing lesions especially malignant tumors from healthy oral mucosa, with a high sensitivity and specificity (Stefano Fedele, 2009). It is a non-invasive aid in the detection of various alterations in the structural and chemical compositions of cells indicating the presence of a diseased tissue. It can be useful in guiding the clinician in identifying the optimal location for biopsy (Sujata Satoskar and Ajit Dinakar, 2006). [4]According to a study, on using violet excitation light, camera-based autofluorescence photodetection technique has presented as a highly promising tool for the diagnosis of oral malignancies (Betz *et al*., 1999)[8]

Elastic Scattering Spectroscopy

ESS is an emerging technique that generates a wavelength dependant spectrum that reflects structural and morphological change within tissues. Elastic scattering implies that the light returns with the same kinetic energy as the incident photons. The incident light can undergo single, or more commonly, multiple scattering events before being collected again at the same surface by an optical probe and the data analyzed. The acquired data reflects both the scattering and absorptive properties of that tissue. This scattering process has been shown to occur at gradients in the optical index of refraction resulting from differences in densities that occur at a cellular and sub-cellular level. The structures that induce the scattering (scattering centres) are the nucleus, chromatin concentration, and sub-cellular organelles. Thus, ESS has been shown to be sensitive to nuclear size, chromatin content, nuclear/cytoplasmic ratio and cellular crowding which are all criteria that the histopathologist looks for when establishing of malignancy within a tissue. ESS has the advantage of being fast, reliable and cost effective and potentially offers a diagnosis in situ, non-invasively and in real time. The technique has not been used only in the diagnosis of dysplasia and malignancy, but can be used to monitor chemotherapy levels and free-flap oxygenation levels. It also can enable guided rather than random biopsies and to assess surgical margins and regional lymph nodes intra-operatively.[11]

Raman Spectroscopy

A Raman spectrum is a form of in-elastic scattering and is generated by shift in frequency of incident excitation light. This is caused by discrete changes in emergent light, above and below the wavelength of incident photons due to the vibrational frequencies of the bio-molecules that constitute the tissue. This technique is extremely sensitive and is the most accurate of the techniques but the signal is extremely weak, in the order of one trillionth of the incident beam.[12] Within biological tissues there are four principle components that contribute to the spectra; water, lipids (cell membranes), nucleic acids (DNA and RNA) and proteins (hormones, iso-enzymes, immunoglobulins and keratins). The resultant spectra from these structures give a characteristic signature for that tissue. The choice of wavelength also enables the operator to probe different depths of tissue due to different wavelength penetrations and the technique therefore represents a true form of optical histo-chemistry. The disadvantage, however, is that it is expensive, complex and difficult to adapt for in vivo use due to superimposed optical fiber and auto-fluorescence complicating the spectra. [13]

Lab on Chip

Microfuidics technology- also referred to as lab-on-a-chip or micro-total-analysis systems (TAS)- is the adaptation, miniaturization, integration and automation of analytical laboratory procedures into a single device or "chip". Microfluidics is often regarded as the chemistry or biotechnology equivalent of the silicon integrated silicon chip that has revolutionized electronics, computers and communications. Microfluidics are by definition suited for handling living cells (whose typical diameter is a few micrometers) in a three-dimensional, biologically relevant environment. This microfluidic chip accepts saliva sample, can be operated by minimally trained personnel, and can provide a diagnostic answer in an automated and timely fashion. The detection of oral pre-cancer (dysplastic) and cancer cells within the chip will take advantage of membrane-associated cell proteins that are singularly expressed on cell cancer cells. The measured profile is compared with archived gene transcription profiles to determine the cancer type and stage.[14]

Pet (Positron Emission Tomography) Scan

Oral cavity is one of the most common site for cancer in the head and neck with a high incidence of regional metastasis. Due to cervical lymph node metastasis, there is significantly reduction in the survival of the patient and which is an important prognostic factor for scientific debate today. It involves the administration of a radioactive tracer that combines a radioisotope, a radioactive compound that is detected by a PET scanner, with a natural body compound, which the body is able to integrate into its system without any negative effects. In oral cancer screening, the radioactive tracer used is Fluorodeoxyglucose (FDG) that combines the natural body compound glucose with the radioisotope Fluorine-18. Although many patients have concerns about the radioactive component contained in PET imaging, Fluorine-18 contains a short half-life and disappears from the body within hours. Consequently, the PET procedure is a safe one for patients.[8]

CONCLUSION

At present, molecular and genetic analysis is not a routine procedure for oral lesions in which biopsy is performed in daily practice. New technology has clearly made a positive impact on the field of dentistry by encouraging clinicians to more routinely perform thorough oral cancer and other oral mucosal

lesions examination. Until recently, surveys had consistently demonstrated a limited understanding of proper oral cancer screening and diagnosis among the dental community. Well-designed and prolonged attempts to educate the dental community as well as their patients may increase overall awareness about these diseases.

Although all these techniques are well helpful in the diagnosis of skin and mucosal lesions, precancers and neoplasms but, still there is a need to establish further new technologies which can be helpful to us in diagnosis not only at cellular, subcellular but also at the molecular level.

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