Benefits of Microwave-Assisted Processing Go Beyond Time Savings

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Introduction

Microwave-assisted processing of biological specimens, from its inception, has been a methodology that promised time savings over conventional processing methods [1]. It has taken almost 30 years to define and control the significant variables associated with microwave processing [2]. Recent research combined with improved technology have helped in the identification and control of the experimental variables associated with microwave-assisted processing [2-7]. Stated simply they are: (1) constant sample temperature control in conjunction with continuous microwave irradiation [3-4], (2) control of wattage in the microwave device (the ability to control microwave power in the same manner as a dimmer switch controls a light) [2-5], and (3) energy uniformity in the microwave cavity [3-4].

Understanding the energy/heat dichotomy, we believe, was the important step in beginning to better understand microwave methods [2]. The precise method of activation that microwave radiation contributes to the acceleration of a wide range of processing applications is unknown. However, with the advent of a better understanding of what appears to be the real variables associated with microwave-assisted processes, the quality of the final product has been improved. This knowledge has led to better techniques and experimental results for the following processes: tissue processing for electron microscopy (EM) [2], immunolabeling [3], formaldehyde fixation [4], and decalcification [5]. As a result the role of temperature in the form of microwave-induced sample heating appears to have a reduced or insignificant role in overall protocol design.

Materials and Methods

The distinct advantage of knowing the true wattage and a uniform microwave environment is that the chemistry used

