Performing mucosal tissue biopsies in general dental practice

Abstract

One of the roles of a dental practitioner is the identification and management of oral mucosal disease (pathology) in its many forms. A tissue biopsy is an important step in the diagnostic process for oral lesions, while the skill required to perform a biopsy should be well within the capability of most practising dentists.[1] The purpose of this article is to help dental practitioners to identify lesions suitable for biopsy in a dental practice setting, and to outline the equipment and explain the techniques used.

Journal of the Irish Dental Association 2011; 57 (4): 203-208.

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Introduction

Biopsy technique is a competence skill that is easily learned. Research shows that only 15% of general dentists would perform a biopsy on their patients.[2] Waiting times for patients to be seen at specialist centres may, however, be long. The heavy workload at these centres, and the delay for the patient, could be alleviated if patients with small and clinically benign lesions could be investigated and treated in the primary care setting. This would have the benefit of improved patient compliance and speedier diagnosis. Dentists providing this service should work closely with their specialist colleagues and the oral pathologist.

Lesions that may require biopsy: clinical description

Whether a lesion is noticed initially by the dental practitioner or by the patient, the first step in the process of diagnosis (as with any pathology) is the taking of a thorough history.

The pertinent information required with regard to a lesion includes the duration of the lesion, its progression (slowly enlarging or recurrent with episodes of quiescence) and any associated symptoms. A full medical history, including medications, dental history and social history are also vital to help determine any systemic or local causes for the lesion.

The clinical examination is a vital step in correctly identifying a lesion, but it is also important to be able to use the correct terminology in describing a lesion. This enables a dental practitioner to communicate effectively with a referral centre or pathologist, as the language used will help the staff at the referral centre to determine the urgency of a referral. The steps for clinical examination should be followed as for any lesion anywhere in the body, and follow the sequence of: 1) look; 2) feel; 3) move; 4) measure; and, 5) record with a photograph. Note should be taken of the:

1. Site, shape, morphology, colour and border.
2. Consistency (soft, firm, lobulated, hard), tenderness, associated symptoms (e.g., discharge) and pulsations (beware).
3 Tethering (fixation to mucosa, skin or underlying structures).
4. Size (using a ruler or callipers for exact measurement).
5. The exact appearance and size recorded by photograph for future reference.

Radiographic images can also aid in the diagnosis of lesions and should be taken when a dental or bony cause for a mucosal lesion is suspected.

It is important at this stage to stress the importance of taking a photographic record

of lesions that one comes across in the dental practice. While a drawing in the patient's notes can be helpful in recalling details, there is no substitute for the usefulness of obtaining photographs of any suspicious lesions. They make the long-term follow-up of benign lesions a simple matter of comparing previous photos to the current state of a lesion, and are also vital when a seemingly benign lesion turns out to be dysplastic or malignant. The pathologist would also welcome a copy of the photographed lesion when receiving a specimen. The relatively low cost of digital cameras and the user-friendliness of modern computer operating systems make the maintenance of a photographic record well within the capability of all dental practitioners.

Lesions that may require biopsy: further steps to diagnosis

It is up the dental practitioner to decide first of all if the lesion in question warrants a biopsy and, if so, if it is a lesion that is suitable to be biopsied in the general dental practice setting.

A presumptive diagnosis is a great help. The surgical sieve (see **Table 1**) approach is taken when the dental practitioner is presented with an oral lesion and makes an initial differential diagnosis of the lesion. Sometimes a diagnosis can be made on clinical grounds alone using a combination of history, examination, knowledge and experience. An experienced clinician can often recognise a fibro-epithelial polyp for what it is and can counsel the patient on whether a biopsy is necessary. Some patients will elect to have the lesion excised due to its nuisance value, while other patients will be reassured and are content to have no treatment.

Patients with lesions that have a history and/or clinical appearance highly suggestive of malignancy (white, red, hard or ulcerated) should be immediately referred to a specialist centre (e.g., dental hospital) by letter and phone. A non-healing ulcer present for more than three weeks must be regarded as a cancer until proven otherwise. This is also true for a hard lump in the neck or sublingual area. Lesions that raise the possibility of significant pathology (such as pemphigus or Stevens-Johnson syndrome) should also be referred urgently to a specialist centre.

Large benign-looking lesions that are unlikely to resolve spontaneously (e.g., a large fibrous polyp >1cm), and that require excision, should also be referred after the initial consultation.

