

# Biopsy of the oral mucosa and use of histopathology services

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## ABSTRACT

Patients often present with intraoral pathology in the general dental practice setting. Therefore, it is important that dental practitioners are aware of how to deal with pathology when this occurs and have an understanding of investigative techniques that might assist in making a diagnosis. Biopsy and subsequent histological examination of the lesion is an important diagnostic tool. Even if dentists refer the patient to another practitioner for the biopsy, the referring practitioner still needs to be familiar with the procedure and results obtained so that the patient can be appropriately managed. This paper reviews clinical issues that may impact on biopsy procedures and the potential pitfalls and problems that may affect the histological assessment of tissue and therefore affect diagnosis. The medico-legal responsibilities of practitioners are also addressed.

**Keywords:** Biopsy, oral mucosa, histopathology.

## INTRODUCTION

Patients often present with intraoral pathology in the general dental practice setting.<sup>1</sup> Therefore, it is crucial that dental practitioners are aware of how to deal with pathology when it presents and have an understanding of investigative techniques that might assist in making a diagnosis. This is important irrespective of whether the dentist is the person actually undertaking the procedure or the investigation. If a referral is made to a specialist for biopsy, the referring practitioner still needs to be familiar with the procedures and results obtained so that the patient can be appropriately managed. The American Academy of Oral and Maxillofacial Pathology recommends that “all abnormal tissue be submitted promptly for microscopic evaluation and analysis”.<sup>2</sup> It is generally accepted that in most instances, microscopic or histopathological examination of tissue is the gold standard for the diagnosis of many lesions that present in the oral cavity and surrounding regions.<sup>2–5</sup> Furthermore, in some types of pathology, histological examination is important not only in diagnosis but also to determine whether there is evidence of malignancy, provide information on the clinical behaviour of the lesion and, in some instances, give prognostic information<sup>6,7</sup> – all of which directly impact on patient management. Deciding whether a lesion needs to be

biopsied is also an important clinical decision. For example, if there is uncertainty whether a lesion may be due to trauma, then the suspected irritant should be removed and the lesion reviewed in 7 to 10 days. If the lesion has not improved markedly then a biopsy is indicated.

Despite the importance of the histological examination of tissue, general dental practitioners do not regularly submit specimens for examination.<sup>8</sup> Suggested reasons for this include the clinician’s perceptions of training deficits and the risk of diagnostic error.<sup>9</sup> Also, the relative infrequency by which dentists encounter pathology compared to other oral/dental problems in general practice has been suggested to contribute to the lack of experience and confidence with respect to the management or further investigation of pathology.<sup>9</sup>

