Mohs micrographic surgery: a technique for total margin assessment in veterinary cutaneous oncologic surgery

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Abstract
Mohs micrographic surgery (MMS) is the gold standard for the excision of locally invasive cutaneous malignancies in human dermatological surgery. Using a unique horizontal sectioning technique, MMS enables 100% surgical margin assessment and provides the lowest recurrence rates for locally invasive tumours. The purposes of this preliminary study were to explore the feasibility of application of MMS in the veterinary setting and to establish practical advantages and limitations of its use in a pilot programme. It was hypothesized that MMS technique could provide 100% tumour margin assessment using frozen and/or formalin-fixed horizontal histopathologic sections. Tumour excision and colour-coded mapping were performed, and specimen tissue was fixed using either frozen sections or formalin-fixed sections. Horizontal sections were assessed for quality and presence and location of neoplastic cells based on the mapped orientation. The MMS technique was used in the excision of six squamous cell carcinomas and five mast cell tumours. In all cases, the MMS permitted 100% tumour margins examination.

Introduction
Mohs micrographic surgery (MMS) is considered the gold standard for the removal of locally invasive, high-risk cutaneous malignancies in human dermatological surgery. Conceived and developed by Frederick E. Mohs, MMS uses a unique horizontal sectioning technique, which enables 100% surgical margin assessment and provides the lowest recurrence rates for locally invasive cutaneous tumours. Colour-coded mapping of excised specimens and microscopic examination of the entire margin permit identification of subclinical tumour extensions for subsequent removal. In human beings, the lesion is excised using local anaesthesia in an outpatient setting. Excised tissue is colour coded and frozen sectioned, with intraoperative histopathology evaluated for completeness of excision. If necessary, further resection is immediately performed and the process repeated until no more tumour cells are identified. MMS is commonly used for the excision of squamous cell carcinomas (SCCs) and certain subtypes of basal cell carcinoma in the human patient. Numerous prospective multicentre case series have demonstrated 5-year and 10-year cure rates ranging from 93 to 99%. Other treatment modalities, including traditional surgical excision with vertical sectioning, fare poorly by comparison. Methods that do not
permit histopathologic margin control can provide excellent results in certain cases (i.e. in situ lesions) but lack the ability to define the boundaries of excised infiltrative tumours.

Excision with traditional histopathologic margin assessment is performed by ‘breadloafing’ the tissue and examining representative vertical sections taken at intervals through the specimen (Fig. 1A). This specimen survey provides assessment of less than 1% of the surgical margin\(^1,10\). Locally invasive tumours like SCCs may have significant subclinical extensions, with the grossly visible tumour being only the ‘tip of the iceberg’ in many cases. Invasion may be irregular, and with only a survey of the margin, extensions can easily escape detection. MMS has the ability to precisely map such extensions for subsequent removal.

One of the indications for the use of MMS for histologically aggressive, non-melanoma skin cancer in human is when tumours excised by traditional methods have recurred. Tumours grow along planes of least resistance, and postoperative fibrosis and scar tissue can provide pathways for tumour extension. Surgical borders that were undermined in previous surgery can provide lateral planes for advancement.

Another indication for the use of MMS is in tumour location. Certain anatomic locations are associated with greater subclinical tumour penetration. Embryonic fusion planes in the ‘H-zone’ of the face (preauricular area, lower eyelids, nose, nasolabial fold, philtrum and temple) provide minimal resistance to subclinical spread\(^11\).

Tumours with perineural invasion are also indications for the use of MMS\(^12\). SCCs are notorious for this, with 64% of SCCs greater than or equal to 2.5 cm in diameter and 11% of those less than 2.5 cm demonstrating such invasion\(^13\). Other indications for the use of MMS include large or grossly poorly defined tumours, tumours arising in scarred or irradiated skin or tumours in areas where tissue conservation is required.