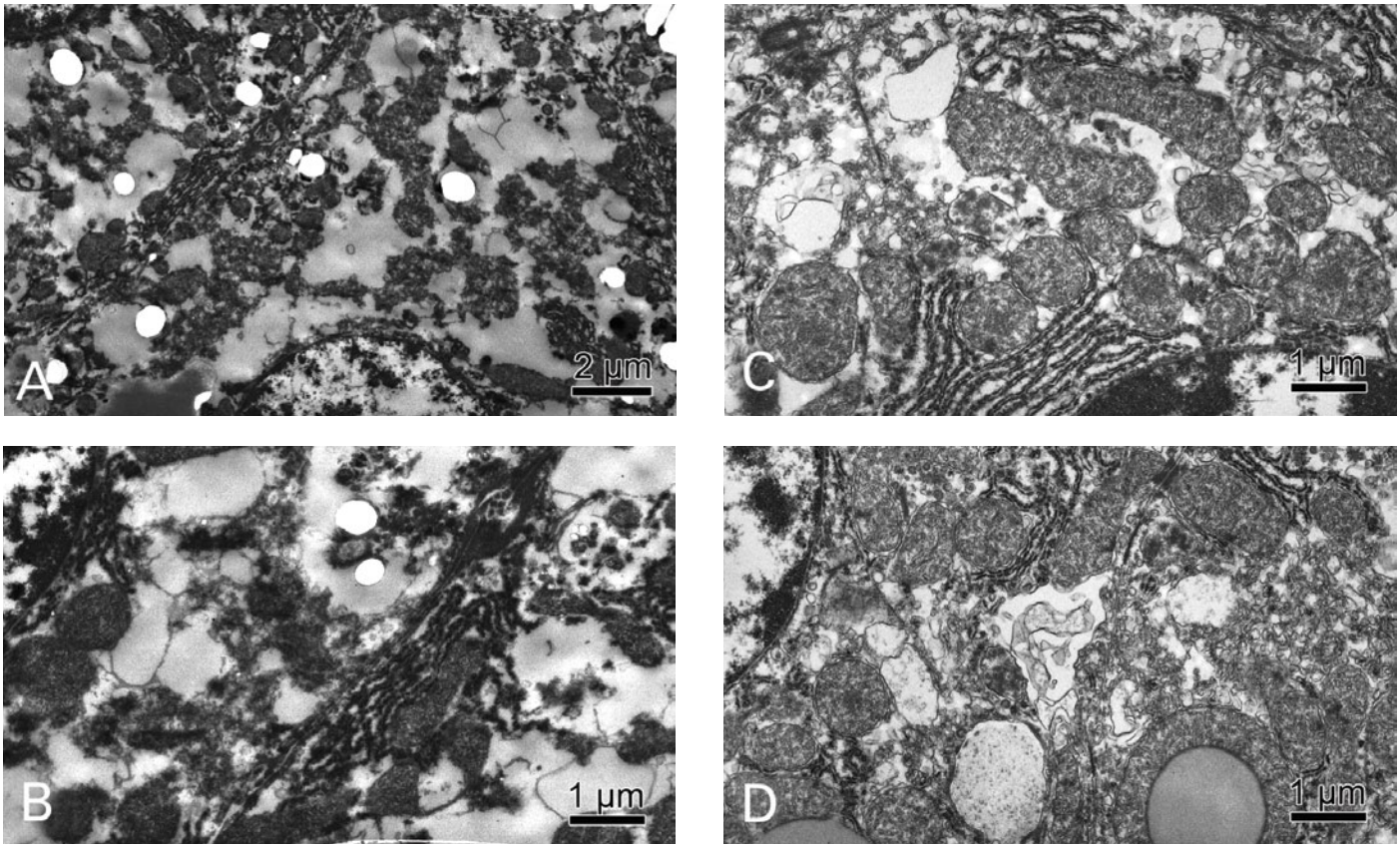

Figure 1: (A-B) Electron micrographs of liver fixed in 10% neutral buffered formalin by conventional and microwave methods. Tissues were fixed conventionally for 3 hours at room temperature. Note the extraction and absence of organelles. (C-D) Tissues were fixed with microwave radiation for 15 minutes at 150 W followed by 5 minutes at 650W. Note the greatly improved ultrastructure [2].

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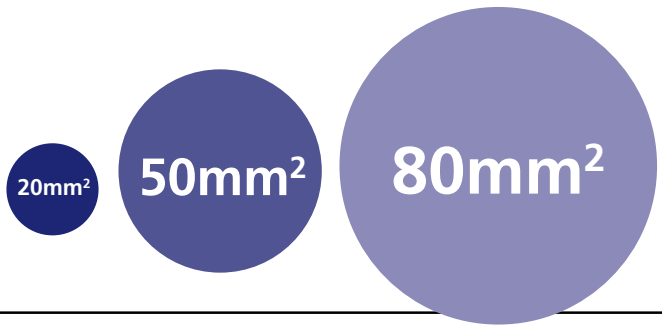

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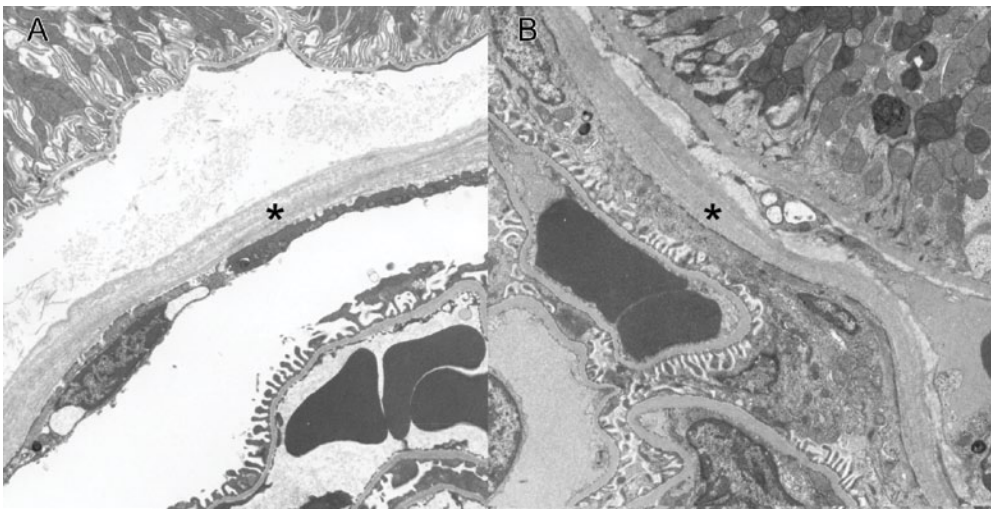