Other lesions, such as a traumatic ulcer or clinical evidence of candidiasis, can be treated (e.g., removal of the cause of trauma, antifungal medication) and following the initial consultation, a review appointment should be made for approximately two weeks' time. If at this stage the lesion is persisting or, indeed, progressing, a decision can be made as to whether a biopsy is necessary and also if referral to a specialist centre is required (see **Table 2**).[3,4]

In many cases it is wiser to perform the biopsy in a specialist referral centre (see **Table 3**).[3,4] Consideration should also be taken of the possibility of complications such as excessive bleeding, scarring and damage to other structures (e.g. nerves) when undertaking a biopsy. These can arise due to the morphology or location of the lesion or the patient's medical history (see **Table 4**). In any individual case, the clinician must decide if performing the biopsy is within their level of experience and ability.

Table 1: Surgical sieve for a lesion: consider as a cause.

Developmental
■ White sponge naevus
Inflammatory
■ Trauma
■ Physical (hot/cold)
■ Chemical (acid/alkali)
■ Irradiation
■ Infection (bacterial, viral, fungal, protozoal, etc.)
■ Immune-mediated
Neoplastic
■ Benign
■ Malignant
Metabolic/endocrine
Degenerative
Miscellaneous
■ Specific to area (e.g., salivary gland disease)
Other

Table 2: Lesions suitable for biopsy in general dental practice.[3,4]

- Fibroepithelial polyp:
- pyogenic granuloma;
- epulis; and,
- mucosal lichen planus (for experienced practitioners; if in doubt, refer).

Biopsy technique

Dental practitioners are adept at many minor surgical procedures and performing a biopsy follows the same basic steps.

Goal

The overall goal of a biopsy is to obtain tissue that can be analysed by a pathologist in order to reach a diagnosis and therefore influence future treatment decisions regarding a patient. To that aim, it is the responsibility of the person performing the biopsy to obtain sufficient tissue and manage that tissue appropriately, so that it arrives to the pathologist in a condition suitable for processing and analysis.

Planning

There are a number of types of biopsy techniques available (incisional, excisional, a scraping and punch) and the dental practitioner, if trained to take a biopsy, must decide on whether an incisional or an excisional biopsy is the most appropriate for the given patient, based on the presumptive diagnosis, the size and location of the lesion, and whether adequate closure of the wound can be made. An excisional biopsy is preferable as it makes the procedure both diagnostic and therapeutic, in that the offending lesion can be completely removed. Excisional biopsies imply that all of the abnormal tissue is removed and are typically performed for clinically

Table 3: Lesions not suitable for biopsy in general dental practice.[3,4]

Any lesion showing clinical features of malignancy (needs urgent referral)
■ Ulcer lasting more than three weeks with no obvious traumatic cause
■ Induration, hardness, recurrent bleeding
■ Verrucous (exophytic type) appearance
Leukoplakia/erythroplakia (refer to specialist unit)
■ All white patches and red patches (velvet like) should be biopsied
■ May need multiple biopsies and follow-up in a specialist unit
Haemangiomas and other vascular lesions (refer to specialist unit)
■ May bleed excessively and require complete excision
Bullous lesions (refer to specialist unit)
■ Requires tissue close to bulla
■ Fresh specimen required for immune-fluorescence
Lesions suspicious for salivary gland pathology other than mucocoeles
■ High likelihood of salivary gland malignancy (needs urgent referral)
Inaccessible lesions
■ Lesions on the soft palate or fauces may cause excessive gagging when attempting to biopsy, making wound closure difficult
■ Increased likelihood of a non-diagnostic biopsy
■ Patient may need a general anaesthetic to achieve adequate tissue sampling

benign lesions less than 1cm in size (in dental practice a conservative maximum size of 1cm should probably be adopted).

Incisional biopsies involve removing only part of the lesion for analysis. It is generally regarded as advantageous to include some normal mucosa in the biopsy specimen to allow comparison with the abnormal tissue, but the majority of the specimen should be lesional. When planning any surgical incision, one must also take account of the surrounding anatomy and plan on how a wound is going to be closed. While wounds on the buccal mucosa will close quite easily, wounds on areas of attached gingiva will not and may need to be left to heal by secondary intention (see **Table 4**).

Preparation

As with any surgical procedure, it is vital to obtain a patient's fully informed consent to having a biopsy.