Limitations of the use of MMS in the human patient include the following: tumours exhibiting satellitosis or early metastasis, tumours too extensive to be amenable to surgical resection, expertise and training of the surgeon and support personnel and the lengthy and sometimes tedious nature of the procedure if numerous intraoperative sections are required\(^14\). Mohs surgeons are boarded dermatologists, who are also board certified in MMS after completion of 1-year and 2-year fellow-

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**Figure 1.** (A) Traditional vertical sectioning – breadloafing can easily miss subclinical extensions of invasive tumours. (B) In MMS, the tissue is divided and cut edges are inked. The peripheral margins are teased into the same plane as the deep margin in order to achieve horizontal sections, incorporating 100% surgical margin assessment. (C) Additional tissue resected from the surgical site can be sectioned in the same horizontal fashion.
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The purposes of this study were to examine the feasibility of application of MMS horizontal sectioning technique in the veterinary surgical setting and to establish the practical advantages and limitations of its use in a clinical pilot programme. It was hypothesized that MMS horizontal sectioning technique could provide 100% margin assessment using either frozen or formalin-fixed horizontal histopathologic sections.

**Materials and methods**

After 1 year of background work, which included training with board-certified Mohs surgeons and procedural trials with lesional and non-lesional cadaver and viable tissues, MMS was used in the excision of six SCCs and five mast cell tumours (MCT).

**Inclusion criteria**

Clinical cases were considered for inclusion if they were admitted for resection of either cutaneous SCC or MCT diagnosed by biopsy or fine-needle aspirate of the tumour. All patients, except one cat and horse, were assessed for metastases by thoracic radiography and regional lymph node aspirate and/or biopsy. All tumours were photographed and measured prior to surgery. Preoperative assessments included a complete blood count, serum chemistry analysis and an electrocardiogram on all animals, except one of the cats and the horse.

**Surgical technique**

Patients were anaesthetized and prepared aseptically for surgery. The planned excision was delineated without altering the usual wide margins currently accepted in veterinary surgery for the excision of both SCC and MCT (3-cm lateral margins and a fascial plane beneath the deep margin). Exceptions to the 3-cm margin minimum occurred in three cases where the need for tissue conservation was at a premium due to tumour location. The excision was made using the scalpel at a 45° angle to the skin surface. The excision was continued around the entire tumour surface, bevelling the incision edge. The tissue was removed in a shape of a bowl.

Peculiar to MMS, this angled excisional method enables the histotechnician to flatten the tissue so that the entirety of the deep and peripheral margins of the tumour may be sectioned in a horizontal plane (Fig. 1B). A two-dimensional map of the wound (Mohs map) was drawn for reference (Fig. 2). In three cases, based on gross assessment by the surgeon, additional tissues were resected from the surgical site using the same bevelled technique (Fig. 1C). In the MMS in human, additional tissues would be taken based on intraoperative frozen section histopathology; but due to the requirement for general anaesthesia, this was not performed in the small animal cases due to the possibility of excessively prolonging the anaesthetic episode.

**Tissue handling – frozen technique**

After the excision was complete, if necessary, the tissue was subdivided into sections small enough to fit on a glass slide. Smaller sections freeze more quickly than large specimens and are easier to manipulate into a horizontal plane. Maintaining the orientation of the tissues with respect to the Mohs map, sections were numbered and cut edges dyed with different colours (Figs. 1 and 2). Colour-coding on the map corresponded to the coloured dyes on each tissue edge. A cryostat was used to obtain horizontal frozen sections. The tissue was flattened so that the peripheral edges were teased or pressed into the same plane as the deeper margin and was partially frozen with freon and transferred to the cryostat for complete freezing. Each specimen was placed upside down on a cryostat chuck surrounded by drops of OCT (optimum cutting temperature) compound (Tissue Tek; Miles Inc., Diagnostics Division, Elkhart, IN, USA). Horizontal sections were cut with the cryotome 5–10 μm in thickness. Cryostat temperature was maintained within the range of −20 to −30 °C.

