

MICROWAVE-STIMULATED FIXATION FOR ELECTRON MICROSCOPY USING A DOMESTIC MICROWAVE OVEN

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Summary

We investigated microwave-stimulated fixation of tissues for transmission electron microscopy using a domestic microwave oven operating at a frequency of 2.45 GHz with an output power of 500W. Microwave-stimulated fixation, in 4% glutaraldehyde, of fresh rat kidney, liver, heart and brain tissues was compared to conventional fixation. Human renal biopsies were similarly studied. Electron microscopy showed excellent ultrastructural preservation comparable to that obtained by conventional fixation. The optimal temperature range for microwave-stimulated fixation was found to lie between 50°C and 55°C. Our results indicate that microwave-stimulated fixation is a rapid and reproducible technique and can be effectively applied to routine diagnostic pathology.

Key words: Microwave, fixation, ultrastructure, diagnosis

INTRODUCTION

Interest in the use of microwave in histopathological techniques has mounted in recent years following the introduction of commercial domestic microwave ovens. Mayers¹ first developed a method to determine whether the theoretical possibility of producing histological fixation by microwave heating could be achieved in practice. He exposed a variety of fresh postmortem tissues including human kidney to the effects of microwave radiation from a microwave generator designed mainly for hospital physiotherapy. The results were promising. Login² first reported satisfactory results of microwave fixation of surgical and autopsy specimens. Subsequently many authors reported encouraging results in tissue fixation for both light and electron microscopy,^{3,4,5,6} immunocytochemistry,^{7,8,9} and histochemistry.^{9,10} These recent studies indicate that microwave fixation may provide satisfactory tissue fixation in a routine diagnostic pathology laboratory.

Our present study investigates the use of a domestic microwave oven for microwave-stimulated fixation of rat tissues and human renal biopsies in a routine diagnostic pathology laboratory. The relevant physical characteristics of the microwave oven were also studied.

MATERIALS AND METHODS

Microwave Oven Calibration

A domestic microwave oven (SANYO EM-1412GYS) operating at 2.45 GHz with a maximum output of 500 Watts was used in this study. Because this microwave oven,

like most domestic ovens, did not have temperature control, it was necessary to first determine the heating curve of the fixative (4% glutaraldehyde) used for each power setting. A rapid response thermocouple thermometer (Digi-Sense, USA with time constant = 0.025 sec) was used to measure the temperature rise in the fixative, taken as the difference in temperature before and after heating by microwave radiation.

The heating curve for 100 ml of 4% glutaraldehyde for the power setting of 20 (equivalent to 100 W) was determined and a calibration graph drawn. Next, a simple method to study the electric field distribution in various regions of the oven was carried out by modifying the method of Washisu and Fukai.¹¹ An insulating plate painted with a reversible thermographic paint (R70 from Thermographic Measurements Ltd., UK) was used to determine the relative distribution of the rising temperature and the electric field. The change in color on the plate indicated the heating pattern and the electric field distribution during microwave heating was determined by studying the heating pattern within the oven.

Microwave-stimulated fixation procedure

Small cubes (about 1 mm³) of fresh kidney, liver, heart and brain from three rats as well as samples from five human renal biopsies were separately placed in a plastic beaker containing 100 ml of 4% glutaraldehyde. The beaker was then placed at the centre of the rotating plate of the oven. The optimal temperature

