Review Article

Use of microwave in diagnostic pathology

ABSTRACT

Conventional tissue fixation and processing is as old as 100 years and still remains the gold standard against which all new technologies and methods need to be assessed. Tissue processing is one of the important steps for obtaining good thin sections without artifacts. Though conventional tissue-processing methods are most commonly followed, they are well-known as very laborious and tedious procedures.

Microwaves a form of electromagnetic wave-induced heat, when applied in histotechnology, reproducibly yields histolologic material of similar or superior quality to that provided by conventional processing methods, making it more popular in the recent years. A laboratory microwave offers features like maximum output of 2000-3000 watts, an in-built source of adjustable temperature probe, facility for ventilation of hazardous fumes, but is expensive. Considering the usefulness of microwave in histotechnology, i.e., reducing the time required for the diagnosis, replacing the conventional equipments of laboratories by microwave-guided ones is a remarkable and an acceptable change.

KEY WORDS: Conventional, electromagnetic, histotechniques, microwave

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INTRODUCTION

The changing situation caused by modernization in the field of medical technology, has led to the replacement of traditional techniques by newer ones. But histotechniques in histopathology more or less still remain the same with just a few changes. For almost 100years, the steps followed to prepare tissues for microscopic evaluation have remained unchanged, but the time consumed by these steps has reduced from several days to merely 1or 2days, and now with the advent of microwave tissue-processing, it has come down to few hours.

A microwaveMicrowave is an electromagnetic non-ionizing wave with a frequency (300 MHz to 300 GHz) and wavelength that can be found about halfway between a radio wave and visible light in the electromagnetic spectrum.[1-4]

MICROWAVE IN HISTOTECHNIQUES

Microwavetechnology became familiar to consumers initially in the form of household microwaveovens that could cook or reheat foods in a fraction of the time required by conventional ovens. The use of householdmicrowaveovens in the histology laboratory started slowly in 1980's, but today,they are commonly used to perform simple procedures such as specimen stabilization, staining, epitope retrieval, and some decalcification procedures.[1]

However, the lack of control over temperature rise and an inability to maintain the temperature at a constant level in domestic ovens, led to the invention of laboratory-grade microwavedevices.[5]

Laboratory-grade microwavedevices are rapidly gaining popularity. They provide sophisticated systems for monitoring and controlling the energy, precise temperature control, agitation to prevent thermal layering, multiple safety features, and most importantly, appropriate ventilation.[5]

Laboratory microwavedevices should be used for any technique that requires precise temperature control or involves the use of hazardous materials, especially toxic, flammable, or caustic reagents.[1]

HISTORY OF MICROWAVES IN HISTO PROCESSOR

It was in 1909that G. Arendt described the first automated histoprocessor. By 1910, all the main techniques had been worked out and many procedures that were in use at that time are still followed in a virtually unchanged form today. Conventional histoprocessors have simply automated the manual procedures without making efforts to reduce the histoprocessing times considerably.[6] Mayers was the first to develop a method to determine whether the theoretical possibility of producing histological fixation by microwave heating could be achieved in practice in 1970. Login was the first to report satisfactory results of microwavefixation of surgical and autopsy specimens. Microwavetechnique was first applied in the processing of tissues in 1985 by Kok andBoon from The Netherlands and Anthony Leong from Australia.[5] It was in 1990's that the

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first microwave histoprocessor was released to the world by Milestone technology.[6]

EFFECT OF MICRO WAVES ON TISSUE SPECIMENS

Routine histology procedures depend on relatively slow infiltration of solutions from the outer surfaces, and if heat is applied, it must work its way into the interior of the specimen by thermal conduction. Exposing thin sections of specimens to microwave energy affects the entire specimen instantaneously and simultaneously, facilitating the exchange of solutions and accelerating the reaction rates due tointernal heat.[1]

Microwavesworks by causing rotation of polar or charged molecules, for example, water,wherein one molecule of water has one big atom of oxygen to which two little hydrogen atoms are attached.[2-4] Water molecules have both positively charged side and a negatively charged side, so, when negative charges are brought near an electromagnetic field, there is repulsion as they are like charges, causing the molecules to rotate as they are asymmetrical.[3] They rotate rapidly through 180° at the rate of 2.45 billion cycles per second. The rotational movement produces heat.[1] Different substances subjected to the same amount of microwave energy will heat up at different rates. For example; 100 ml of water needs 2.2 times more heat to warm up than 100 ml of alcohol.Materials which heat up the fastest are composed of non-symmetrical polar molecules, which are easily rotated by microwave energy.[7] Acquired rotational energy is transferred into random motion on collision with other molecules. Oscillating dipoles are hindered by their own inertia and by frictional retarding forces from their surroundings. As the molecules collide, they absorb the microwaves and convert the energy into kinetic andthermal energy. Unlike conventional heating, heating in microwave is from within (internal heating) and its effect occurs throughout the material being irradiated.[2-4] The greater the dipole moment of a molecule, the greater the influence of the alternating electrical fields on it, and the faster is the heating process. Water has a dipolar value of 6.17, ethanol-5.64, and 2-propanol-5.54. On the other hand, pure paraffin has a dipolar moment of 0, which means that its molecular structure is not influenced by contact with microwaves.[7]

