Abstract

The biopsy may very well be the most utmost procedure. Although the obtainment of biopsies is widely used in all medical fields, the practice is not so pervasive in dental world. Biopsies establish evolutive control of disease processes, and are able to document healing or relapse. A biopsy is often the only way to diagnose oral lesions and diseases. General dentists must be able to perform simple oral biopsies for the diagnosis of oral lesions. The ability to differentiate between benign and premalignant or malignant oral lesions is essential for establishing a correct diagnosis. Although most biopsies are performed in hospitals, a recent study has shown that many dental practitioners felt able to perform biopsies but are short of some necessary skills. The intention of this review is to acquaint a general dentist with some of the principles of oral biopsy technique.

Keywords:
Oral biopsy, Premalignant lesions, Malignancy, Toludine blue, FNAC.
Introduction

The biopsy may very well be the utmost procedure brazen out to the dentist and successful tissue diagnosis depends on an appropriate surgical biopsy technique. The practitioner who endeavor this procedure without being sentient of principles involved may avert more proficient hands from diagnosing a malignancy until it is too late. Although the obtainment of biopsies is widely used in all medical fields, the practice is not so pervasive in dental practice - fundamentally because of a lack of awareness of the procedure among dental professionals.

The word biopsy instigated from the Greek terms bios (life) and opsis (vision): vision of life. A biopsy consists of the obtainment of tissue from a living organism with the rationale of examining it under the microscope in order to establish a diagnosis based on the sample. Biopsies establish evolutive control of disease processes, and are able to document healing or relapse. Consecutively, the biopsy findings are of indisputable legal medical value.

A biopsy is often the only way to diagnose oral lesions and diseases and as with most of the procedures there is often more than one method of undertaking the surgery successfully. An incongruous, deceptive sample is of no use to the pathologist, clinician or most importantly the patient who would be ill served by a redundant repeat procedure. If dentists are not sure about the most appropriate site to biopsy, they should refer the patient to a clinician specializing in the field because a biopsy from an inappropriately selected site could give both the patient and the dentist a false sense of security. General dentists must be able to perform simple oral biopsies for the diagnosis of oral lesions. The capacity to differentiate between benign and premalignant or malignant oral lesions is essential for establishing a correct diagnosis.

Whatever is the method used; the aim is to provide a suitably representative sample for the pathologist to interpret, while minimizing preoperative discomfort for the patient. Many factors may make a biopsy problematic and be the reason for not undertaking it in general practice. These include: fear of medico-legal implications, unfamiliarity with biopsy technique, a lack of faith in personal diagnostic skills and the contention that biopsy is a specialist procedure.

Although most biopsies are performed in hospitals, a recent study has shown that many dental practitioners felt able to perform biopsies but are short of some necessary skills. The intention of this work is to acquaint a general dentist with some of the principles of oral biopsy technique so as to give confidence and to
provide required skills for the procedure of oral biopsy.

**THE USES OF ORAL BIOPSY ARE:**

- Diagnostic - Verifying or establishing a diagnosis of a clinically suspicious lesion.
- Planning proper treatment - Local or radical, surgery or irradiation.
- Checking progress of treatment - To elicit its effectiveness.
- Checking the extension of disease - Whether invasive or not.
- Evaluating end results - Whether the lesion is free of recurrence or is persistent.

Biopsy should not be reserved for obviously malignant lesions, but should be used in ruling out malignancy in seemingly insignificant ones. A biopsy is indicated in all those conditions of oral region which show alteration from natural appearance.

**THE INDICATIONS FOR ORAL BIOPSY SHOULD INCLUDE:**

- Any progressive ulcerated lesion which has been present for 3 weeks or one which fails to respond to therapy in 3 weeks.
- Any growth which has been present for 3 weeks or more.
- White or red patches in mucous membrane especially those having a warty or corrugated appearance.
- Regions which are intra-osseous arid produce rarefaction and expansion of the cortical plates.
- Any inflammatory lesion that does not respond to local treatment after 10 to 14 days (that is after removing local irritant).
- Persistent keratotic changes in surface tissue (ex: lips or oral mucosa).
- Any persistent tumescence, either visible or palpable beneath relatively normal tissue.
- Any lesion that has the characteristics of malignancy.

