Cytology is a quick, easy, and inexpensive diagnostic tool. It is commonly used for the diagnosis of neoplastic processes. Cytology has several benefits:

- The risks of fine-needle aspiration are minimal
- Anesthesia or sedation is often not required
- Aspirated tissue does not require any processing—results can be obtained more quickly than with histopathology.

However, cytology is not without limitations: due to lack of tissue architecture and variability of cellularity, cytologic diagnoses can be misinterpreted or inconclusive. Cytology is often used in conjunction with biopsy or additional cytochemical or immunocytochemical stains to improve specificity of diagnosis.

**CYTOLOGIC CLASSIFICATION**

Many neoplasms present as discrete masses in the skin or subcutaneous tissue or within an organ. It is important to understand the cytology of healthy tissue cells in order to recognize a pathologic process, such as a tumor.

Classification is based on cellular morphology and tissue of origin, if recognizable. Cytologically, neoplasms are classified into 1 of 3 categories, with rare exceptions:

1. **Epithelial neoplasia**
2. **Mesenchymal neoplasia**
3. **Round cell neoplasia.**

Benign neoplasms should consist of a uniform population of cells; however, it is essential that specimens be thoroughly evaluated for criteria of malignancy, which include:

- Anisocytosis
- Anisokaryosis
- Prominent/multiple nucleoli
- Variation in the nucleus to cytoplasmic ratio (N:C)
- Multiple nuclei
- Abnormal mitotic figures.
Epithelial Neoplasia

Appearance
Epithelial cells commonly are cohesive; therefore, cytologically, epithelial tumors are arranged in tightly adhered clusters. The cells are often round with round nuclei, though some epithelial neoplasms, such as squamous cell carcinomas, can have atypical shapes.

N:C varies greatly depending on cell type and tissue of origin:
- Epithelial cells from healthy tissue (liver, pancreas, lung, etc) are uniform in size and shape with distinct cell borders.
- Neoplastic epithelial cells from glandular tissue may have cytoplasmic vacuoles or produce a cytoplasmic product, appearing as discrete granules or as a large globule that displaces the nucleus.
- Carcinomas will generally have marked criteria of malignancy and, in the absence of an inflammatory process, can be easy to distinguish from healthy tissue.

Types of Tumors
Some of the more common cutaneous epithelial tumors include:
- Sebaceous adenoma
- Trichoblastoma
- Squamous cell carcinoma.

Sebaceous adenomas are benign and fairly common in dogs but less so in cats. Surgical excision should be curative. See Table 1 for a list of cytologic characteristics.

Trichoblastomas (Figure 1, page 14) in dogs were previously known as benign basal cell tumors; they were reclassified as trichoblastomas because, histopathologically, the tumor shows differentiation of the hair germ of the developing follicle. They occur most commonly around the head and neck and occasionally metastasize. See Table 1 for a list of cytologic characteristics.

Table 1. Cytologic Characteristics of Epithelial Neoplasia

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Cytologic Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sebaceous Adenomas</td>
<td>- Clusters of uniform glandular epithelial cells</td>
</tr>
<tr>
<td></td>
<td>- Cytoplasm: Abundant and filled with discrete cytoplasmic vacuoles</td>
</tr>
<tr>
<td></td>
<td>- Nucleus: Often obscured by vacuoles</td>
</tr>
<tr>
<td></td>
<td>- Difficult to differentiate from sebaceous hyperplasia due to lack of tissue architecture</td>
</tr>
<tr>
<td>Basal Cell Tumors</td>
<td>- Small, round cells that occur in clusters or linear rows or ribbons</td>
</tr>
<tr>
<td></td>
<td>- Cytoplasm: Scant rim of pale basophilic cytoplasm</td>
</tr>
<tr>
<td></td>
<td>- Nucleus: Large and round, with clumped chromatin pattern</td>
</tr>
<tr>
<td></td>
<td>- Have high nucleus:cytoplasmic ratio</td>
</tr>
<tr>
<td>Trichoblastomas</td>
<td>- Cells are often larger than those of basal cell tumors</td>
</tr>
<tr>
<td></td>
<td>- Cytoplasm: Scant rim of basophilic cytoplasm</td>
</tr>
<tr>
<td></td>
<td>- Nuclei: Prominent, large and round</td>
</tr>
<tr>
<td>Squamous Cell Carcinomas</td>
<td>- Round to polygonal shaped cells</td>
</tr>
<tr>
<td></td>
<td>- Cytoplasm: Basophilic in varying amounts; often keratinized</td>
</tr>
<tr>
<td></td>
<td>- Nuclei: Round; vary greatly in size and chromatin pattern, from coarsely stippled to pyknotic; perinuclear vacuoles common</td>
</tr>
<tr>
<td></td>
<td>- Often marked variation in nucleus:cytoplasmic ratio because cells vary greatly in level of differentiation</td>
</tr>
</tbody>
</table>

WHAT ABOUT MELANOMA?