## Biopsy of mucosal lesions

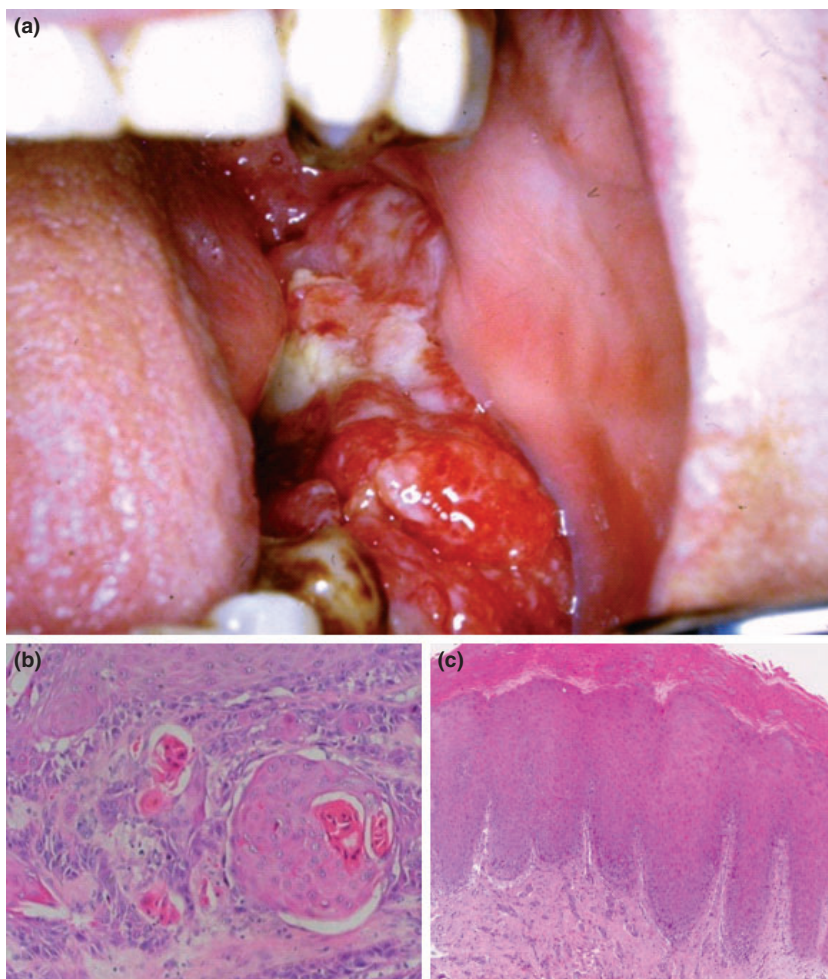
Franklin and Jones stated that *adequate and appropriate* collection of tissue is essential for accurate examination, diagnosis and ultimately treatment.<sup>10</sup> Therefore, for dentists who undertake biopsy procedures, understanding what is adequate and appropriate is important. As discussed by Poh *et al.*, an *appropriate* biopsy contains tissue that is representative of the lesion; this is dependent on three main factors, namely,

selection of the biopsy site, the type of biopsy and finally the adequate submission of the specimen to the laboratory.<sup>4</sup> The amount of tissue submitted is important. Whilst a biopsy does not necessarily have to be large, very small or superficial biopsies can be inadequate and not diagnostically useful – small biopsies can also be lost or become distorted during processing. It is important that there is an adequate amount of tissue for assessment. Finally, an accurate and relevant clinical description of the lesion can assist the pathologist in the diagnosis.<sup>2</sup>

### **Selection of biopsy site**

For large lesions there may be discrepancies in the histological features found from one site within the lesion to another. For example, with respect to a large squamous cell carcinoma there may be areas which demonstrate obvious invasive disease, whilst others may indicate epithelial dysplasia (Fig 1). The site that is selected for a biopsy, particularly in larger lesions, must

be representative of the overall pathology that is present in the lesion. In these situations, multiple smaller biopsies of the lesion may be appropriate in order to provide such representative tissue to the pathologist for examination. This was supported in a recent study by Lee *et al.* who demonstrated that when histological diagnoses from single-site biopsies of oral leukoplakias were compared with the histological diagnoses after resection of the same lesions, the “agreement rate” between these diagnoses was only 56%. Of more concern was that 29.5% of patients were actually underdiagnosed. The authors found that the rate of underdiagnosis was reduced to 11.9% when multiple biopsies were taken initially.<sup>11</sup> For smaller, discrete lesions (Fig 2), an excisional biopsy may be appropriate. From a clinical perspective, there are other factors that may influence the type of biopsy or the selection of tissue. These might include surrounding anatomical factors which could potentially complicate surgery and the clinical nature of the lesion (e.g., if clinically it demonstrates marked vascularity).



**Fig 1.** Squamous cell carcinoma involving the left retromolar area (A). Histology from the posterior aspect of the lesion demonstrates invasive tumour (B), whilst anterior aspect of lesion demonstrates dysplastic epithelium only (C).

