

# Microendoscopy: a clinical reality in the intraoperative margin analysis of head & neck lesions

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## Abstract

### Introduction

The tumour margin is an important surgical concept that significantly affects morbidity and mortality. We describe the clinical application of intraoperative microendoscopy in defining the surgical margins during head and neck surgery.

### Method

A clinical margin is first marked by the operator followed by its microendoscopic assessment. Biopsies are taken from areas suggestive of close or positive margins on microendoscopy. The histological margins are further analysed formally, and the resection is revised accordingly, if necessary.

### Discussion

The advantage of this technique is the possibility of sampling a large area of mucosa whose histomorphological changes can be visualised in real time, thereby allowing the operator to make important informed decisions regarding the resection margins at the time of surgery. Further, shrinkage as well as thermal and orientation artefacts can also be avoided.

### Conclusion

We used a microendoscope to aid intraoperative decision-making during surgery.

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## Background

The mucosal surface of the upper aerodigestive tract is bathed in a 'milieu' of toxins which can give rise to disease when the host repair processes are overcome. These disease processes may be discrete or multifocal and can occur anywhere within the aerodigestive mucosal blanket. The usual epithelial response to chronic injury is metaplasia and hyperplasia, which can manifest as keratosis and leukoplakia. Further disruption of this already unstable mucosa can entrench carcinogenic changes leading to the development of squamous cell carcinoma. This may be multifocal and difficult to differentiate from the surrounding unstable mucosa by simple observation. Several areas of mucosa can co-exist along this pathway to malignancy; hence, any examination of the mucosa must be detailed and comprehensive in order not to miss subtle lesions<sup>1-3</sup>.

The diagnosis of dysplastic lesions cannot solely be based on clinical findings. Therefore, histological evaluation of a representative specimen is necessary. Dysplasia and carcinoma *in situ* may herald invasive oral cancer<sup>1,2</sup>; however, carcinomas can occur in areas with no previous signs of dysplasia. This may be because of the rapid emergence of invasive cancer, or it may be that earlier biopsies were taken from unrepresentative sites of the lesion or before morphological changes could be detected. Furthermore, the grading of dysplasia suffers from interobserver variability<sup>3</sup>.

There is no reliable method applicable to the upper aerodigestive

tract that can replace biopsy for a more definitive diagnosis of malignancy, but some methods may be used as supplements. Exfoliative cytology carries the risk of false-positive or false-negative results; a biopsy is still necessary for final diagnosis. Vital dyes have been used to identify a suitable site for biopsy, but literature has shown that false-positive staining may be as high as 30%<sup>4</sup>. This is mainly caused by enhanced staining of the hyperplastic edges of ulceration and filiform papillae of the tongue.

Histological assessment of a tissue sample is regarded as the most reliable criteria for correct diagnosis; accordingly, the specimen must be taken from the most representative area. In cases involving the uterine cervix (which can also undergo squamous metaplastic changes), microcolposcopy and colposcopy are used to examine the mucosa. These procedures consist mainly of assessment of the vascular pattern, intercapillary distance, surface contour, colour, tone, and clarity of demarcation. However, the accuracy of colposcopy for the detection of mucosal change is between 70–98%. Furthermore, by using colposcopic techniques, direct oral microscopy of pre-stained mucosal lesions (with magnifications of up to  $\times 8$ ,  $\times 12$  and  $\times 20$ ) has been shown to offer an advantage in selecting more representative sites for biopsy than routine clinical examination alone<sup>5-7</sup>.

We believe that this can be achieved using the microendoscope. Microendoscopy was first popularised by Hamou in 1979 as a technique to study the uterine epithelium. The scope was later



### Background (Cont.)

modified for use in the upper aerodigestive tract by Andrea et al<sup>8</sup>. A microendoscope is used to obtain an accurate microscopic assessment during clinical examination to facilitate an informed surgical decision with respect to the nature of the cellular characteristics of the mucosal lesion and its edge, thereby aiding biopsy yield and excision margin<sup>7</sup>.