Some text books classify melanomas as round cell tumors. However, the cells can exhibit features similar to epithelial, mesenchymal, and round cell tumors, occasionally in the same tumor, which is why they can be difficult to characterize.
- Benign melanomas generally contain so much pigment within the cytoplasm that it is difficult to fully evaluate the cellular detail.
  Figure A. Benign melanoma from a cat: The cells contain so many granules that individual cellular features are difficult to discern. (Wright-Giemsa stain; 1000x)

- Malignant melanomas occur most commonly in the oral cavity or nail bed. They often have much less pigment than benign melanomas, making criteria of malignancy, such as a single large prominent nucleoli, easy to spot.
  Figure B. Malignant melanoma from a dog: The cells exhibit obvious criteria of malignancy, including large prominent nucleoli, and have minimal granulation in the cytoplasm. (Wright-Giemsa stain; 1000x)
Squamous cell carcinomas (Figure 2) are the most common head and neck tumor,¹ one of the most common epithelial tumors of the nose and paranasal sinus in cats,² and a fairly common oral and nasal tumor in dogs. Cytologic diagnosis of squamous cell carcinoma can be straightforward; see Table 1 for a list of cytologic characteristics. These tumors are often ulcerated and necrotic so secondary suppurative inflammation is common.

MESENCHYMAL NEOPLASIA

Appearance

Mesenchymal tumors originate from connective tissue. Connective tissue includes fibrous, adipose, bone, cartilage, and muscle connective tissue.

- Mesenchymal cells can vary in shape, from round to spindle, and have wispy cytoplasmic borders.
- The nuclei are often oval but occasionally round.

Healthy mesenchymal tissue does not exfoliate well but malignant mesenchymal tissue exfoliates readily; cellularity can assist with a diagnosis of neoplasia.

Types of Tumors

- Sarcomas, such as osteosarcoma and hemangiosarcoma, exhibit obvious criteria of malignancy.
- However, other mesenchymal tumors (classified as soft tissue sarcomas on histopathology) display minimal criteria for malignancy.
- This histopathologic subheading includes a number of tumors, including:
  » Perivascular wall tumors, such as myopericytomas and hemangiopericytomas

<table>
<thead>
<tr>
<th>Table 2. Cytologic Characteristics of Mesenchymal Neoplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Osteosarcomas</strong></td>
</tr>
<tr>
<td>• Majority consist of plasmacytoid cells</td>
</tr>
<tr>
<td>• <strong>Cytoplasm:</strong> Basophilic in varying amounts; often contains eosinophilic granules (common to find eosinophilic product within background; ie, osteoid)</td>
</tr>
<tr>
<td>• <strong>Nuclei:</strong> Large, prominent; often eccentrically placed in cell</td>
</tr>
<tr>
<td>• Often marked anisocytosis and anisokaryosis plus multinucleation</td>
</tr>
<tr>
<td>• Erythrophagia occasionally observed in neoplastic osteoblasts;¹ low numbers of osteoblasts observed in these specimens</td>
</tr>
<tr>
<td><strong>Hemangiosarcomas</strong></td>
</tr>
<tr>
<td>• Spindle-shaped, plump cells</td>
</tr>
<tr>
<td>• <strong>Nuclei:</strong> Large, prominent</td>
</tr>
<tr>
<td>• Moderate anisocytosis and anisokaryosis</td>
</tr>
<tr>
<td>• Erythrophagia occasionally observed</td>
</tr>
<tr>
<td><strong>Soft Tissue Sarcomas</strong></td>
</tr>
<tr>
<td>• Unusually shaped cells</td>
</tr>
<tr>
<td>• <strong>Cytoplasm:</strong> Very long, often bipolar, projections; can contain low numbers of discrete vacuoles</td>
</tr>
<tr>
<td>• <strong>Nuclei:</strong> Prominent and round, with a stippled chromatin pattern; multinucleated cells containing 5 to 6 nuclei are common</td>
</tr>
<tr>
<td>• Red blood cells often identified in background</td>
</tr>
</tbody>
</table>

¹ Barger A, Skowronski M, MacNeill A. Cytologic identification of erythrophagocytic neoplasms in dogs. Accepted for publication; Vet Clin Pathol.
Peripheral nerve sheath tumors, such as neurofibromas and schwannomas.

