Scanning Electron Microscopy of Thyroid Cells Under Fully Hydrated Conditions—A Novel Technique for a Seasoned Procedure: A Brief Observation

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Technical information for handling fine-needle aspiration samples from thyroid lesions for WETSEM™ electron microscopy is presented. The use of wet SEM technology maintains cytological features of the thyroid cells, in the atmospheric electronic microscope chamber without the need for solidification. Images are presented from normal and pathological thyroid specimens showing subcellular elements unavailable to the cytopathologist by light microscopy. Of 24 samples, 18 were adequate for clinical evaluation. In 16 of these 18 specimens, we could find features compatible with the final histological or cytological diagnosis (post-hoc). In two cases, the cell features were too unique to be interpretable. Because this procedure is relatively simple, there is potential for the use of this technology as an adjunct to light microscopy in clinical and research settings.

Fine-needle aspiration (FNA) for cytologic evaluation of thyroid lesions is currently the cornerstone for differential diagnosis of thyroid nodules (1,2). Its wide use is credited with a decrease in the number of patients requiring thyroidectomy while doubling the yield of cancer diagnosed in those who eventually undergo thyroid operation (3).

The reported sensitivity and specificity rates of thyroid FNA vary from 65% to 98% (mean 83%) and 72% to 100% (mean 92%), respectively (4,5). The false-negative rate varied from 1% to 11.5%, and the false-positive rate varied from 0 to 7.7% (3–5), with accuracy of cytological diagnosis ranging from 70% to 97% (3–5). However, the ability to utilize EM of fresh cytological specimens obtained by thyroid FNA was technically limited. Only recent innovations (13) allow routine and reproducible imaging of fully hydrated samples at room or body temperatures, thus eliminating the need to solidify the samples (usually by freezing), thus preserving the internal structure and lipid droplets of cells. The advancement was in the development of a polymer membranous partition that protects the sample from the vacuum, used in EM, while being transparent to the electronic beam, referred to as WETSEM™ technology (Fig. 1) (13).

The objective of the present study was to characterize thyroid cells derived from malignant and benign nodules with wet SEM, and to investigate whether this technique can potentially generate useful information regarding thyroid pathology.

Materials and Methods

Patients

FNA samples were obtained from nonselected 24 patients (4 male), age 53.2 ± 21.7. In six patients, FNA specimens were

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obtained *ex vivo* immediately after surgical extraction of the thyroid, and in 18 specimens were obtained during routine cytological procedure. The cytological or histopathological diagnoses were as follows: Graves’ (1), Hashimoto’s (2), lymphoma (1), papillary carcinoma (5), follicular variant (1), benign nodules (9), C-cell hyperplasia (1), and inadequate samples (4).

**WETSEM capsules**

The sample holder centers on a rigid enclosure with a window that consists of a thin, electron-transparent partition membrane. Beam electrons penetrate the partition polyimide membrane of 145 nm in thickness, probe the sample, and scatter back to the backscattered electron detector placed above the sample. Cells from cytological samples are allowed to be attached directly on the membrane, which is coated with fibronectin to enhance the affinity of cells (Fig. 1).

**Isolation of mixed suspension**

Cells were aspirated from thyroid glands using a 23-gauge syringe needle. Part of the sample was used for conventional cytologic examination, and the rest of the sample was spilled into RPMI culture for wet SEM. Red blood cells (RBC) were lysed with RBC lysis solution, and the remaining cells were resuspended in culture medium and placed on the fibronectin-coated capsules overnight at 37°C.

**Fixation and staining protocol**

For fixation, the samples were washed four times with phosphate-buffered saline (PBS), and then incubated with 1% glutaraldehyde and 2% paraformaldehyde at room temperature for 30 min. For staining, the samples were washed twice with PBS and twice with water. Incubation of the samples with 0.1% osmium tetroxide (OsO₄) for 30 min at room temperature was followed by washing four times with distilled water and incubation with 0.01% uranyl acetate for 10 min. After an additional three washes with distilled water, the samples were ready for imaging in the wet state.