**Tissue handling – formalin-fixed technique**

After excision, the tissue was oriented with respect to the Mohs map by the placement of sutures. Tissue
was oriented to the site map and then fixed in formalin overnight. The tissue was subdivided into sections small enough to fit on slides, and cut edges were dyed with different colours to correlate with the map of the site. During paraffin embedding, the peripheral margins were pressed down so that they were in the same plane as the deepest margin. While it would have been possible to cut and dye the tissues prior to formalin fixation in the same manner as the frozen sections, it was decided not to do this to simulate the way in which a referring veterinarian might in the future be able to submit an excised tumour for horizontal sectioning.

Histopathologic assessment

The prepared sections were stained routinely with haematoxylin and eosin (H&E). In the case of one MCT, special staining was performed with toluidine blue. Each section was assessed for quality and presence and location of neoplastic cells based on the mapped orientation. Quality was scored as poor, moderate or excellent based on the degree of fragmentation and folding noted. Assessment was performed by the lead investigator and by a pathologist scanning with the ×20 and ×40 magnification and then further searching on middle to higher magnification (×200 to ×400). Microscopic findings and location of residual tumour cells were indicated and denoted on the Mohs map with red ink (Fig. 2).

Results

Signalment

SCC cases included three domestic shorthair cats, one miniature schnauzer and one quarter horse. Anatomic locations of the SCC included pinnae (cats, $n=3$), periocular area (cats, $n=1$), mandible (dog) and vulva (horse). One feline patient had bilateral pinnal SCC and the other had unilateral pinnal SCC. The periocular SCC on a

Figure 2. A Mohs map is a schematic two-dimensional representation of the surgical site, combining measurement and charting of the tumour excision site. The excised tissue dimensions are recorded and also sectioning and inking pattern are recorded. Sections with clean margins are typically denoted with red check marks, while a red dot denotes an area of residual tumour.
third feline patient was a recurrent lesion after a previous medial canthoplasty to remove a primary SCC. All the patients underwent a preoperative assessment for metastases, except for the cat with bilateral pinnal SCC, which was to be humanely euthanased (feline immunodeficiency virus [FIV]-positive stray), and the horse.

MCT cases included five dogs: an Australian terrier, a Rat terrier, a Jack Russell terrier, a Labrador retriever and an American bulldog. Anatomic locations of the MCT included caudal thigh (n=1), stifle (n=1), dorsal muzzle (n=1), medial hock (n=1) and ventral thorax (n=1). The thorax and the hock tumours had both been treated previously and recurred locally. The hock lesion was located at the border of a previous irradiation field. The thorax lesion arose within the scar of previously surgically excised tumour.

Surgical technique

Surgery was successfully performed using the described MMS technique. The exceptions were the pinnectomies, when there was no need to orient the blade in a 45° bevelled fashion in order to achieve the margins. Notable departure from standard MMS was in accommodating current veterinary protocol of 3-cm margins (or the widest margins available) where possible. The three cases where the 3-cm margins were not possible due to tumour location were the muzzle MCT and the periocular and mandibular SCCs. One-centimetre margins were taken for the periocular and mandibular SCCs and 2-cm margins taken for the muzzle and thigh MCT. The largest sample of resected tissue that was cut and sectioned was from the caudal thigh MCT (7×4 cm). The surgeons chose to perform additional resection of tissue in three cases where it was determined that more tissue was desired to meet the wide margin requirement.

Tissue handling and histopathology

Horizontal frozen sectioning of surgical margins was performed in five of the SCC cases. One SCC case had a combination of frozen and formalin-fixed sectioning (the bilateral pinnectomy). All the MCT cases used horizontal formalin-fixed sections.

The assessment of frozen sections was completed within 1 h of excision, while processing of formalin-fixed sections required 2 days from excision to assessment. The time required to assess the slides in all cases was less than 15 min. In all cases, MMS allowed 100% surgical margins assessment.