Figure 2: (A) Kidney glomerulus fixation results for a 24-hour conventional fixation with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer compared to (B) 24-hour fix in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.06M phosphate buffer. Both primary fixations were followed by microwave-assisted fixation in 2% aqueous osmium tetroxide and processed to polymerized resin blocks by the microwave methods of Giberson et al. (2003). When both primary fixations are microwave-assisted, the results mimic B (no shrinkage seen in the glomerulus or between tubules as seen in A). The asterisks in A and B denote Bowman's Capsule. Scale bar = 2 µm.

on the bench is freely transferable to a microwave-processing environment. This is especially true for fixatives containing formaldehyde. Galvez et al. [2] elucidated the role that wattage and sample temperature control play in microwave-assisted fixation of fresh tissue in formaldehyde. These same factors were also the key components to reproducible microwave-assisted immunolabeling [4]. The significance of true wattage on embryo viability after microwave exposure was demonstrated experimentally by Sanders and Gartner [8]. Early microwave-assisted processing techniques for electron microscopy did not have the advantage of true wattage or control of the microwave environment as did the later techniques [3, 6]. Tingling et al. [6] used a microwave-assisted technique for EDTA decalcification that controlled sample temperature external to the microwave device being used. This was the first demonstration of continuous microwave radiation in combination with no microwave-induced sample heating. Microwave devices for the present research were from the PELCO BioWave® line of microwave technology (Ted Pella, Inc. Redding, CA).

Results

For the four techniques that clearly benefit from the presence of microwave radiation (EM fixation and tissue processing, immunolabeling, formaldehyde fixation and decalcification), a reduction in turnaround times has been a significant component. These time savings can be summarized as follows:

- EM processing (fixation through resin infiltration): reduced to < 40 minutes
- EM resin polymerization (epoxy resins and LR White): reduced to < 75 minutes
- Single labeling protocols (fluorescence or DAB): reduced to < 60 minutes
- Formaldehyde fixation for histology (tissues <5mm thick) reduced to 60 minutes
- Decalcification with EDTA (based on conventional methods): >10X rate increase.

Consistent fixation results are concurrent with the noted time savings. Figure 1 demonstrates the effect of 20 minutes of microwave exposure on the rate of ultrastructural preservation with 10 percent neutral buffered formalin. Microwave involvement resulted in a significant improvement in ultrastructural detail when compared to 3 hours on the