**Results**

**Morphological features**

Normal tissue (Fig. 2)—The cell is elongated with an eccentric nucleolus. Peripheral granules 2–6 μm in diameter are situated near the apical plasma membrane with polarity. Larger granules (10 μm in diameter) are dispersed uniformly throughout the cytoplasm. Larger vacuoles are osmium negative and vary in size between 10 and 60 μm in diameter.

Benign colloid nodule with degenerative changes (Fig. 3)—Similar to Figure 1 with less prominent vacuoles.

Well-differentiated papillary carcinoma of thyroid (Fig. 4)—cluster of cells with no peripheral granules. Few central located small granules, 0.5–1 μm in diameter are present (mitochondria?).
Well-differentiated papillary carcinoma of thyroid (Fig. 5)—prominent large irregular and nonuniform cytoplasmic vacuoles probably from cisternae of the endoplasmic reticulum, numerous mitochondria, and no peripheral secretory granules. Light microscopy of the cytological specimen and the histopathology are presented for comparison.

Well-differentiated follicular carcinoma of thyroid (Fig. 6)—cells with no peripheral granules and few mitochondria.

Hürthle (oxyphilic) cell (Fig. 7)—Round-shaped, rich in mitochondria.

Clinical evaluation

We were able to obtain adequate samples for the clinical evaluation of wet SEM specimens from 18 of the 24 patients. Compatibility with malignancy was considered if the specimen was poor in secretory granules, had abnormal vacuoles, or had nuclear inclusions or evaginations. In 16 specimens, we could find features compatible with the histological or cytological diagnosis (post hoc). In the case of the follicular carcinoma (Fig. 6), although the cytological diagnosis was of a follicular lesion, the wet SEM specimen had features of a
malignant cell. The postsurgical tissue diagnosis was of follicular carcinoma. In two cases, the cells had unique features, and thus we were not able to interpret them.

Discussion

Wet SEM has an advantage over standard SEM techniques in the preservation of lipids, especially in large aggregates such as lipid droplets. Its advantage over conventional cytological light microscopy lies in its ability to identify additional subcellular elements. Utilization of EM in the daily practice of cytopathology was therefore advocated as an ancillary technique for diagnosis, for narrowing differential diagnosis, and for confirmation of diagnosis (14).

The osmium-avid secretory granules apparent in thyrocytes from normal tissue and from benign pathological states such as Hashimoto’s thyroiditis and Graves’ disease can indicate normal or increased synthetic activity as demonstrated by TEM (11), whereas the decrease in the granule density as detected in the malignant cases might point to an abnormality in the thyroid hormone synthesis associated with the dedifferentiated state of the malignant cell. The large osmium-negative granules are most probably colloid droplets within a dilated cisternum of the endoplasmic reticulum; again, the significance can be related to cell activity and the dedifferentiated state of the malignant cell. That malignant nodules are predominately dormant (“cold nodules”) fits the paradigm described.

The precise structural counterparts of the diverse morphological objects as seen by wet SEM are still a matter of investigation. Several approaches are currently being pursued such as Gold-antibody conjugates for wet SEM immunohistochemistry and the assessment of vesicle iodine content.

Current trends in thyroid cancer epidemiology point to an increasing prevalence of the disease with lower stage at presentation. This could partly be due to increasing diagnostic vigilance (6,16) where smaller, incidental sonographically diagnosed nodules are investigated by cytology. Thus, in addition to improving diagnostic yield form FNA, there is a potential need for obtaining additional prognostic information regarding the nodule. Recently, examination of cytological material for BRAF point mutation and RET/PTC rearrangements were shown to be of diagnostic value in equivocal cases (7,8) but are limited to less than 40% of papillary carcinomas. Immunohistochemistry staining of FNA specimens have not shown significant clinical value in separating benign from malignant thyroid tumors. The use of electronic microscopy can delineate ultrastructural components not available to the cytopathologist by light microscopy assisting in diagnostics and prognostics.

We report the technology and its potential, and not its diagnostic and prognostic yields because the series is too small for meaningful conclusions. Only collaborative large-scale work will ascertain whether ultrastructural changes identified by wet SEM can be integrated into clinical useful information.

References


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