The quality of frozen sections ranged from poor to excellent, with the most common artefacts being fragmentation and folding (Fig. 3). These artefacts are most commonly encountered in larger individual sections with more fat and fascia. Longer freezing times (5–10 min) were used to help minimize such artefacts in these specimens. The only sections that were scored as poor were from the SCC pinnectomy, which used frozen sections. This was likely due to cryotome difficulty in sectioning the cartilage. The vulvar SCC section quality was scored as excellent and the others as moderate. A ‘muddy’ H&E colour differential was noted in some of the frozen sections and was ascribed to the frozen sections typically being thicker than the formalin-fixed sections.

Formalin-fixed sections were generally of better histologic quality, ranging from moderate (2) to excellent (3). The most common artefact resulting in quality scores of moderate for occasional individual sections was fragmentation of larger sections due to separation of deep fat and fascia from fatty areas with thick dermis. More problematic than histologic quality in the formalin-fixed cases was accounting for changes in sample dimensions during fixation and processing and difficulty in pressing the peripheral margins into the same plane as the deep margin during embedding. Despite
Neoplastic cells were noted at the surgical margin in the equine vulvar SCC (Fig. 4) and three of the MCT cases (Fig. 5). These subclinical extensions were mapped and the information was used to direct further adjunctive treatments by oncologists. Radiation was chosen for two of the MCT cases, chemotherapy with lomustine and prednisone for one of the MCT cases and intralesional cisplatin in sesame seed oil emulsion was chosen for the equine vulvar SCC. Additional aggressive surgical resections were not options in these cases due to the lack of available tissue after the initial surgeries. This can occur in MMS in human of large and/or aggressive tumours, and the use of a multidisciplinary approach using adjunctive therapies, most commonly radiation, is well documented. All other cases were assessed as having margins free of neoplastic cells. In the case of the mandibular SCC, the tumour was positioned directly over the mental nerve foramen, and horizontal sections enabled visualization and assessment of the nerve, which was free of perineural invasion (Fig. 6), making a hemimandibulectomy unnecessary. Perineural invasion, a well-documented phenomenon in human SCC cases, has been infrequently discussed in the veterinary patient but has been noted in both naturally occurring clinical cases and experimental models.

Case follow-up

Though the objective of this study as a pilot programme was purely descriptive and did not include a prospective study of recurrence data, case follow-up was considered valuable for future work. Case follow-up was obtained for all the cases, except the FIV-positive feline with SCC, which was humanely euthanased postoperatively. Follow-up ranged from 5 to 19 months without recurrence for all the MCT at the time of submission of this article. The equine vulvar SCC was lost to follow-up after 7 months without recurrence. The periocular feline SCC was followed without local recurrence for 1 year until the patient was reported deceased by the client. Unfortunately, no necropsy had been performed to confirm cause of death. The canine mandibular SCC and the unilateral feline pinnal SCC experienced no recurrence at 19 and 6 months, respectively.

Figure 4. Residual SCC (A, broadly indicated by the arrow) is evident in this horizontal section from an SCC resection from a mare vulva (magnification of ×20, scale bar is 500 μm, H&E staining). (B) magnification of ×200).

Figure 5. Small clusters of neoplastic mast cells (arrow) are noted in the specimen near an inked edge (magnification of ×200, scale bar is 100 μm, H&E staining).
Discussion

MMS technique was successfully used in the veterinary setting. The primary advantage of its use is 100% margin assessment for tumour excision. The limitations of frozen sections included variable section quality and cryostat operator inexperience, while the advantage was the speed of results. However, until greater proficiency and speed can be achieved using the frozen technique, the use of general anaesthesia for most small animal veterinary surgical resections will limit the value of intraoperative frozen sections. Its use in the future in the large animal patient may be more readily achieved as many of these surgeries are performed as standing procedures under sedation.

Another possible way to use intraoperative frozen sectioning would be to wake the small animal patient from anaesthesia and bandage the surgical site pending the results of the histopathology. An additional anaesthetic episode would be required for either closure or additional tissue resection. The ability to bandage the surgical site would be highly dependent on the extent of the defect and its anatomic location. Given that frozen sections were available within an hour in this study, there may be some cases in which a prolonged anaesthetic episode would be desired, provided there is consent of the client and anaesthesiologist.

