Microwave Processing in Diagnostic Electron Microscopy

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Introduction

Transmission electron microscopy (TEM) continues to play an important role in diagnostic surgical pathology [1,2], particularly in such areas as kidney pathology and tumor diagnosis, among others. Diagnostic TEM is subject to unique time constraints, quality control regulations, and other problems not seen in other TEM applications. The diagnostic TEM laboratory must produce high-quality electron microscopy on small samples which frequently are suboptimal in fixation and tissue quality due to the pathology involved and time factors associated with biopsy and surgery. In the vast majority of cases, a second sample is not obtainable if the first one is inadequate. Despite these problems, the diagnosis must be done as rapidly as possible, and rapid "turnaround" time of samples is the highest priority, even in conditions of high caseload. Any technology which reduces the long processing procedures for TEM samples could be of significant benefit in reducing turnaround time in the diagnostic TEM laboratory. However, it is equally essential that any new processing technology not result in alterations in ultrastructural morphology which differ from accepted morphology, or loss of ultrastructure features essential for diagnostic purposes. Concomitantly, the procedure must consistently yield high quality results to facilitate diagnosis and meet mandated standards. We therefore compared diagnostic pathology cases processed using traditional methods with those processed using a microwave oven for all tissue processing stages (fixation, dehydration, embedding, polymerization) from the standpoints of processing time, turnaround time, and comparative ultrastructure of the final product.

Microwave Process Development

The microwave assembly used was the Pelco 3440 MAX programmable laboratory microwave oven equipped with a load cooler (Ted Pella, Inc., Redding, California), which cools and recirculates water to beakers within the microwave chamber which serve as heat sinks to prevent specimen heating. A temperature probe inserted into the fluid the specimen is in at any step controls the specimen temperature via the programmable oven by switching the microwave generator off and on to maintain temperature below a pre-programmed set point.

In initial trials, tissue samples from pigs were used to develop and optimize the microwave protocol and for comparison between conventional and microwave processing. Subsequently, human diagnostic samples were used from approximately 200 cases in which sample size was adequate to carry out both routine and microwave processing. In these cases, samples to be microwaved were kept in buffer after aldehyde fixation until the final diagnosis was made. The samples were then processed by microwave, sectioned, stained and photographed to allow ultrastructural comparison between samples processed using the two different methods.

Processing Times Comparison

As shown in Figure 1, microwave processing significantly reduced every stage of tissue processing compared to the conventional methods in use in our laboratory. Diagnostic specimens are always received in aldehyde in our laboratory, but the time in aldehyde is not always known. On fresh porcine samples, we found 40 sec in the microwave to provide excellent aldehyde fixation, and this is routinely done on diagnostic specimens for which time in aldehyde is not known. As can be seen from Table 1, the total processing time (time specimen is in various steps) is reduced from 24 hr in conventional processing to 2.3 hr in the microwave schedule we used. The actual processing time (time between receipt of specimen and ready for sectioning) in reality cannot be achieved in much less than two normal 8 hr work days using conventional pro-