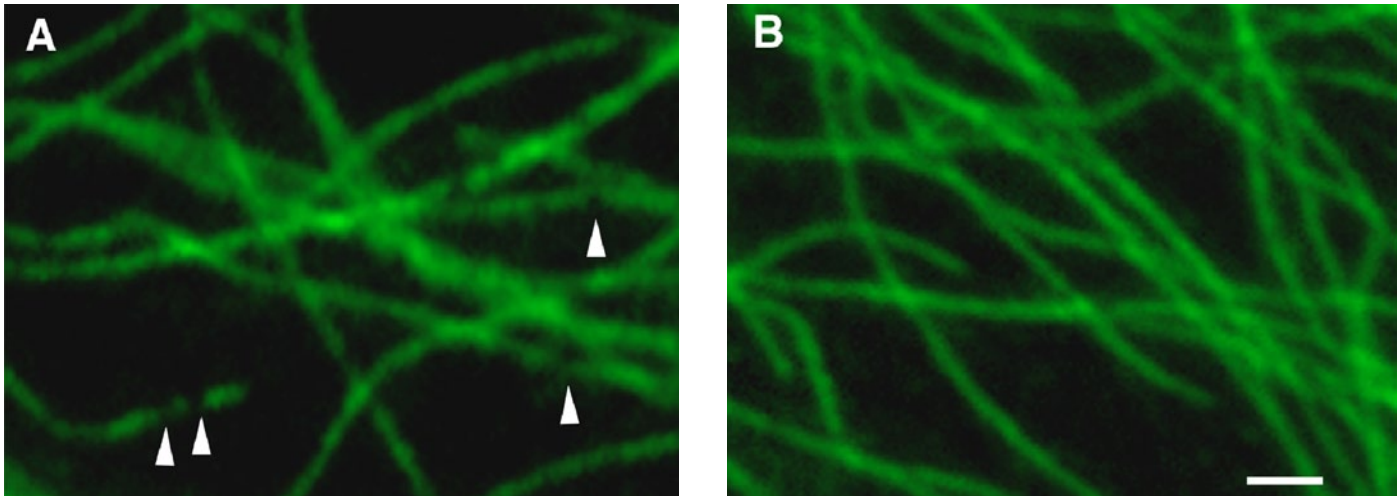

Figure 3: HeLa cells transfected with CellularLights, tubulin GFP (Invitrogen, Carlsbad, CA) fixed in 3% paraformaldehyde and then labeled with an anti-GFP antibody followed by Alexa 488 secondary detection. (A) Cells fixed conventionally for 30 min. at 37 C. The continuity of label down the microtubules is not uniform (arrows). (B) Transfected Hela cells fixed in the presence of 150 W of microwave radiation for 1 min. at 37 C. The continuity of the label is superior as compared to A above. Bar = 2 µm.

