Intraoperative Microwave Processing of Bone Margins During Resection of Head and Neck Cancer

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Objective: To develop an accurate method for performing histopathologic analysis for a full cross-section of cortical bone within 2 to 3 hours.

Methods: Microwave technology was used to augment and to more rapidly perform fixation and decalcification of cortical bone.

Results: Using the methods described, slides suitable for histopathologic analyses regarding the presence or absence of malignant tumor were prepared in 2 to 3 hours and, in 10 patients studied, had a 100% correlation with slides prepared in 7 days using the standard decalcification technique.

Conclusion: Microwave technology allows accurate assessment of a full cross-section of cortical bone regarding the presence or absence of malignant tumor within the time limits required for resection of a malignant head and neck tumor and reconstruction of the surgical defect.


The ability to assess bone involvement by type of tumor has become increasingly important in today's field of head and neck reconstructive surgery. When ablation of a tumor requires composite resection of a bone such as the mandible that has a relatively thick cortex, standard methods of cross-sectional histopathologic analysis include a lengthy period for decalcification that can require 7 to 10 days. Forrest et al1 have reported intraoperative analysis of cancellous bone curetted from the mandibular marrow space. However, errors can occur from omitting a full cross-sectional analysis of the bone margin.

RESULTS

The microwave-facilitated “quick” bone margins were deemed positive for tumor in one instance and suggestive of tumor in another. They were negative for tumor in 9 additional resections. In the case in which a margin was suggestive of tumor, an additional segment of bone was resected and the second margin was negative for tumor. In other words, the margin suggestive of tumor was treated as a positive result. There was a 100% correlation of the quick bone margins prepared by microwave processing with those prepared using traditional decalcification techniques and permanent sections. In all instances, the pathologist judged that sufficient cytologic detail had been preserved to allow for adequate analysis (Figure). This included the ability to appreciate malignant neoplasm invading large neural structures. Extension along the course of the inferior alveolar nerve can be an important avenue for the spread of malignant neoplasms in the oral cavity.

COMMENT

One of the cardinal principles regarding surgical resection of malignant neoplasms is the achievement of negative margins as an indication of adequate removal of tumor. Frozen-section analysis using cryofixation has been a reliable means of analyzing the margins of soft tissues. However, tissues that contain a significant amount of hard calcium matrix, such as cortical bone, cannot be sectioned by the cryotome and, therefore, cannot be evaluated using standard frozen-section techniques.

Malignant neoplasms in the oral cavity can invade contiguous bony structures. When this occurs, the prognosis for cure is diminished and resistance to ra-
PATIENTS AND METHOD

A technique of processing a full cross-section of mandibular or maxillary bone was developed that allows an analysis of the histopathologic condition within 2 to 3 hours. Nine patients with biopsy-proven squamous cell carcinoma of the oral cavity and oropharynx and 1 patient with adenoid cystic carcinoma of the maxillary sinus underwent composite resection of the primary tumor and adjacent mandible or maxilla. An additional patient underwent 2 separate composite resections. Patient demographics along with the histologic features and primary sites of the neoplasm are given in the Table. In all cases full cross-sectional intraoperative bone margins were assessed using the process herein described that is facilitated via microwave technology.

The entire surgical specimen containing the bone to be analyzed was placed in 10% formalin and treated in a conventional microwave oven (model 8500 X5; Whirlpool “Time Master,” Benton Harbor, Mich) at a medium setting (corresponding to approximately 40°C) for 10 minutes. This initial fixation allowed thin (1- to 2-mm) full cross-sections of the bone margins to be cut with a 0.5-mm-thick, diamond-bladed band-saw (model 1180; Mar-med Inc, Cleveland, Ohio) without fragmentation. The sections were then microwaved in formalin at a medium setting for an additional 10 minutes. They were then microwaved in rapid bone decalcification solution containing a proprietary mixture of hydrochloric acid and formalin (Apex Engineering Products Corp, Plainfield, Ill) at a medium-low setting (approximately 37°C) for 20 minutes. The specimen was then evaluated for pliability (ie, the ability to distort or bend it by a laboratory technician’s digital pressure). The achievement of pliability was the end point. This step was repeated, using 10-minute periods of microwave irradiation until the end point was achieved. This usually required 4 to 7 repetitions regarding mandible margin sections. The sections were dried by microwaving them at a medium setting in absolute alcohol for 10 minutes and then cleared by microwaving them at a medium setting in isopropyl alcohol for 15 minutes. The sections were microwaved at a medium setting in paraffin for 3 cycles of 15 minutes each and then embedded, cut, stained with hematoxylin-eosin, and placed on a cover glass. The entire process, from receipt in the laboratory to interpretation by the pathologist required from 2½ to 3 hours.