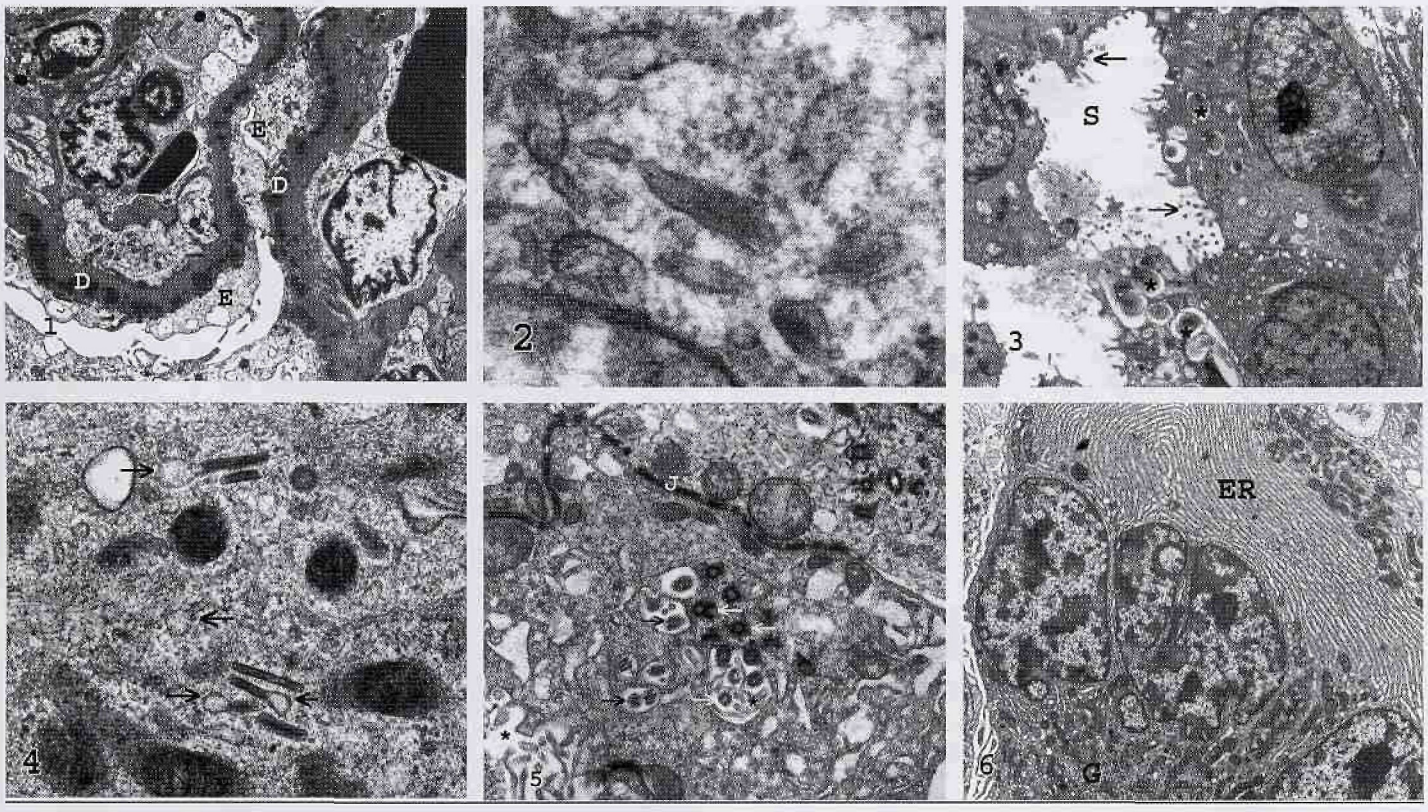


Table 1: Processing Time: Microwave vs. Conventional Processing

	Microwave	Conventional
Aldehyde Fixation	40 sec	2 hr
Buffer Rinse	5 min	5 min
Osmium Fixation	40 sec	2 hr
Buffer Rinse	5 min	5 min
Dehydration	50 % acetone 80 sec 70 % acetone 80 sec 90 % acetone 80 sec 100% acetone 80 sec	50% EtOH 30 min 70% EtOH 30 min 95% EtOH 30 min 100% EtOH 60 min
Transitional Fluids	None	EtOH:EtOH=1:1, 30 min 100% EtOH, 30 min
Infiltration	Acetone:Resin[1]=1:1, 15 min 100% Resin, 30 min	EtOH:Resin[2]=3:1, 1 hr EtOH:Resin=1:1, 1 hr EtOH:Resin=1:3, 1 hr 100%:Resin, 1 hr
Polymerization	75 min	12 hr
Total Processing Time	2.3 hr	24 hr
Actual Processing Time	3.5 hr	2 days

[1]Spurr's/Epon 1:1. [2]Spurr's

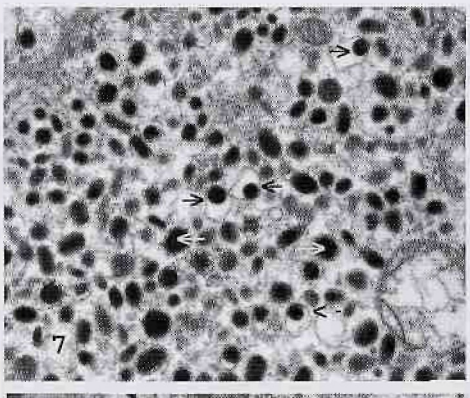
cessing, whereas even taking account of time for solution changes, microwave processing can be completed in 3.5 hr. This means a specimen received at the beginning of the workday can be ready for sectioning by noon and, in many cases, can be completed by the end of the same day. We also found that microwave techniques can be applied to some or all of the processing steps and can be interchanged depending on the needs and priorities of the day. For example, specimens arriving mid-afternoon can be processed by microwave to the polymerization stage, then polymerized overnight at 70°C in a conventional polymerization oven. Microwave processing also has the added bonus of reducing fixative and buffer costs, as both aldehyde and Osmium fixation can be carried out in microtubes using 600 µl of fixative.