A pre-operative photograph of the lesion should be taken. Local anaesthetic (with adrenaline to aid haemostasis) is then administered with a number of small infiltrations around but not into the lesion to be biopsied. A field block can also be used to aid anaesthesia.

Relatively little equipment is required to perform a biopsy (see **Table 5**) but it is important to lay the equipment out ready to be used before commencing the biopsy.

Table 4: Locations where caution is needed when taking oral biopsies.

Hard palate midway between molar teeth and midline
■ Risk of haemorrhage from the greater palatine artery
■ Manage bleed with direct digital pressure for 10-20 minutes
■ If uncontrollable, will need emergency referral to a maxillofacial unit
Lip
■ Risk of distortion of the lip contour if biopsy comes close to the external skin
■ Lesions close to the external surface of the lip should be referred to a specialist centre
■ The labial artery runs between the mucosa and the orbicularis oris and is easily cut
Areas of attached gingival/mucosa
■ Primary closure may not be possible
■ Wounds generally heal very well by secondary intention
■ May need prolonged direct pressure for haemostasis
■ Risk to gingival contour if near the gingival margin
Floor of mouth
■ Difficult access due to nearby teeth and tongue
■ Highly vascular area and can bleed heavily and/or develop a haematoma
■ Thin friable mucosa, which is difficult to close
■ Lesions in this area should be referred to a specialist centre
Buccal mucosa
■ Generally a safe area to take a biopsy from but take care to avoid the parotid duct orifice adjacent to the upper second molar
Tongue
■ Highly mobile and vascular structure
■ Requires a good assistant to hold the tongue using a piece of dry gauze throughout the procedure
■ If well anaesthetised, a 2.0 or 3.0 silk suture can be passed through the tip of the tongue to aid retraction
Buccal sulcus adjacent to lower premolar teeth
■ Risk of injury to mental nerve
■ Perform superficial biopsies only

Table 5: Equipment needed to perform a biopsy.

- Sterile gloves
- Fine-toothed forceps
- 15-blade scalpel
- 4mm or 5mm punch biopsy (if a punch biopsy is going to be performed)
- Fine sharp scissors
- Sterile gauze
- Sterile saline
- Suture material
- Procedure drape (for maintaining a clean field and protecting the patient's clothes during the biopsy)
- Sterile specimen container containing formalin

Table 6: Punch biopsy technique.

- The area to be biopsied is identified and, if mobile, should be held under tension using the opposite hand. The assistant can retract the lip or tongue using fingers, a dental mirror or wooden spatula if required.
- The punch should be held perpendicular to the lesion.
- Using a gentle twirling motion and almost no downwards pressure, the punch should be allowed to cut through the mucosa and just into the submucosal tissue.
- The punch blade should not be buried to its full depth (oral mucosa is quite thin).
- It will be clear if a deep enough punch was performed as the tissue sample will be freely mobile from its surrounding mucosa and only attached to the underlying submucosal tissue.
- The tissue sample can now be gently held with forceps while the submucosal tissue at the base of the biopsy specimen is cut using a sharp, fine scissors or scalpel.
- While the assistant puts direct pressure on the wound, the freed biopsy specimen is then examined to assess whether it is of suitable size and condition.
- The specimen is then placed in a container of formalin (see section on specimen).
- The wound is inspected for haemostasis and one or two sutures should be placed to aid haemostasis and also to aid identification of the biopsy area when reviewed.

Table 7: Incisional biopsy technique using a scalpel.

- The area to be biopsied is identified and held under tension (skin tension is vital when performing biopsy with a scalpel).
- The blade should be held in a pen grip and the incision should be made using a light, even stroke at right angles to the mucosa (avoid undercutting the mucosa specimen).
- The incision should be deep enough to pass through the mucosa and into the submucosal tissue. This allows the mucosa to pull away from the biopsy and makes the technique very simple.
- If the wound is going to be closed primarily a trapezoidal or elliptical incision is made, making sure that the length of the ellipse is at least twice as long as the breadth.
- If the wound is going to be left to close by secondary intention, then the incision should be tailored to the size and morphology of the lesion.
- If performing an excision biopsy, cut around the lesion with a small margin of normal tissue (cancer is not suspected so a large margin is not necessary).
- Once the mucosa has been incised, the specimen can be gently picked up at one end using a forceps and the underlying soft tissue can be cleared using the scalpel.
- A 3.0 silk stitch can be passed through polyps during excision to aid retraction.
- For flat mucosal lesions, a silk stitch can be passed through one end of the specimen to aid handling and to provide orientation.
- Once the biopsy specimen is removed, the wound can be closed as normal.