Microendoscopy allows *in vivo* and *in situ* examination of the epithelium. The scope allows monitoring of the whole mucosal surface, both normal and pathological, and enables the detection of patterns specific for pathology, e.g. inflammation, metaplasia, dysplasia and neoplasia. The advantages of the scope are that once the microendoscope is acquired at a relatively low initial cost, it can be used like any autoclavable Hopkins rod, and maintenance costs are similarly low. The scope can be used to guide further surgery, biopsy or simple surveillance of large areas of suspect mucosa. The microendoscope enables on-table analysis to identify choices of further surgery even when the biopsy specimen (frozen section) is not available.

We present the head and neck surgical oncologist with a workable protocol for using the microendoscope in the assessment of the margins of excised lesions.

### Method

The operating Storz Hopkins II autoclavable microendoscope (Figure 1) was attached via an adapter to a camera system (3 chip Olympus/Sony) linked via a video recorder with outputs to a monitor and photo printer. Video encoding was done primarily using a Xenon light source (Karl Storz 300 attached via a Storz fibre optic cable), a 3 CCD camera (Karl Storz Tricam) and a Sony DV Cam system (DSR-20P), thus enabling a high-resolution playback.



**Figure 1:** Storz Hopkins II autoclavable microendoscope

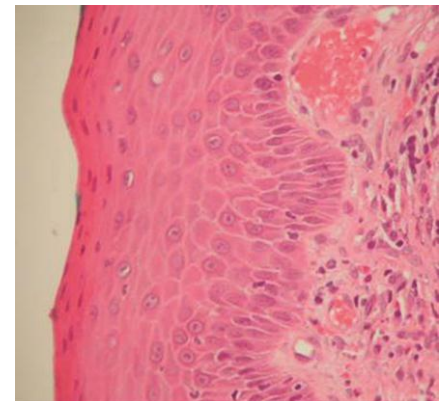
Static documentation was performed in the form of simple (Sony printer/dpi) as well as dynamic photography (in the form of a video clip stored on a DV magnetic tape, which required editing). High quality photographic images could be extracted from the edited video. The scope is available in different sizes, which can be used for different anatomical sites. The dimensions of the microendoscope were 5.5 mm in diameter and 23 cm in length, and the vital stain used was methylene blue.

The microendoscope scope had a fitted rotating screw which allowed magnification to be changed from  $\times 0$  to  $\times 60$  to  $\times 150$ , and minor movements permit focusing and de-focusing at specific depths of field. Having prepared the patient, the clinician would mark out the proposed margin for excision (Figure 2). The generalised area would be surveyed at  $\times 0$  magnification until an area of abnormality is found. This

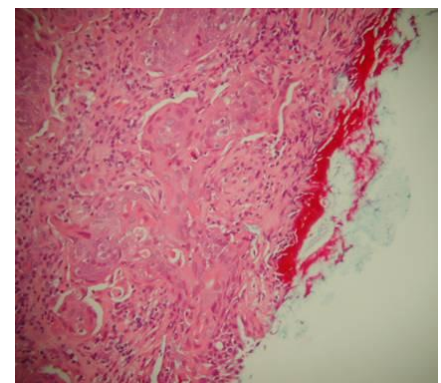


**Figure 2:** Clinical margin of an oral lesion

area would then be examined at  $\times 60$  and then at  $\times 150$ . The examination would always proceed from an area of normality (Figure 3) to abnormality (Figure 4); the entire surface of the lesion would be reviewed to determine any heterogeneity. Subsequently, the microendoscopic margin of the lesion would be delineated, and any areas of discrepancy between the clinical and microendoscopic margins would be photo-documented. The clinician would then excise the lesion, which would be sent for frozen-section analysis as would be the noted areas of discrepancy between the clinical and microendoscopic margin. A later analysis is performed between the histological photomicrograph and microendoscopic image. When a frozen section is not available, a