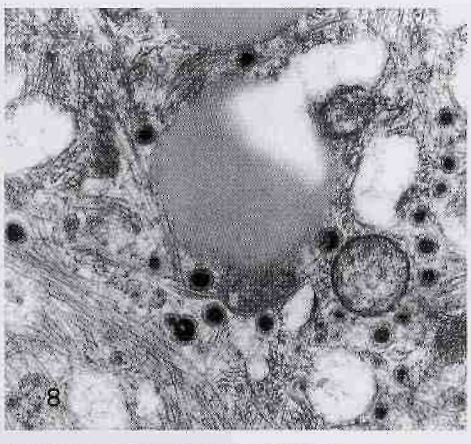
Turnaround Time Comparison

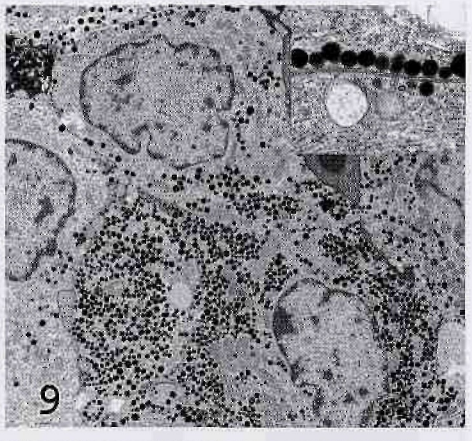
Between 1997 and 2002, we had 2,543 cases which were completely or partially processed using microwave techniques, 70% of which (1,780 cases) were processed solely using microwave technique for all steps. Of these, 174 cases were excluded from the turnaround time study because of factors affecting turnaround time not associated with processing (i.e., microscope breakdown), leaving a total of 1,606 microwave cases for this portion of the study. A total of 1,606 other cases, derived in the same way, but processed by conventional methods during the same time span were used for comparison. In both groups, turnaround time (days from receipt to completion) for each case has been corrected for non-work days (holidays and weekends).

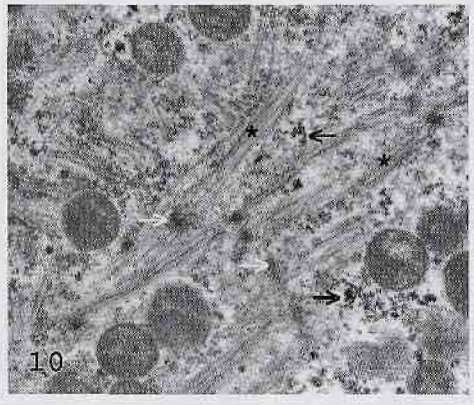
In Table 2, the turnaround times of the 1,606 cases in each group are compared. The average turnaround times calculated from these data are 2.4±1.04 days for microwave samples, and 3.4±1.05 days for samples processed with conventional methods. Although, the difference in means is not statistically significant, 94% of microwave cases were completed in 4 days or less, 85% in 3 days, 44% in 2 days, and 15% in 1 day, compared to 80%, 58%, 24%, and 0%, respectively, for conventionally-processed samples (Table 2). Additionally, only 6% of microwave cases took 5 or more days to complete, compared to 20% for conventional processing.

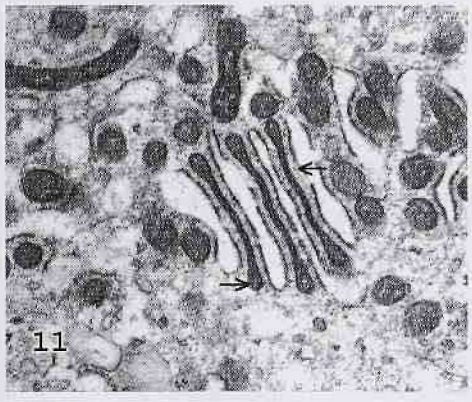
Thus, from a practical standpoint, microwave allowed 94% of cases to be completed in 4 days or less, and increased the number of cases completed in 2 days or less by almost 3-fold. It also allowed a significant number (15%) of cases to be turned around back to the pathologist the same day, which is not possible using conventional processing.