Microwavescan either pass through something with little or no effect, or they can be reflected or absorbed. Some substances, such as plastics, glass, and paraffin pellets, are considered "microwave transparent" because they remain unaffected when exposed to microwave energy. Other substances, such as metal, will reflect microwaves. When substances absorb microwave energy, they become excited and generate internal heat. It is widely accepted that as the microwave energy is absorbed in tissues, it is converted into kinetic and chemical energy.[1]

The three types of substances based on the above principle are:

1. Microwavetransparent, e.g.: Plastic, glass, paraffin pellets
2. Microwavereflectant, e.g.: Metals
3. Microwaveabsorbent e.g.: Tissues, proteins

Microwaves have been applied in various fields of histopathology like fixation, histoprocessing, rapid staining (routine, metallic, and fluorescent) for both light and electron microscopic studies, immunolabeling, and antigen retrieval.[2,4]

COMPARISON OF DOMESTIC MICROWAVE OVEN WITH LABORATORY MICROWAVE OVEN

Both domestic and laboratory devices can be used to perform many of the procedures in a routine histology laboratory, but safety, reproducibility, and sample quality are important considerations when selecting the best device for your operation. The frequency of 2.45 GHz was selected for household microwave ovens because it is the frequency at which polar molecules, especially water molecules, respond strongly and the microwaves maintain good strength even at great depth. This capability is essential for cooking food and is also practical for histology laboratory work. Domesticmicrowave oven is quite economical than the laboratory oven and gives almost the same results as that of the latter one.[8] Calibration of domestic ovens is essential for optimum results and the accuracy of the temperature probe, duration of cycle time, and net power levels at various settings must be determined before the oven is used to process tissues, wherein the laboratory ovens are preprogrammed for various procedures.[1]

Unlike domestic microwave ovens, the laboratory microwave oven does not produce hotspots or uneven heating in tissues due to the presence of magnetic stirrer kept beneath which provides an even field of irradiation.[8] Toxic and flammable solvent vapors generated during processing cannot always be adequately vented from domestic ovens and present an ignition hazard if the electrical system is unprotected, unlike laboratory ovens wherein adequate ventilation is created for the escape of fumes.[1]

Application of microwave infixation

The main aim of fixation is to prevent, or atleast arrest the autolysis of tissues and thereby maintain the tissues close to their living state. This can be achieved by cross-linking of proteins, which makes the proteins insoluble.[7,9] Application of heat causes partial denaturation of proteins, thereby helping in the histological fixation of tissues.[7]

Mayers first developed a method to determine whether the theoretical possibility of producing histological fixation by microwave heating could be achieved in practice.[10] Leongfirst reported satisfactory results of microwave fixation of surgical and autopsy specimens.[11] Subsequently, many authors reported encouraging results in tissue fixation for both light and electron microscopy,[12-15] immunohistochemistry,[16-18] and histochemistry.[18,19] Marani *et al*. introduced the term 'stabilization' when chemical fixatives arenot used in the microwave method. The word 'Fixation' is applied 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" has been suggested.[10] In most of the cases, microwave irradiation has been used to enhance the diffusion of a chemical fixative into tissue.Since the time of discovery of microwave-assisted fixation, there has been no chemical fixative that could be directly microwaved which would give morphological preservation equivalent to formaldehyde. The use of formaldehyde in microwave ovens is strongly discouraged, even in microwave devices designed to vent hazardous fumes while the solution is inside the cavity, due to the inhalation risk from evaporating hot formaldehyde fumes as they are being removed from the instrument. Novel glyoxal-based fixatives (*Tradename*: ID XL Plus) which do not evaporate, even at the elevated temperatures, have been introduced in microwave fixation.[8]

Microwavetechnology was first applied in the field of histopathology in a study of histological fixation of fresh specimen using microwave heat. There was no loss of microscopic detail and the staining was uniform throughout. The shrinkage noted was slight and customary artifacts were slight or absent. The approximate time taken for fixation of tissue less than 5 mm in size is 20 min.[20]

The speed with which MWs can accomplish fixation of both large and small biopsy specimens is a major asset. The following procedures can be adopted for large throughput laboratories with requirements of a high speed of turnaround.[21]

1. Specimens continue to be sent to the laboratory in 10% buffered formalin, a necessary precaution to avoid autolysis which may result from delays and other mishaps that occur during the transportation of fresh specimens.
2. Following examination and sampling, 2 mm-thick specimens are placed in cassettes, completely immersed in normal saline, irradiated to a temperature of 62°C, and held at this temperature for 30 s. For convenience, 20 cassettes are placed in each of three beakers of saline, equidistantly located at the periphery of the oven's rotating dish. Although morphological preservation is slightly better at higher temperatures, 62°C appears to allow optimal preservation of tissue antigen.