Characteristics that raise the suspicion of malignancy in an existing lesion are red or speckled red appearance of the lesion, discontinuity of the surface, persistence of lesion more than 2 weeks, signs of speedy growth, frequent bleeding on gentle manipulation and indurations of lesion and surrounding tissue.

**WHEN ORAL BIOPSY IS NOT NEEDED**

There is no need to biopsy normal structures. A biopsy would be needless in normal anatomical variations such as physiological gingival pigmentation, geographic tongue, linea alba, lingual indentations, protuberances and exostosis.

It is not required to biopsy irritative/traumatic lesions that respond to the removal of a presumed local irritant. On the other hand, a biopsy is
contraindicated in seriously ill patients, in those subjects with some systemic disorder that may worsen, or where secondary complications may develop. Likewise, a biopsy should be avoided in the case of lesions located in deep regions or in areas of difficult access where the surgical technique proves complicated or hazardous, with the risk of damage to neighboring structures. The same considerations apply in the case of suspected vascular lesions such as hemangiomas, due to the risk of massive and persistent bleeding. Biopsy is not advised in the case of multiple neurofibromas, due to the risk of neurosarcomatous transformation, or in tumors of the greater salivary glands. 2,10

PRECAUTIONS IN ORAL BIOPSY: 1

- Avoid the use of dyes and antiseptics that discolor tissue because they prevent proper preparation of the specimen.
- Plan incisions to avoid or minimize disfiguring the patient. Where possible, keep incisions parallel to Langer's lines of tension.
- Take care in the use of tissue forceps so as not to crush or mutilate the tissue specimen.
- Encapsulated lesions should be removed en toto so as to prevent breaking the natural barrier set up.
- In the oral cavity, be careful that tumor tissue does not spill into the mouth from where it may be aspirated or swallowed.
- Remove the entire malignant mass without cutting into it so as to minimize opportunities for metastasis and prevent implant nodules from being scattered in the tissue.
- Carry the incision deep enough, but avoid penetration of underlying periosteum which may act as a natural barrier to cancer cells.
- The surgeon should not section the specimen because he will not always choose the best plane.
- Avoid drying out the specimen by placing it in 10% formalin solution (4% formaldehyde) that is approximately twenty times the volume of the specimen.

HOW TO OBTAIN A GOOD ORAL BIOPSY:

A biopsy technique can be reduced to six steps: selection of the area to biopsy, preparation of the surgical field, local anaesthesia, incision, handling of the specimen and suturing of the resulting wound.

1. Selection of the area to biopsy

The selection of the site of biopsy is of the utmost importance because upon this hinges the accuracy of the diagnosis. A site should be selected along the most progressive portion of the lesion because this will show the potentiality of the lesion for invasion and also will give a picture of the emerging portion of the lesion rather than the dying or necrotic portion. Areas
nearest bone, cartilage should be avoided if possible because this is where tumor beds are shallowest and are frequently sites of necrosis. 6,11

In white patches since the prickle cell can either form keratin or divide, and it cannot do both at the same time, the part which is most likely to show advanced precancerous change is any area that rather resembles granulation tissue, and may only be flecked with keratin. 6

A colored lesion which is dark brown or black in color where color is believed due to melanin should not be incised into but the entire lesion should be removed en-mass and serve as the biopsy specimen. Lesions which are dark bluish or purplish in color and if small, should be totally excised because, they usually, are hemangiomatis and the hemorrhage produced on incision may be difficult to control. 11,12

In stalked lesions, the entire lesion should be removed by completely excising the base from surrounding normal tissue and should not be removed by snipping the pedicle. Entire thickness of an ulcer should be taken because the superficial portion is usually exudate, necrotic tissue or granulation tissue, the true nature of the lesion always exist at the base. In large lesions which vary in character, multiple specimens should be taken. 6,13

When dealing with undersized lesion, an excisional biopsy will be performed, whereas incisional biopsy should be carried out in the most illustrative area of the large lesion (more than 1 cm). If there is any suspicion about the malignant countenance of the lesion, vital staining with toludine blue can be use as an adjunct to select representative areas. Toludine blue is a basic dye that fixes to nucleic acids and stains the nuclear content of malignant cells; in these cases samples should be taken from areas with deep blue patches, as light blue areas are not significant. 14,15 Toludine blue is used in three steps:

• wash the area with 1% acetic acid
• apply a 1% Toludine blue water solution for 1 minute
• mouth wash with 1% acetic acid

The sample must include healthy tissue at the margin of the lesion.