Using formalin-fixed sections was more practical due to better experience and training, but it yielded results much later and made tissue orientation and preparation (flattening of edges) markedly more difficult. The physical changes that excised tissue undergoes in the process of formalin fixation and processing also make its use inherently less accurate than frozen sectioning. The dimensions of tumours calculated from formalin-fixed specimens must be adjusted to take into account a 30% postfixation shrinkage in order to compare them with in vivo clinical measurements. The use of formalin-fixed horizontal sections in this study represented a departure from typical MMS technique using frozen sections; however, formalin-fixed technique has been described previously in the human literature (‘Slow Mohs’).

The authors recognized that the key difference between the human and the veterinary Mohs case is the ability of the human Mohs surgeon to perform the procedure using local anaesthesia in an outpatient setting. This enables removal of residual tumour in successively staged serial horizontal frozen sections after intraoperative examination of the histopathology. The requirement for general anaesthesia in the small animal veterinary patient would tend to preclude this advantage of MMS at the current time but does not negate a key aspect, the complete margin assessment provided to the surgeon.

Tumours of the skin and subcutaneous tissue are the most common neoplasms in the canine and equine species and the second most common in the feline species. SCC in animals represents perhaps the closest analogue to the human malignancy. As in humans, there is a recognized actinic (solar) relationship with older patients, with unpigmented and hypopigmented skin being most at risk. The tumours are locally aggressive but slow to metastasize. The most common locations, sparsely haired areas of the pinnae, nares, lips and eyelids, and the high risk of recurrence in these areas are remarkably analogous to the human tumour pathology. Numerous treatment modalities have been used in treatment of the veterinary patient including traditional surgical excision with vertical sectioning, cryotherapy, photodynamic therapy, radiation and various forms of chemotherapy. The best cure rates have been associated with surgical excision; yet, the literature demonstrates that...
veterinary recurrence rates compare poorly to those provided by human MMS: nasal planum in felines, 30%; pinnae in felines, 23%; periocular in equines, 30–40%28–33. These are dramatically worse than the 1.0–6.7% recurrence rates for primary SCC reported in human MMS prospective case series29,39,54. Given the ready analogy between the animal and the human tumour, it is the hypothesis of the authors that 100% margin assessment would be valuable in treatment of the animal SCC and long-term prospective case series using MMS should be performed.

The ultimate value of MMS in the resection of MCT is more controversial. MCT are one of the most common neoplasms in veterinary cutaneous oncology; yet, the biological behaviour of these tumours remains poorly understood. The association between prognosis and histopathologic margin evaluation has been called into question35. Although surgery remains the treatment of choice for cutaneous MCT in areas amenable to wide excision, the presumed potential for satellitosis or ‘skip metastases’ (i.e. non-contiguous growth) demands that the veterinary surgeon consider that every MCT could be incompletely resected36. The extent of surgical margins necessary to obtain tumour-free margins as well as numerous other prognostic factors have been subjects of much ongoing research and debate37–41. It is noteworthy that none of the extant research has used horizontal sectioning in assessment of the margins. Without 100% margin assessment, serial vertical sectioning at variously proposed intervals has been used to determine the completeness of margins. It is the authors’ assertion that neither an improved understanding of the value of complete margins nor a clear picture of the behaviour of MCT may be achieved without the benefit of total margin assessment provided by MMS.

In conclusion, this study represents the first pilot programme using MMS technique in the veterinary field. The hypothesis that 100% margin assessment could be achieved was confirmed. It has been asserted in the veterinary literature and commonly accepted that the pathologist cannot examine the entire surgical margin42. This is false. The technique has been exquisitely described in detail in the human dermatological literature for over half a century, and its value as the standard of care firmly established. It is the authors’ conclusion that long-term prospective studies using this technique are indicated to determine if recurrence rates of selected cutaneous malignancies in veterinary patients can be improved.

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**References**