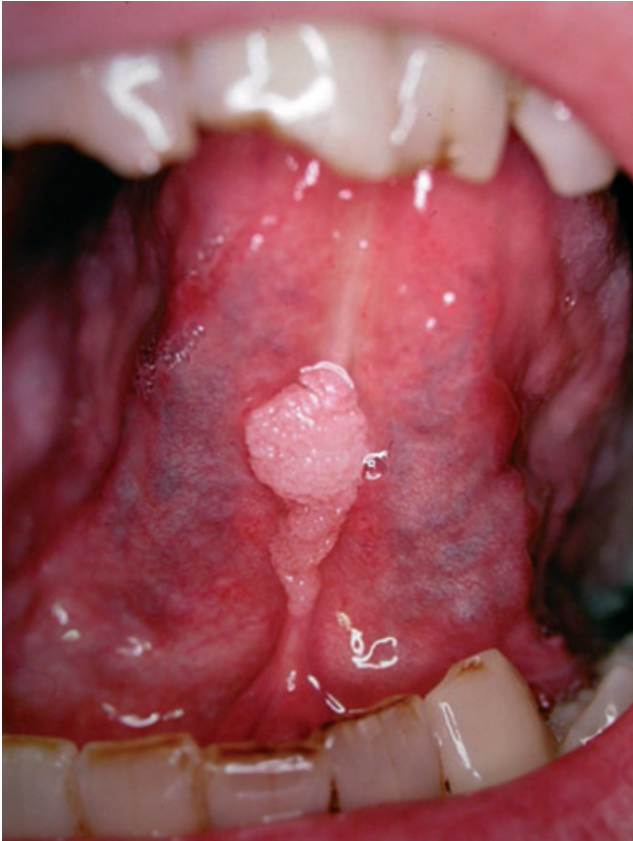


Fig 2. Squamous papilloma involving the floor of mouth. A localized lesion such as this can be easily excised. In this case the close proximity of the lesion to anatomical structures in the floor of the mouth also needs to be considered.

### **Type of procedure**

For many lesions, surgical biopsy is the primary choice in order to attain adequate tissue for histological examination. Other techniques for sampling tissue, such as cytology or fine needle biopsy, may have clinical applications. However, potential pitfalls with these techniques need to be understood so that information from these techniques can be meaningfully interpreted.

### **Surgical biopsy**

The traditional means of collecting tissue for histological examination is via scalpel biopsy of tissue (Fig 3). This may be achieved by taking a sample of the lesion (incisional biopsy) or removal of the entire lesion (excisional biopsy). The choice between incisional or excisional biopsy is dependent on many factors. Some of these have been mentioned previously including the anatomical site of the lesion and proximity to other structures, the size and nature of the lesion itself. A small, clinically benign lesion can be easily removed in its entirety, an excisional biopsy as opposed to a large



Fig 3. Scalpel biopsy of the lower lip to remove a mucocele.

leukoplakic lesion that is broadly distributed across the tongue.

Alternative methods to scalpel biopsies that are reported in the literature and frequently used include punch biopsies. Disposable punch biopsies are available in different sizes and can usually be provided on request from the pathology laboratory. Punch biopsies are a useful and often easy method for undertaking biopsies depending on the type of lesion and also clinical access to the lesion. This type of biopsy generally produces few artefacts within the tissue.<sup>12</sup>

### **Exfoliative cytology/brush biopsies**

This is a non-invasive method that, in some situations, may be useful in the evaluation of mucosal pathology, particularly in evaluating superficial cellular features of lesions for features of atypia which may indicate malignancy.<sup>5,13</sup> This type of investigation may have a place where the patient declines a surgical biopsy. Whilst the accuracy of cytological analysis has improved with the advent of computer-assisted analysis,<sup>5</sup> diagnosis based on examination of cytological features alone is not recommended for definitive diagnosis of malignant lesions. In the instance of clinically suspicious lesions, because of the limited sensitivity and specificity of cytological examination,<sup>5</sup> surgical biopsy techniques should be considered.

### **Problems with biopsies**

Despite the importance of submission of tissue for histological examination as an important investigative technique, there may occasionally be difficulties in the interpretation of the histology or there may be a lack of correlation between clinical signs and symptoms and the histological features observed. As previously mentioned, this may occur if unrepresentative tissue samples are submitted for histological examination.

However, there may be other factors that may affect the histological features of the submitted tissue.