2. Preparation of the surgical field.
The site of biopsy should be disinfected with a quaternary ammonium compound. Iodine-containing surface antiseptics are contraindicated, as they may stain the tissues. A 0.12- 0.20 % chlorhexidine solution is preferred.

3. Local anaesthesia:
An amide-type local anaesthetic with vasoconstrictor should be used and infiltrated away from the lesion are to avoid institute artefacts in the sample.

4. The incision:
Oral tissues should be immobilized far from the area to biopsy with non-toothed
tweezers. A sterile and distinct incision is acted upon to obtain a sliver of tissue when aiming at incisional biopsy. Soft tissues incisions should be elliptical in shape producing a “V” wedge that includes both the lesion and healthy margins. If various lesions are present, multiple biopsies should be taken.

5. Tissue handling

The specimen is handled gently to avoid crush artefacts and introduced in the fixing solution. The role of the fixing agent is to preserve the cellular architecture of the tissues. There are authors that suggest the placement of the specimen on a sterile paper with the mucous surface facing upwards to avoid distortion and curling of the sample margins. The best fixing agent is a 10% formalin solution, as it induces less ultrastructural alterations in the samples. 70% ethanol can also be used. The samples should never be put in isopropyl or methyl alcohol, saline or distilled water - as severe alterations may be provoked. The volume of the fixing agent should exceed 10 to 20-fold the volume of the sample. When immunofluorescence or immunostaining are needed, specimens should not be fixed, but sent as soon as possible to the laboratory for freezing or put in Michel’s solution. When the material is sent to the pathologist, it should be accompanied with a detailed report that includes identification of the patient, clinical records, clinical signs and a probable diagnosis as well as the orientation of the sample. An explanatory diagram of the biopsy area may be useful for this purpose. 2,6

6. Suture

The suture should achieve good haemostasis, facilitate healing and should be removed after 6-8 days.

The most repetitive errors that should be avoided when taking oral biopsies

In order to achieve a worth, artefact-free oral biopsy that permits the oral pathologist establish a histological diagnosis, the clinician should avoid:

• Pressing the sample with the tweezers, particularly if toothed, as may produce tissue tears and “pseudomicrocysts”
• Infiltrating anaesthetic solution within the lesion, as it can cause sample alterations
• Applying products to the lesion that induce tissue modifications
• Using an insufficient volume of fixing solution
• Inclusion of undesired material in the sample: glove powder, calculus, restorative materials, etc.
• Taking insufficient amount of tissue in extension and depth. 16

TYPES OF ORAL BIOPSY 17

• Exfoliative Cytology: It is the study of cells that have been shed or removed from the epithelial surface of various organs.
These cells can be recovered either from natural secretions such as sputum or by artificial means such as paracentesis or lavage. The cells can be collected from the epithelial surfaces by lightly scraping the surface, by swabbing, or washing the surfaces. During malignant conditions or during infection, the exfoliation becomes exaggerated and the epithelial cells show variation in morphology. Such exfoliated cells, when collected and appropriately stained (Giemsa or PAP), give information on the living epithelium from which they are derived. These characteristic cellular and nuclear appearances in cells thrown off from healthy epithelium differ distinctly from those, derived from inflamed or malignant lesions. Thus by studying the alterations in morphology of the exfoliated cells and their pattern, the diagnosis of various pathologic conditions can be made.

- **Oral brush biopsy:** The oral brush biopsy, using a specially devised circular bristled brush, has been designed to access and sample all epithelial layers, in conjunction with the basal cell layer and the most superficial portion of the lamina propria. Thus, the cellular material acquired should be positive with all epithelial layers in a disaggregated form spread over the surface of a glass slide. The debate over the efficiency of oral brush biopsy raises two questions. One question is whether indications for oral mucosal biopsy should be expanded to include certain “benign-appearing” lesions, either in high-risk patients (e.g., current or former smokers, heavy drinkers), or in all persons regardless of risk. A second question is what is the most effective and efficient method of biopsy of oral mucosal lesions. Now a days oral brush biopsy coupled with computer-assisted analysis (OralCDx, OralScan Laboratories, Inc., Suffern, NY) has been developed as a technique for evaluating unexplained clinically detectable alterations of the surface epithelium of the oral mucosa (cancer or precancer).