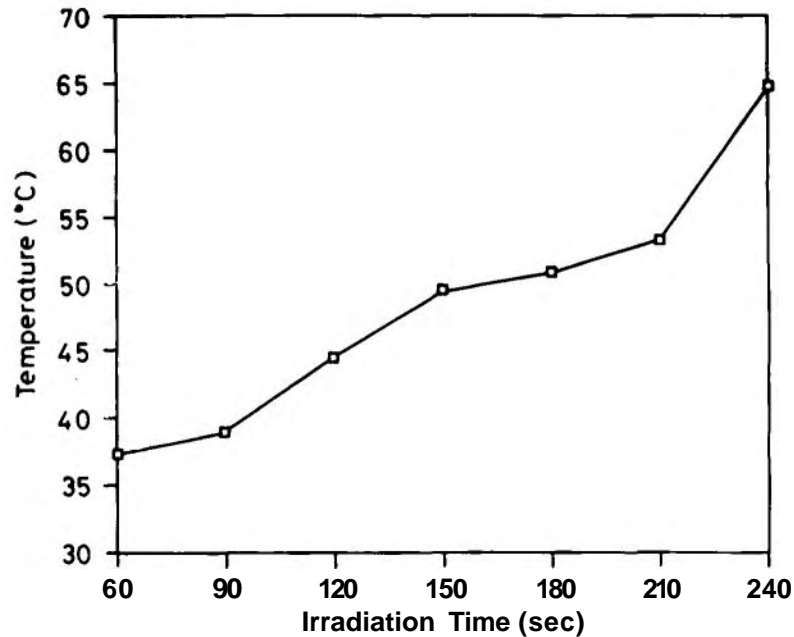


FIG. 1: A calibration graph showing heating curve for 100 ml of 4% glutaraldehyde for the power setting of 20 on the SANYO EM-1412GYS microwave oven.

for fixation was determined by irradiation for various time intervals (120 – 230 sec) to achieve a corresponding final fixation temperature of 44 – 60°C respectively. The fixative temperature was measured with the thermocouple thermometer immediately after the power was switched off. A fine needle temperature probe was used to measure the *in-situ* temperature of the tissues after irradiation. The specimens were subsequently post-fixed in 1% osmium tetroxide for 2 hrs. and dehydrated in graded alcohol before embedding in Epon.

A control group of similar tissues from three rats and five human renal biopsies were fixed in 4% glutaraldehyde overnight and processed according to routine methodology for electron microscopy.

Thick epoxy sections were cut, stained with toluidine blue and examined under the light microscope to check the quality of the fixation and to select areas for ultrathin sectioning. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with a Philips CM 10 transmission electron microscope operating at 60 KV.

RESULTS

The heating curve for the fixative used (4% glutaraldehyde) is as shown in figure 1. This graph revealed the heating pattern of the oven to be useful in the determination of reproducible irradiation time and temperature.

The electric field distribution as determined by the heating pattern was found to be uniform at the centre region of the rotating plate where the beaker was placed.

Under the light microscope, toluidine blue stained epoxy thick sections from both rat and human tissues revealed that microwave-stimulated fixation yielded as good details as those prepared by the routine method.

Electron microscopy of microwave-stimulated fixed rat tissues showed well-preserved ultrastructural architecture and fine cellular details comparable to those fixed by the conventional technique (Figs. 2 – 5). Cellular and nuclear membranes were sharply demarcated. No irradiation artefacts were observed.

Figure 6 shows an electron micrograph of human renal biopsy microwave-stimulated fixed at 50°C in 4% glutaraldehyde. There is excellent preservation of ultrastructural features including glomerular basement membrane, foot processes and cell organelles. In general, the diagnostic quality of the electron microscopic images obtained by the microwave technique was comparable to those obtained by the conventional technique (Fig. 7). We found that the optimal temperature for fixation lies between 50 – 55°C. This was also observed by others.^{2,3,5} We also noted that the optimal temperature range for good fixation was not critical for the various types of tissues studied.

DISCUSSION

The use of domestic ovens for laboratory use suffers from the lack of control over temperature rises and an inability to maintain the temperature at a constant level. There are now dedicated microwave processors which provide excellent temperature control. Nevertheless, the use of domestic ovens for microwave fixation of tissues could still attain reproducible results of diagnostic quality. We emphasise the need to calibrate the oven for different types of fixatives and fixative volumes to ensure reproducible and predictable results. Reasonably consistent temperature rise is possible if care is taken with the irradiation time and the use of water (dummy) load as a temperature buffer.²

Our study of the electric field distribution showed that it was uniform within the central region of the rotating plate where the beaker was placed for irradiation. Thus the problem of non-uniform fixation due to the presence of 'hot' and 'cold spots' in the oven did not arise.

Electron microscopy of microwave-stimulated fixed tissues showed well-preserved ultrastructural architecture and fine cellular details comparable to those fixed by the conventional technique. Cytoplasmic structures and nuclear membranes were sharply demarcated. These results confirm previous reports on the good preservation of cell morphology observed in the electron microscopic studies of tissues irradiated by microwave.^{3,4,5,9} The present study also indicates that microwave-stimulated fixation is a suitable method for sample preparation in routine diagnostic ultrastructural renal pathology.

Microwave treatment of unfixed tissue is often called 'fixation',^{9,13} but Marani et al¹⁴ introduced the term 'stabilization' when no chemical fixatives were used in the microwave method. 'Fixation' applies to when chemical fixatives are used, and 'stabilization' if only physical effects of microwave heating are applied. When a combination of both chemical fixative and physical effects are used, then the term "microwave-stimulated fixation" seems to be more appropriate.⁹ At present there is no general consensus amongst researchers as to the usage of these terms, hence standardisation of correct terminology will ensure more effective dissemination of knowledge in this rapidly expanding field.