### **Surgical considerations**

Surgical technique is very important. For example, the crushing of tissue with tissue forceps during the procedure or rough handling of the tissue can destroy the histological features, rendering accurate microscopic assessment useless (Fig 4).<sup>14</sup> In one study it was reported that “crush artefact” was more frequently encountered in specimens submitted by general dental practitioners.<sup>14</sup> Although surgical technique may be a factor in this, the authors also suggested that inflammatory lesions were more likely to be submitted by general dentists and such lesions were more prone to this sort of damage.<sup>14</sup> Other confounding factors may be introduced by previous biopsy or surgery in the area of the lesion(s) and, in that situation, consequent healing and inflammation may mask diagnostic histological features.

Other surgical factors that can introduce artefacts or tissue distortion include removal of tissue by laser or electrosurgery.<sup>15</sup> These techniques can induce thermal artefacts including carbonization, nuclear elongation and vacuolar degeneration of tissue that, in some instances, may affect the ability of the pathologist to accurately assess the tissue, particularly in small specimens (Fig 4B). Hence it is best to excise with a scalpel and use electrosurgery to control haemorrhage at the biopsy site.

Incorrect handling of tissue following its removal can introduce artefacts or render tissue non-diagnostic. For example, fixation of the specimen is important. For routine histological examination, 10% formalin is the fixative of choice. However, if other diagnostic techniques are required other specific fixatives may be required. Formalin and other fixative agents are usually supplied by the pathology laboratory. Incorrect fixation of tissue can introduce artefactual changes, thereby hampering the pathologist’s ability to accurately assess the tissue.

### **Type of lesion**

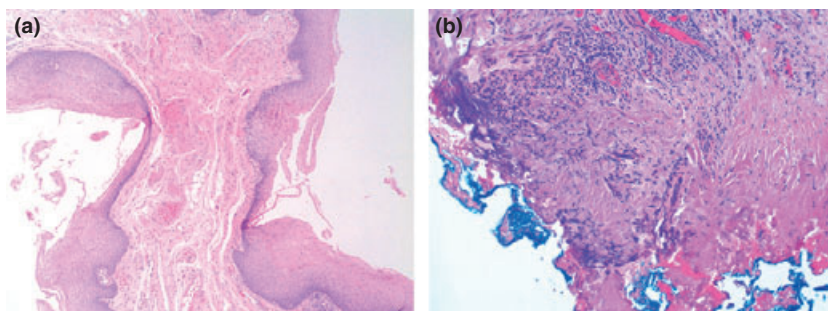
The type of lesion can also be a factor in how the biopsy specimen is treated; as mentioned previously, certain diagnostic tests require specific fixatives. Immunofluorescent techniques increase the accuracy of diagnosis for benign dermatologic conditions of the oral mucosa (e.g., lichen planus, pemphigus etc.).<sup>16</sup> After removal the specimen is bisected; one piece is placed in transport medium for immunofluorescence and the other is placed in formalin for routine histological examination. Treating the specimens in this order reduces the risk of the “fresh” specimen becoming contaminated by formalin, thereby making immunofluorescence studies difficult.

### **Medico-legal responsibilities**

Dentists have a clear professional obligation to diagnose and manage oral mucosal pathology or to appropriately refer. Once a biopsy has been taken there is a clear responsibility to note the result, inform the patient and take appropriate management steps. Failure to act, particularly with biopsies involving malignancy, has resulted in legal actions against the health professional.<sup>17</sup>

### **CONCLUSIONS**

Within the literature it is suggested by many authors that general dental practitioners should have adequate training to undertake simple biopsy procedures of apparently clinically benign lesions.<sup>18</sup> The fact that this does not necessarily occur may be due to many factors, most likely due to the relative infrequency of the clinical presentation of pathology in general dental practice. However, dentists should be cognizant of the occurrence of pathology in their patients and even if not undertaking investigative techniques themselves, they should understand the principles of investigative techniques relating to oral pathology and have strategies in place so that diagnoses can be made in a timely manner.



**Fig 4.** (A) Crush artefact caused by squashing the tissue with tissue forceps during removal. This has caused alteration of the gross structure of the tissue and altered the architecture of the epithelium. (B) Destruction of normal histological features at the periphery of a specimen caused by the use of electrosurgery. In small lesions the entire histological features may be obliterated, in larger lesions it may make examination of the surgical margins difficult as in this case.

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