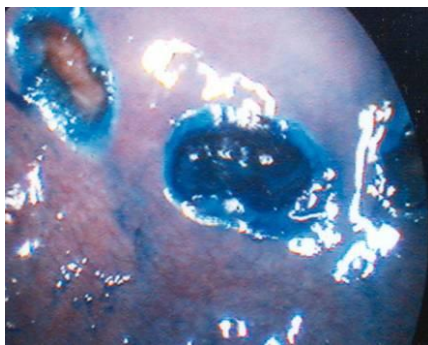
**Figure 3:** Normal mucosal histology of the previous lower margin ( $\times 200$ )



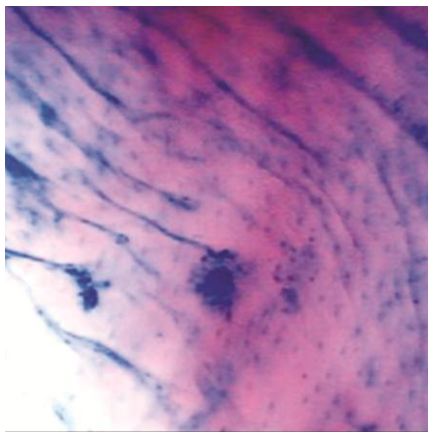
**Figure 4:** Abnormal histological features in the right mucosal margin ( $\times 150$ )

**Method (Cont.)**

microendoscopic margin is taken, and the biopsy sent for formal histological analysis. After suction clearance, the tip of the microendoscope is firmly applied to the prestained (0.1% methylene blue) area of interest to obtain an occlusive contact and then moved for dynamic assessment of the lesion (Figure 5), its margins, the local tissue and the underlying mucosal vasculature and blood flow. The examination is found to be reproducible between operators.



**Figure 5:** Stained margin



**Figure 6:** Normal mucosal microendoscopic image of previous lower margin, corresponding to histology ( $\times 150$ )

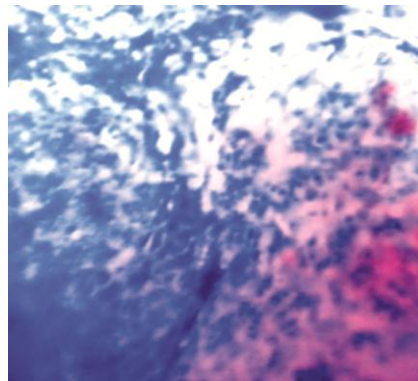
Staining is repeated if further assessment is required (Figures 6 and 7).

The examination can be performed at operation *in situ* and repeated on the excised *ex vivo* specimen (with the clinical margin) to assess involvement of the macroscopically

Table 1	Diagnostic criteria assessed to determine abnormality
Cellular level	Cell- observed in the longitudinal tissue plane rather than in transverse/depth
	Cellular morphology, expected histology
	Nuclear staining pattern- orientation, size, shape and limits
	Nucleolar staining morphological pattern, orientation
Tissue Level	Cell-cell regularity
	Extracellular matrix- homogeneous, heterogeneous
	Margin- discrete, blurred
	Underlying cells, microvessel density

clear tissue. A large area of mucosa can be quickly assessed and accurately sampled.

Adverse microendoscopic predictors of abnormality (Table 1) would include the presence of a large nuclear to cytoplasmic ratio in cells, together with evident bizarre-shaped nuclear staining, the presence of numerous mitotic figures per field, punctate staining of cells and the heaping up of bizarre sheets of stained cells suggesting rapid cell turnover. There may even be pseudo-tissue borders in unexpected sites, e.g. not at traditional squamocolumnar junction sites. Using these criteria, a high sensitivity and specificity in



**Figure 7:** Abnormal microendoscopic features in right mucosal margin, corresponding to histology ( $150\times$ )

determining abnormal mucosa compared with formal histopathological examination may be obtained.

**Discussion**

Microendoscopic assessment of mucosal lesions can facilitate an

informed surgical decision with respect to the nature of the cellular characteristics of a lesion and its edge, aiding biopsy yield and the adequacy of the laser excision margin.