The exact mechanism of microwave-stimulated fixation is not known. The current understanding is that rapid oscillation at a frequency of 2.45 GHz of bipolar molecules

such as water present in the tissues in the microwave field produces instantaneous and uniform heat. This in turn enhances diffusion of chemical fixatives and reaction rates. It is also possible that rapid oscillation of water molecules may result in stable bonding of soluble and structural proteins which can better withstand the deleterious effects of subsequent processing.

Finally, microwave-stimulated fixation offers the distinct advantage of completing the process of fixation in a greatly reduced time of minutes compared to that by conventional methods of hours. It also produces less tissue shrinkage.^{3,9} Our results indicate that microwave-stimulated fixation is reproducible and of sufficiently good quality to be useful in a routine diagnostic laboratory.

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NOTE: Microwave-stimulated fixation for all the specimens illustrated in figures 2 to 6 were carried out at 50°C in 4% glutaraldehyde.

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FIG. 3: Rat liver showing well-preserved ultrastructural morphology. In particular, note the nuclear membrane, mitochondria and rough endoplasmic reticulum. (Microwave-stimulated fixation. Uranyl acetate, lead citrate x 14000)

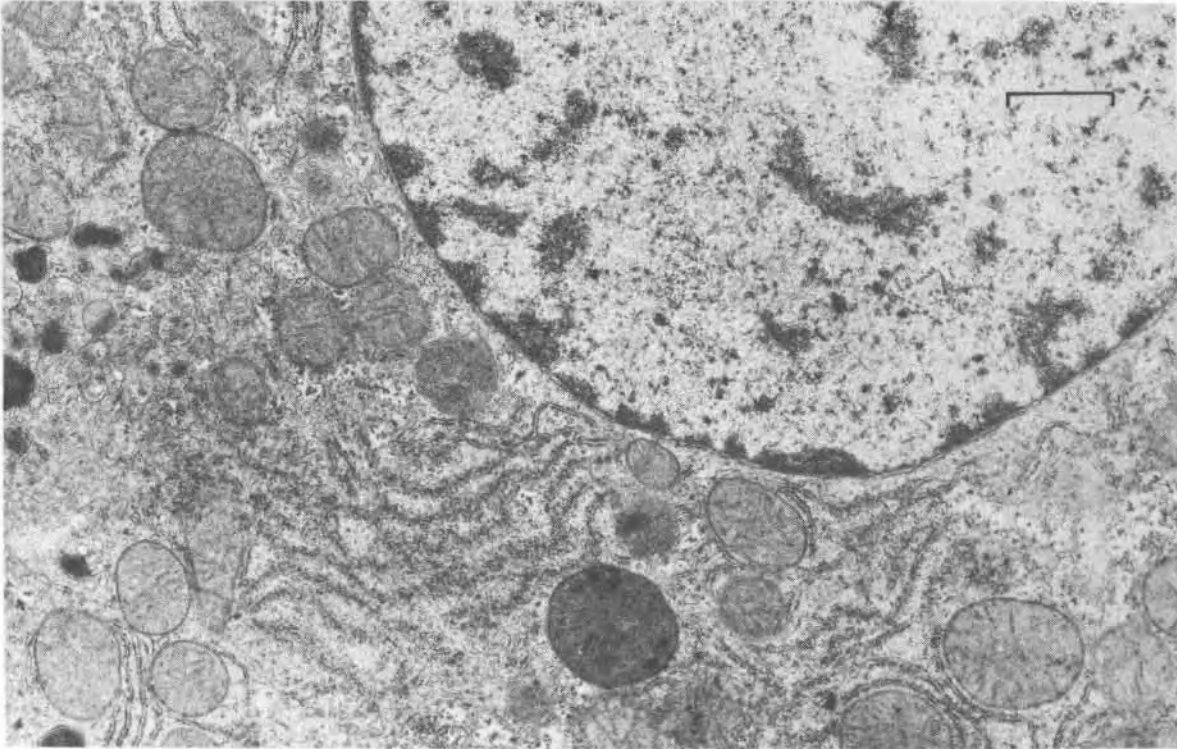


FIG. 2: Rat kidney showing excellent preservation of ultrastructural features comparable to those obtained by conventional fixation. (Microwave-stimulated fixation. Uranyl acetate, lead citrate x 7800)