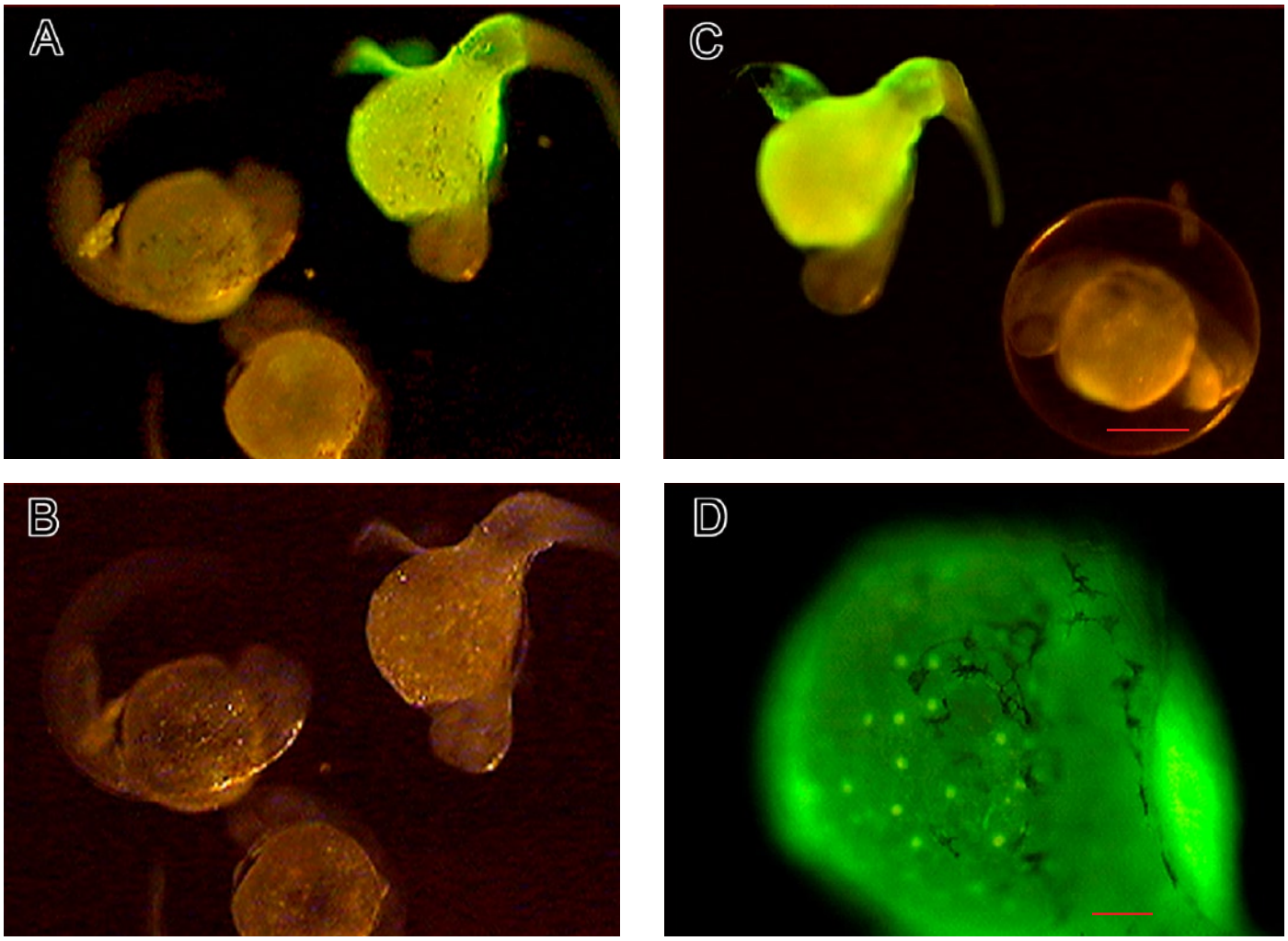

Figure 4: Fluorescence microscopy of living GFP/nRap1 expressing zebrafish embryos before (A) and after (B) fixation with 3% paraformaldehyde and 0.5% glutaraldehyde in PBS. Conventional fixation times were evaluated from a few minutes up to an hour with similar results. The GFP fluorescence was maintained when the fixation was microwave-assisted (2 minutes at 150W, 2 minutes at no power, followed by 2 minutes at 150 W). Images A, B, and C are all at the same magnification. Scale bar in C = 0.15 mm. The GFP expression in embryos following microwave fixation in C is shown at a higher magnification in D (bar = 50 μm).

bench at the same temperature [4]. The evaluation of the results from a 24-hour bench fixation with two EM fixatives, paraformaldehyde/glutaraldehyde and glutaraldehyde alone, indicate the difficulty in standardizing fixation with time (Figure 2). The study indicated that a 24-hour time period produced the best results for the mixed aldehyde, whereas 2 hours was best for glutaraldehyde alone (results not shown). The shrinkage artifacts seen in Figure 2A were eliminated when the fixation was done in the microwave, and the quality of fixation for the mixed aldehyde was maintained as well (Figure 2B) [4,7].

Figures 3A-B present the results of a conventional fixation and labeling contrasted with a microwave-assisted fixation and labeling protocol. *HeLa* cells transfected with Cellular Lights Tubulin-GFP (Invitrogen, Carlsbad, CA) were conventionally fixed for 30 minutes (Figure 3A) or 1 minute at 150 W true wattage (Figure 3B). The microwave-assisted processes (fixation and labeling) resulted in improved immunohistochemistry as evidenced by the continuity of the microtubule structure. The importance of a microwave-assisted fixation is demonstrated in Figures 4A-D. A conventional fixation results in the loss of the GFP fluorescence (Figure 4B). When the same fixation is microwave-assisted, GFP fluorescence is maintained (Figures 4C-D).