- **Fine needle aspiration biopsy:** Aspiration or FNA Biopsy is performed with a fine needle attached to a syringe. Aspiration biopsy is often referred to as Fine Needle Aspiration (FNA). FNA biopsy is a percutaneous (through the skin) biopsy. FNA biopsy is typically accomplished with a fine gauge needle (22 gauge or 25 gauge). FNA is the fastest and easiest method of biopsy, and the results are rapidly available. One disadvantage of FNA is that the procedure only removes very small samples of tissue or cells. If the sample is benign fluid (for example, a cyst), then the procedure is ideal.

- **Punch biopsy:** Punch Biopsy is typically used by dermatologists to sample skin rashes, moles and other small masses,
in a similar way this technique is useful in oral mucosal biopsies. Generally it is used in an incisional fashion for diagnostic purposes; however, larger punches may be used to excise small lesions. After a local anesthetic is injected, a biopsy punch, (3 mm to 4 mm or 0.15 inch in diameter), is used to cut out a cylindrical piece of mucosa. Punch biopsy may be tricky on freely movable oral tissues and probably offers no weightage against with scalpel biopsy. The technique may be appropriate in the hard palate and other sites with better support and tissue that is bound down. The wound heals by secondary intention, two "dog ear" defects can result in punch biopsies much larger than 5 mm, thus an incisional biopsy is preferred on larger lesions and discomfort may persist longer than anticipated by the clinician and the patient.

**Incisional biopsy:** A punch biopsy is essentially an incisional biopsy, except it is round rather than elliptical. Incisional biopsies can include the whole lesion (excisional), part of a lesion, or part of the affected mucosa plus part of the normal mucosa (to show the interface between normal and abnormal mucosa) and removed for purposes of diagnosis. Incisional biopsy is consistently recognized as the gold standard. While it is relatively easy to perform, a certain degree of surgical skill is necessary and the procedure is time consuming. The disadvantage includes tumor spillage and leaking of residual disease.

**Excisional biopsy:** The excisional biopsy is analogous to incisional biopsy, with the exception of that entire lesion or tumor is included. This is the superlative method of diagnosis of small melanomas (when performed as an excision). Ideally, an entire melanoma should be submitted for diagnosis if it can be done safely and cosmetically.

**Bone biopsy:** Biopsy is usually required to attain a provisional diagnosis, which may need to be confirmed by examination of the full lesion if excision follows. Biopsy may be necessary to differentiate between benign bone tumours, metastatic tumours, degenerative or congenital lesions.

*Open incisional biopsy:* This method often provides a good large piece of tissue, and therefore a good chance of obtaining an accurate diagnosis. Complications such as an increased risk of haematoma formation, wound break down, infection, seeding of the incision with tumour cells and post-biopsy pathological fractures are all major disadvantages.

*Closed needle or trephine biopsy:* This is the method of choice since although it does provide a smaller specimen, postsurgical complications are greatly reduced. Both are easy, quick and safe and an accurate diagnosis can be made in up to 94% of cases. Either a Jamshidi
needle or Michelle trephine technique can be used. 17

**Conclusion:** It is not easy to procure a good biopsy specimen, nor is it very intricate, but the procedure must be carefully premeditated and expeditiously carried out, and the provisional diagnosis must be borne in mind prior to biopsy. Inadequate care at any stage could consequence in a non-diagnostic biopsy and may necessitate the patient having a repeat procedure with its subsequent physical and psychological morbidity. 21 Dentists must therefore be aware not only of where, when and how to perform a biopsy, but also of when to refer to the patient to a specialized center. Finally, emphasis must be placed on the importance of postgraduate training in oral medicine and in diagnostic oral biopsy procedures for general dentists. This may be because some of them pay little attention to oral pathology (due to unawareness, a lack of training, etc.). Diagnosis and risk assessment of oral lesions (premalignant or malignant) require a team effort from both clinicians and pathologists.

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