Forty patients undergoing resection of oral squamous cell carcinoma were recruited by Upile et al. The surgical margin was first marked by the operator, followed by microendoscopic assessment. Biopsies were taken from areas suggestive of close or positive margins after microendoscopic examination. These histological samples were later scrutinized formally and the resection margins revisited accordingly when necessary. Using the microendoscope, Upile et al. reported their experience in the determination of surgical margins at operation and later comparison with frozen section and paraffin section margins 'gold standard'. They obtained a sensitivity of 95% and specificity of 90%. Interobserver kappa scores comparing the microendoscope with formal histological analysis of normal and abnormal mucosa were 0.85<sup>9</sup>.

The same group used the microendoscopic technique for microvascular monitoring of the free autologous jejunal flap by the direct visualisation of the flow of erythrocytes through the capillary vasculature on both mucosal and serosal surfaces. Blood flow was seen to be pulsatile, with individual erythrocytes visible in the capillaries. The best view was obtained when the scope was focused directly on the capillary

### Discussion (Cont.)

rather than the graft surface. The view of the unstained mucosal surface was bland apart from the fine capillary loops which were seen to fill with each pulsatile event. The microendoscopic examination of the serosal surface revealed much larger calibre vessels with obvious blood flow<sup>10</sup>.

A fundamental knowledge of regional histology and pathology is obviously assumed on part of the clinician. We believe that there must be a high degree of cooperation between the surgeon and histopathologist. However, initially, correlation of microendoscopic stills or video footage with formal histology slides must be performed until confidence is gained. This correlation exercise should continue until a measure of proficiency is gained in topographical histopathologic interpretation. Local specificity and sensitivity in detecting areas of abnormality should be ascertained to develop skills of having a raised index of suspicion of when to biopsy. The potential pitfalls of the technique are that it is a new skill and the equipment is still relatively expensive (although costs could be offset against the saving of potentially residual disease and failed grafts). Costs can be reduced by the local manufacture of the various adaptors necessary to connect the microendoscope to existing camera systems (Laparoscopic, Cystoscopic and Otolaryngological).

The disadvantages of this technique are that the microendoscope does not provide three-dimensional information to provide depth of invasion; however, this may be ascertained by a series of 'MOHR'S'-like 2-dimensional analyses using the microendoscope. A degree of depth field information may be obtained by varying the focus to allow visualisation of underlying structures such as cells or blood vessels. Despite this, cellular details can be determined up to tens of cell layers deep depending on

illumination. Intra-wound interpretation using the microendoscope for assessing deep margins is difficult and requires a thorough understanding of the topographical histopathological appearance of the area particularly when observing the oblique cuts made with excision. It also requires surgeons to have intimate knowledge of the histology of the area on which they are operating.

The technique of microendoscopy will no doubt be improved by advances in optical systems, illumination, recording and image processing. The microendoscope has a range of exciting applications in Oral Medicine, Otolaryngology, Oral and Maxillofacial Surgery and Plastic Surgery; however, the main advance with the scope is that we have more informed choice of the state of the *in situ* epithelial margin taken when excising squamous cell carcinoma. We found that the microendoscopic examination of lesions had great utility in the excision of mucosal lesions. This combined with the recent advancement of vital or immunologically tagged antibody-targeted staining will advance the type of surgical margin we take from the standard clinically visible margin compared with the histopathological margin during surgery<sup>9,11</sup>.

### Conclusion

We have described the clinical application of this once-orphaned technology and provided a practical guide to its everyday use by the surgeon in both clinical and surgical fields. The advantage of this technique is that a large area of mucosa can be sampled, whose histomorphological changes can be visualised in real time, allowing the operator to make important informed decisions with respect to the intraoperative resection margin during the operation. Shrinkage as well as thermal and orientation artefacts are also avoided. For the technique to be successful, the surgeon, pathologist and cytopathologist will need to be

familiar with the subtleties of microendoscopy, and this will require close collaboration among specialties.

### Consent

Written informed consent was obtained from the patient for publication of this report and any accompanying images.

Institutional ethical approval was obtained.

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