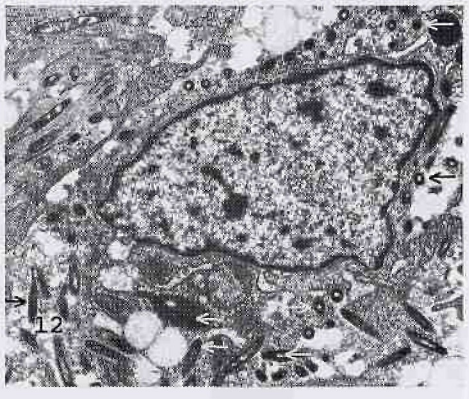


Table 2: Turnaround Time: Microwave vs. Conventional Processing

	Number of Days (% of Total Cases)					Total Cases
	1	2	3	4	5+	
No. Microwave Cases	239(15)	706(44)	413(26)	154(9)	94(6)	1606
No. Conventional Cases	0(0)	378(24)	551(34)	354(22)	324(20)	1606

Tissue Ultrastructure

In our hands, microwaved blocks were indistinguishable from conventional blocks in terms of sectioning, lead citrate and uranyl acetate staining, and stability in the beam. One micron sections stained avidly with methylene blue/azure II and basic fuchsin. Of greatest importance, ultrastructural features essential for diagnostic purposes were indistinguishable from those seen in conventionally-processed samples, as is demonstrated in Figures 1-12. Figure 1: renal tissue from a patient with systemic lupus erythematosus, showing subepithelial dense deposits (D) in the basement membrane, and effacement of foot processes in the epithelial layer (E). Figure 2: melanoma, showing striated structure of Stage 2 melanosome essential for diagnosis. Figure 3: pulmonary adenocarcinoma showing microvillus processes (arrows) extending into acinar space (S). Cells surrounding acinus are joined by junctional complexes, and contain granules containing myelin forms of surfactant granules (*), indicating Type II epithelial cell differentiation. Figure 4: histiocytosis X, showing classic "tennis racquet"-shaped Birbeck (Langerhans) granules (arrows) diagnostic for this condition. Figure 5: brain tumor, showing multiple intra- and intercellular neolumina containing microvillar processes (*) and cilia (black arrows) with cytoplasmic basal bodies (white arrows), and elaborate junctional complexes (J) between cells bordering lumina. Features are diagnostic for ependymoma. Figure 6: plasmacytoma, showing massive arrays of endoplasmic reticulum (ER), numerous mitochondria and large Golgi apparatus (G) typical of plasmacyte differentiation, together with abnormal multiple nuclei. Figure 7: adrenal phaeochromocytoma containing large numbers of two types of granules of 100-500 nm diameter. Round to oval granules with tight limiting membranes are adrenaline granules (white arrows); granules acentrically located within limiting membrane with large halo are noradrenaline granules (black arrows). Figure 8: brain tumor of unknown origin containing multiple arbovirus particles. Figure 9: pituitary adenoma showing excellent preservation of electron-dense secretory granules, and alignment of granules along plasma membrane (inset). Figure 10: poorly-differentiated rhabdomyosarcoma showing electron-dense particulate glycogen (arrows) and randomly-oriented arrays of thick and thin myofilaments (*) attached to Z-bands in primitive sarcomere differentiation. Figure 11: chordoma, showing alternating lamellae of ER and attenuated, compressed mitochondria (arrows) typical of chordoma cells. Figure 12: Abdominal lymph node of patient with Whipple's disease showing extracellular (black arrows) and phagocytosed (white arrows) Tropheryma whippleii organisms.

Summary

Microwave processing applied to diagnostic ultrastructure cases significantly reduced the maximum turnaround time and increased the number of cases completed in 2 days or less by 2.5-fold. It also allowed a significant number (15%) of cases to be completed in less than 1 day, which was not possible using conventional processing. This corroborates earlier findings by Giberson et al. (3) who showed microwave processing times comparable to ours, and when used in conjunction with digital imaging to eliminate photographic steps, were able to achieve turnaround of 4 hours. Even without digital imaging, it is feasible to complete cases in less than 1 day, which puts diagnostic electron microscopy turnaround time in the same time frame as routine histology and immunohistochemistry. More importantly, this was achieved with no loss of quality of product or loss/alteration of ultrastructural features essential for diagnosis. Moreover, in cases difficult to embed, such as skin punch biopsies and tumors with high lipid and collagen content, microwave embedment was superior to that obtained using conventional processing. In our hands, this could be further improved by using vacuum in conjunction with microwave methods, as has also been discussed by Giberson et al. (3). The only major drawback of microwave processing in the diagnostic laboratory is that it is so rapid that cases can be prepared more rapidly than they can be sectioned and examined. During processing, a technician must be committed full-time to the microwave and is thus unavailable for other duties, including sectioning. Thus, to optimize the benefits of microwave processing, it must be scheduled according to the technical priorities of the day. ■

References

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