Discussion

The real benefits of microwave-assisted methods go beyond time savings. Those benefits, we demonstrate, reside in a microwave-assisted fixation step (Figures 1-4). Microwave methods provide the tool to standardize the fixation process to a matter of minutes versus hours, and the benefit of that approach can be seen in Figure 2. It is further demonstrated by high consistency in immunohistochemistry (Figure 3). Figure 4 provides further evidence of the uniqueness of microwave methods. GFP fluorescence is maintained only after a microwave-assisted fixation (Figure 4).

Improved microwave technology has provided the ability to define and control the important experimental parameters

associated with the science: sample temperature control, energy uniformity, and true wattage. A better understanding of the energy/heat dichotomy [2] has provided the knowledge to better design microwave-assisted protocols for a wide range of applications.

In conclusion, we believe it is evident that microwave radiation provides important improvements for many conventional processes [2-8] and that the radiation produces experimental results that are largely independent of a temperature component. MT

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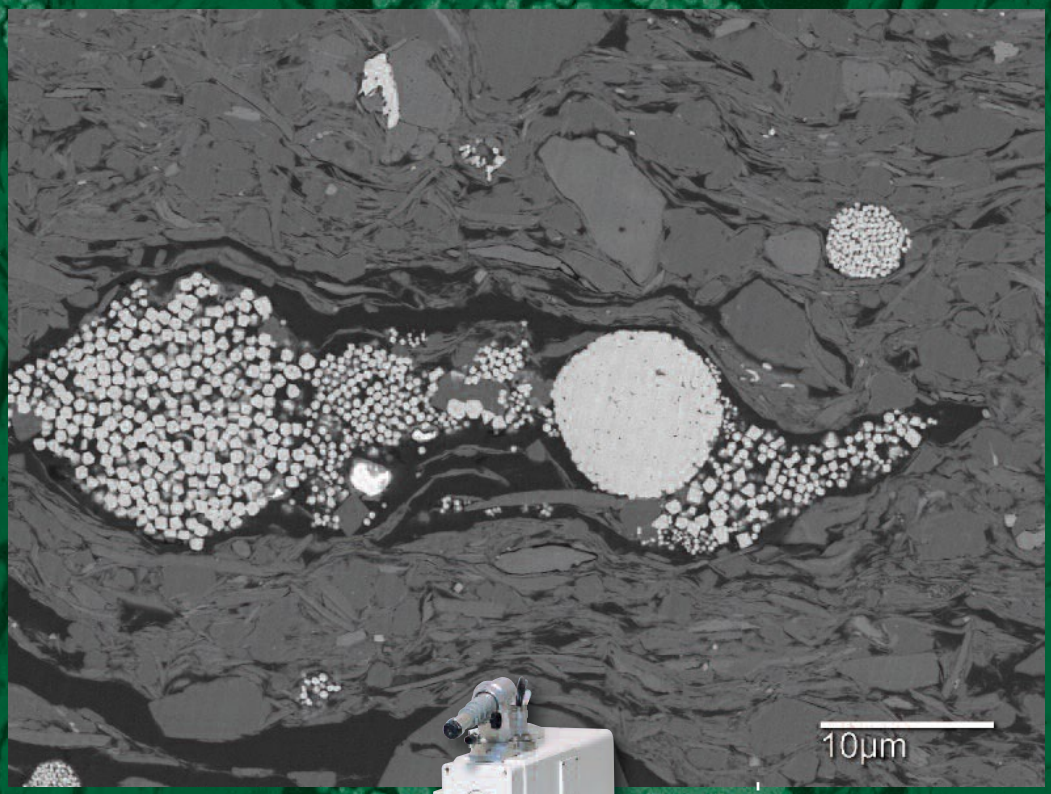

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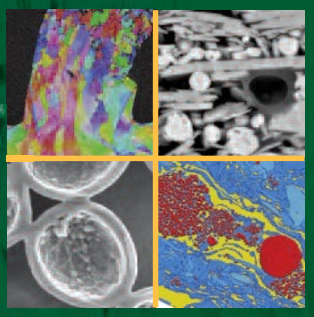

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