Standard processing of a mandible for histopathologic analysis of cross-sectional margins requires decalcification in Curtis Matheson Scientific protocol solution B (0.135 g of sodium tartrate as a buffer in 1 L of 1% hydrochloric acid; Biochemical Sciences Inc, Swedesboro, NJ) for approximately 7 days.

The accuracy of this analysis, in terms of correctly identifying the presence or absence of tumor in the bone margins, was evaluated by comparison with the analysis of the histopathology obtained from the examination of the bone specimens prepared using standard techniques that required 7 days for decalcification.

Several authors have identified 2 distinct patterns of mandibular bone involvement by oral cavity squamous cell carcinoma. There is the erosive pattern with a sharp interface between tumor and bone and the infiltrative pattern where fingerlike projections of tumor invade, on an irregular front, with unpredictable lengths of mandibular involvement, especially regarding the marrow space. The infiltrative pattern is associated with a significantly lower prognosis for cure of cancer and with a significantly higher rate of positive margins, identified on permanent sections of the decalcified specimen. In patients with the infiltrative pattern, Wong et al noted positive margins for tumor in 47.6% of their patients. No intraoperative histologic monitoring of bone margins was performed.

Microwaves have a frequency between 300 MHz and 300 GHz. The conventional domestic microwave oven has a frequency of 2.45 GHz, corresponding to a wave length of 12.2 cm.

Microwaves are generated by a magnetron and propagate at the speed of light in a vacuum. They are reflected by most metals but pass through glass and deeply into tissue samples. They cause rapid rotation (approximately 2.45 billion cycles per second) of dipolar molecules and of any molecules having an asymmetric charge such as the polar side chains of many proteins. This increased intramolecular and intermolecular motion produces heat, enhancing chemical interaction, and facilitates diffusion of fluids in and out of tissues. Because the microwave energy is not strong enough to alter covalent bonds or to break hydrogen bonds, cellular architecture remains intact. Unlike more conventional heating, which begins at the surface and de-
pends on conduction to reach deeper levels, microwave heating occurs anywhere the microwaves have passed into the tissue and is more uniform throughout a greater volume of tissue. Microwaves effect deeper levels more quickly.7,8 Microwaves facilitate important processes needed to prepare tissues for analysis by the pathologist including fixation, dehydration, staining, and decalcification. Microwave technology has been previously used to speed up and to improve immunohistochemical reactions, special staining techniques, and decalcification of cadaveric temporal bones.9-13

Decalcification of bone is necessary to allow cutting thin sections from a tissue specimen using a microtome. This is accomplished by displacing calcium using an acid solution. The rate at which this occurs depends on several factors including the thickness of the bone involved. The thick cortical bone of the mandible requires about 7 days for decalcification using standard processing techniques. As we have shown, this can be reduced to about 2½ to 3 hours when facilitated with microwave technology.

We are evolving our protocol so as to provide an answer regarding the bone margin earlier in the procedure. For example, when the neck is N0 or nodal disease is not bulky, the primary tumor can be resected en bloc with the mandible and submandibular glands (ie, level I nodes). Preparation of the bone margins can begin at this point while the extirpative surgical team completes dissection of level II to V nodes, as indicated. Also, after initial fixation, we have begun carefully cutting the bone marrow as a separate bloc from the cortex and analyzing the marrow bloc first. Because less decalcification is required for the thin bone spicules of the marrow bloc, analysis can be completed in about 1 hour. The bone marrow is also the compartment most likely to be positive for tumor involvement. If the bone marrow is shown to be positive for tumor, decalcification of the cortical portion becomes irrelevant and additional margins can be resected from the patient immediately.