Punch or knife

There are two main methods of performing a biopsy. A punch biopsy involves using a cylindrical, very sharp, sterile blade of a given diameter (usually 4 or 5mm) to incise an even circle into the epithelium (see **Table 6**).

A knife biopsy involves using a scalpel (usually 15 blade) to make an incision in the mucosa (see **Table 7**).

In our opinion, the punch biopsy is ideal for performing intra-oral biopsies as it is very user friendly, easy to judge the depth of the biopsy, offers an adequate size of tissue for the pathologist, and the resulting wound is easily closed with one or two sutures. The small dog ears formed when closing a punch biopsy wound are not an issue with intra-oral biopsies as they heal well and are almost invisible after two to three weeks.

Regardless of the type of biopsy performed, haemostasis can be achieved by direct pressure with damp gauze. The wound can then be closed with size 4.0 silk or vicryl sutures.

Some biopsy locations are not amenable to direct closure such as on attached gingiva or on the attached mucosa of the hard palate. Once haemostasis is achieved in these areas by direct pressure, the wound can be allowed to heal by secondary intention. Materials such as Coe-pack and the patient's denture can be useful in some circumstances.

The specimen

It is important that a tissue specimen arrives to the laboratory in a condition suitable for analysis by the pathologist. To achieve this goal the specimen should be large enough to be processed, and must be handled as gently as possible while being collected. The gentle use of a fine forceps is necessary while it is being collected (in one study, crush artefacts were found to be present in 27% of biopsies taken by general dental practitioners).[5] Some clinicians recommend the use of a fine hypodermic needle or suture passed through the specimen during retrieval to avoid crushing it with forceps, but note that this can also damage the specimen.

For all excision specimen sizes, it is useful to orientate it by placing a fine black silk suture through one end. The specimen could also be placed on blotting paper to prevent curling.

Once taken, the specimen should immediately be placed in a sealable container filled with 10% neutral buffered formalin with a volume at least 10 times that of the specimen volume. The formalin works by creating intermolecular bridges between proteins, thus preventing autolysis. This process renders a specimen unsuitable for evaluation by immuno-fluorescence and therefore, in situations where this is required, the biopsy should be performed at a specialist centre.

If an instrument contacts the formalin, put it aside for sterilisation to avoid contaminating the wound with formalin.

A pathology request form must be adequately completed. Details that must be entered include: the date of the biopsy; the patient's name, address and date of birth; and, the name, location and contact details of the referring dental practitioner. Clinical details should include a description of the lesion, its location in the mouth, the provisional diagnosis or differential diagnosis, the type of biopsy (incisional or excisional, punch, etc.) and the orientation if a location stitch has been placed. A drawing and/or photograph is often extremely useful to the pathologist. In some circumstances, contacting the pathologist directly would be appropriate, particularly if there is a complex history.

Sending the specimen

There are regulations in place governing the transport of human tissue (Agreement Dangerous Routier [ADR], UN/ECE European Agreement Concerning the International Carriage of Dangerous Goods by Road, and the WHO Guidance on Regulations for the Transport of Infectious Substances 2007-2008, for example). The specimen must be transported to the hospital pathology laboratory in a manner that will ensure that it arrives intact and minimising any chance of leakage or loss of the specimen. The specimen must be sent by courier or taxi in a package with the following components:

- leak-proof primary container (filled with formalin and specimen, and labelled with patient details);
- secondary sealable package to enclose the primary container (sealable plastic specimen bag with sufficient absorbent material to absorb the entire content of the inner container); and,
- outer packaging (a rigid container able to withstand a free fall drop and containing material to protect the inner packaging).

The outer container must be marked as follows:

Adjacent to the diamond-shaped mark, the shipping name 'BIOLOGICAL SUBSTANCE, CATEGORY B' must also be written.

Specimens contained as outlined and marked as above can be transported by any driver and do not need any ADR documentation. In Ireland, tissue specimens must not be sent by post.

There are several companies that provide suitable containers and their details are usually available from the local laboratory. Most laboratories in Ireland are staffed by general pathologists who are competent in interpreting the pathology of the more common oral mucosal lesions, particularly if the clinical details are adequate. Where necessary, they may seek the advice of a pathologist with a specialist interest in oral and maxillofacial pathology.