Osteosarcomas (Figure 3) can be difficult to differentiate from other primary bone tumors. A cytotoxical stain is available to help differentiate osteosarcoma from other mesenchymal tumors. One limitation of this stain is that normal or reactive osteoblasts will also stain positively; therefore, close examination of the aspirate for clear evidence of malignancy is recommended before this stain is performed. See Table 2 for a list of cytologic characteristics.

Hemangiosarcomas (Figure 4) can occur in multiple locations, including the spleen, liver, bone, skin, muscle, and heart. Thoracic and abdominal hemangiosarcomas are often accompanied by significant hemorrhage. Masses are often cavitated, making it difficult to obtain a diagnostic sample.

If possible, aspiration of a more solid portion of the mass is beneficial. See Table 2 for a list of cytologic characteristics. Histopathologically, hemangiosarcomas are occasionally described as epithelioid and have solid areas, which result in a more cellular aspirate.

Soft tissue sarcoma (Figure 5) aspirates are often cellular and consist of aggregates and individualized mesenchymal cells. See Table 2 for a list of cytologic characteristics. However, these sarcomas cannot be classified as malignant based on cytology. Instead, histopathologic grading and immunohistochemistry are recommended to predict the behavior of these tumors.

Lipomas (Figure 6) are benign tumors composed of mature adipocytes. Cytologically, a lipoma cannot be distinguished from subcutaneous adipose tissue. Grossly, the aspirate will consist of droplets of lipid that often rinse away during the staining process. Intact adipocytes are large cells with voluminous amounts of cytoplasm and small round nuclei.

**ROUND CELL NEOPLASIA**

**Appearance**

These tumors have many similar features that include:

- Round cell shape
- Arrangement of cells individually or in sheets

These tumors exfoliate very well, resulting in highly cellular cytologic specimens. Each round cell tumor has distinct features.
Types of Tumors

Round cell tumors consist of several different tumor types; the most common include:

- Plasma cell tumors
- Histiocytomas
- Mast cell tumors
- Lymphoma
- Transmissible venereal tumors.

**Plasma cell tumors** (Figure 7) can be difficult to distinguish from histiocytoma, especially if a prominent golgi apparatus is not visible. See Table 3 for a list of cytologic characteristics.

**Histiocytomas** (Figure 8) are only identified in dogs and commonly diagnosed in dogs less than 4 years of age. The neoplastic cell is a Langerhans cell or epidermal dendritic cell and often resolves on its own. See Table 3 for a list of cytologic characteristics.

**Mast cell tumors** (Figure 9) are the most common skin tumor in dogs. They can occur as single or multiple skin tumors and occasionally infiltrate the abdominal organs and bone marrow. Mast cell tumors cannot be graded on cytology; histopathology is required. Aspiration of the draining lymph nodes may be beneficial to determine the potential behavior of
In summary

Cytology is a useful diagnostic tool, especially in the evaluation of neoplasia. It is a noninvasive technique with sometimes immediate results. Identification of a malignant process can be done easily in many cases and may assist veterinarians in planning the next diagnostic step.

N:C = nucleus to cytoplasmic ratio

References

Anne Barger, DVM, MS, Diplomate ACVP, is a clinical associate professor in pathobiology at the University of Illinois College of Veterinary Medicine and the section head of clinical pathology for its Veterinary Diagnostic Laboratory. Her research interests include immunocytochemistry, osteosarcoma, and cytology, and she is a prolific writer on these topics. Dr. Barger is a member of the American College of Veterinary Pathology’s exam committee and the American Society for Veterinary Clinical Pathology’s membership committee. She received her DVM and MS from University of Illinois and completed a residency in clinical pathology at North Carolina State University.