Other methods of evaluating for the presence of bone involvement and for the extent of bone that should be resected regarding tumors of the oral cavity have been used. Radiographic techniques that have been used include the following: standard pantographs of the mandible, computed tomography (CT), and magnetic resonance imaging.14 Of these, CT and magnetic resonance imaging scans have proved to be the most accurate.14,15 Computed tomographic scans can reliably detect erosion of cortical bone but can fail to show the extent of involvement of the bone marrow. Also, CT scans are compromised when dental fillings are present, resulting in significant metallic artifact. Magnetic resonance imaging is better for showing abnormal marrow changes.

Radionuclide bone scans are of some value in predicting bone involvement but are probably not as accurate as CT and magnetic resonance imaging scans.14 Clinical assessment by an experienced extirpative head and neck surgeon can be of great value and at some institutions has been remarkably accurate. Brown et al14 used direct inspection of the resected specimen after periodontal stripping to judge the adequacy of resection. Schusterman et al16 noted a 2% incidence of positive bone margins when clinical assessment alone was used. However, as noted earlier, others have experienced a significantly higher rate of abnormal bone margins with Wong et al17 noting abnormal bone margins in 47.6% of their patients with an infiltrative pattern.

Forrest et al1 showed that curettage of the bone marrow space had an overall accuracy of 97% in 33 patients. However, of the 3 patients with margins shown to be positive for tumor on final decalcified permanent section, only 2 (67%) of 3 were correctly identified on the basis of intraoperative curettage (ie, there was a false-negative rate of 33% for this subgroup).

### Patients With Oral Cavity Cancer Who Had Microwave Processed Bone Margins Performed Intraoperatively

<table>
<thead>
<tr>
<th>Sex/Age, y</th>
<th>Primary Cancer and Site*</th>
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<tbody>
<tr>
<td>M/74</td>
<td>SCCA retromolar trigone, SCCA floor of the mouth (separate operative procedure at age 70 y)</td>
</tr>
<tr>
<td>M/39</td>
<td>SCCA lip</td>
</tr>
<tr>
<td>M/51</td>
<td>SCCA base of the tongue</td>
</tr>
<tr>
<td>M/49</td>
<td>SCCA floor of the mouth</td>
</tr>
<tr>
<td>M/37</td>
<td>SCCA mandible</td>
</tr>
<tr>
<td>F/69</td>
<td>SCCA retromolar trigone</td>
</tr>
<tr>
<td>F/69</td>
<td>SCCA floor of the mouth</td>
</tr>
<tr>
<td>F/62</td>
<td>SCCA floor of the mouth</td>
</tr>
<tr>
<td>F/69</td>
<td>SCCA retromolar trigone</td>
</tr>
<tr>
<td>F/39</td>
<td>Adenoid cystic cancer maxillary sinus</td>
</tr>
</tbody>
</table>

*SCCA indicates squamous cell carcinoma.

A, Bone marrow space and bone without tumor (hematoxylin-eosin, original magnification ×150). B, Bone with squamous cell carcinoma in bone marrow space (hematoxylin-eosin, original magnification ×150).
We have used microwave technology to allow a quick analysis of a full cross-section of the bone margin. This technique can also provide information regarding involvement of neural structures and periosteum.

The microwave processing of bone margins requires some experience on the part of the laboratory technician preparing the specimen. Using temperatures that are too high during processing can result in the loss of cytologic detail. While we were able to achieve satisfactory results with a conventional domestic microwave oven, there are laboratory-grade microwave ovens available that offer potential advantages such as more accurate assessment of the temperature using temperature probes, and more accurate power cycles. Also, these ovens often provide a venting system that reduces the risk of exposure of laboratory personnel to potentially toxic fumes. The special bone saw used was equipped with a diamond blade that provided thin sections, facilitating processing.

CONCLUSIONS

Microwave technology can speed up and enhance processing and decalcification of full cross-sectional margins of bone. This allows accurate analysis within the time limits of a surgical extirpation of an oral cavity cancer along with microvascular reconstruction. This might improve the surgical eradication of tumor and preserve a microvascular reconstruction that could be jeopardized if a bone margin is found to be positive for tumor 1 week after the extirpative surgery.

Accepted for publication April 6, 2001.


REFERENCES


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