Follow-up

A biopsy report may be available within two to three days of the specimen's arrival at the laboratory, but since most reports are sent to the general practitioner by post, this extends the turnaround time. Delay also occurs in some cases due to the need to cut further sections, to do special stains, or where there is difficulty in interpretation and the case needs to be examined by more than one pathologist. An appointment should be made with the patient for approximately two weeks after the procedure to inspect the wound and discuss the biopsy results. At this stage the patient can either be reassured that no further treatment is needed or, if required, he/she can be referred to a specialist centre for further management. If there is any concern about the lesion, do not remove the sutures until the patient is seen in the appropriate centre (with an accompanying photograph of the original lesion).

Example of a punch biopsy

The images overleaf illustrate the steps taken when performing a punch biopsy for a lesion with the clinical appearance of a small fibro-epithelial polyp on the dorsum of the tongue. The lesion was excised as it was a nuisance for the patient. After consultation, consent was given for the procedure by the patient.

The relevant clinical details are entered on the histopathology form and the specimen is sent to the pathology laboratory for processing. The patient is booked for a review appointment for two weeks' time.

Conclusion

There are a myriad of possible diagnoses when one is presented with a patient with oral soft tissue pathology and these patients often first present themselves in general practice. A combination of clinical knowledge and experience will enable the dental practitioner to determine if the lesion warrants a biopsy and whether the lesion is of serious concern.

Biopsy of small clinically non-suspicious lesions can be performed relatively easily in the dental practice and is often swifter and more convenient for the patient than referral to a hospital unit.

References

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Further information on histological specimen handling and transport is available at:

www.dentalschool.ie/oral-pathology/www.stjames.ie/GPsHealthcare Professionals/LaboratoryPolices/transportation.pdf

www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2007 2cc.pdf

Example of a punch biopsy

The following images illustrate the steps taken when performing a punch biopsy for a lesion with the clinical appearance of a small fibro-epithelial polyp on the dorsum of the tongue.

FIGURE 1: A fibro-epithelial polyp is deemed suitable for biopsy.

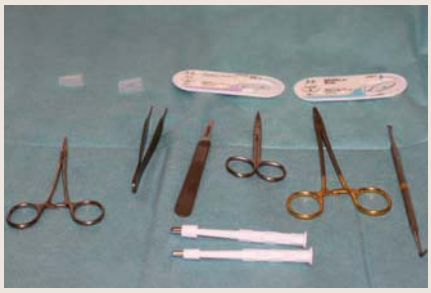

FIGURE 2: The required equipment is laid out.

FIGURE 3: Local anaesthetic is administered around the lesion.

FIGURE 4: A 5mm punch is selected.

FIGURE 5: The punch is positioned perpendicular to the lesion.

FIGURE 6: The punch is 'twirled' into the lesion.

FIGURE 7: The specimen is held in by the underlying tissue.

FIGURE 8: The specimen is cut free from the underlying tissue.

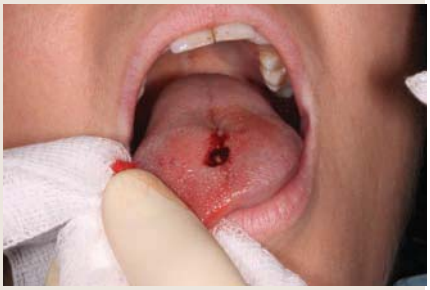

FIGURE 9: The wound is ready for primary closure.

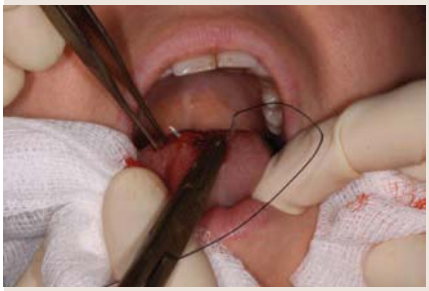

FIGURE 10: A 3.0 silk suture is used to close the wound.

FIGURE 11: Haemostasis and adequate wound closure.

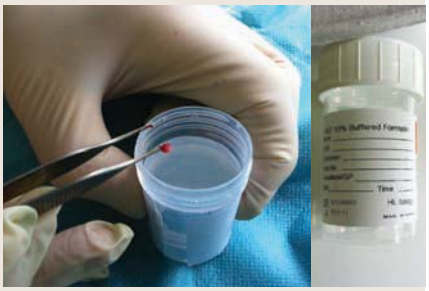

FIGURE 12: The specimen is placed in formalin.