By simultaneously affecting the complete tissue block, it is able to heat within the sample, thus avoiding time-dependent gradients of fixation. The increased temperature coagulates and precipitates the proteins and the microwaves stabilize the proteins' non-covalent secondary bonding. It minimizes long-term fixation artifacts such as extraction of cellular components. Immunohistochemical staining performed to demonstrate more stable cytoplasmic antigens revealed no significant difference between microwave fixations and formalin fixation. Electron microscopy of microwave-stimulated fixed tissues shows well-preserved ultrastructural architecture, fine cellular details, and sharply demarcated cytoplasmic structures and nuclear membranes compared to those fixed by the conventional technique.[12]

Application of microwaves in the processing of tissues

Tissue processing performed permits sectioning of tissue into thin sections so as to be visualized microscopically. This consists of a series of steps wherein tissues pass through various reagents, which will finally permit sectioning.[7,9] Diffusion is the key to processing. Diffusion of reagents can be increased by the application of heat, which in turn, reduces the time.[4,5] Microwave histo processing relies on the principle of using microwave energy to speed up the process of the diffusion of liquids into and out of the specimens. As opposed to conventional tissue processors, which use a graded series of alcohols, a clearing agent, usually xylene and paraffin wax in an overnight process, microwave histo-processing employs just three reagents as mentioned below in four step processinvolving single change in ethyl alcohol andisopropanol and two changes in paraffin.[22]

1. 100% ethyl alcohol for dehydration
2. Isopropanol for the intermedium
3. Liquid paraffin for infiltration

The alcohol can be used several times and the paraffin can be reused many times, possibly for months.Clearing solutions are not necessary because the alcohol is evaporated from the tissue before paraffin infiltration.[1] Paraffin must be added to the microwave in liquid form as microwave energy will not melt paraffin pellets.[7]

The microwave tissue processing reproducibly yielded histological material comparable in quality to conventional tissue processing. Moreover, use of microwave tissue processing enhanced safety by eliminating formalin and xylene from the procedure.The approximate time taken for processing is[23]

1. Short Schedule-15 min and
2. Long Schedule-60 min

The effect of conventional and microwave tissue processing on cytoplasmic and nuclear details of various tissues like epithelial, fibrous, and glandular tissues, showed no statistically significant variation.[8,24,25] According to Boon *et al.*, in microwave-processed tissues, epithelium was of better quality than the conventionally processed one, however, stroma showed focal condensation.[24]

According to Panja *et al.*, tissue processed by microwave method showed statistically significantly less shrinkage compared to tissues processed by conventional techniques. However, Kok *et al.*, showed no significant difference in the amount of tissue shrinkage in the conventional and microwave-processing techniques.[24]

Application of microwave instaining

Obtaining good histological images for successful interpretation is largely governed by good sample preparation

and staining.[7,9] Staining of tissue sections and cell preparation is based on diffusion of dye into the tissue and its binding to the substrate.[9] Microwave irradiation has been beneficial for both. Microwave irradiation can be applied for accelerating routine, special, metallic, as well as immunofluorescent stains.[5] Staining methods that normally take minutes can be done in a microwave oven in seconds; those that take hours, in minutes; and those that take days or even weeks can be completed in a matter of hours using microwave techniques. The optimum temperature for most non-metallic stains is between 55° and 60°C and for metallic stains between 75°C and 95° C.[7]

Microwave-accelerated processing is as effective as slower traditional staining, reduces the time up to 70% and sections stain identically with several methods such as Periodic acid–Schiff's, Van Gieson, Congo red, Masson's trichrome, Alcian blue, Mayer's mucicarmine, and silver methods.[1]

ANTI GENRET RIEVAL

- Shi *et al*. in 1991, first established the use of microwave heating for antigen retrieval. Buffers used are citrate buffer, EDTA etc. Epitope retrieval is a complex subject. It should be stressed that the retrieval method employed must be tailored to the antibody markers to be demonstrated and the detection system used.[9]
- FDA-Food and Drug Administration approved methods must be followed precisely or a disclaimer must be included in the pathology report indicating that the results reflect a departure from the approved method. Despite this, many have found microwave technology to be beneficial in achieving epitope recovery in formalin-fixed tissues for some markers.[9]
- Various researchers have found microwave technology to be beneficial in achieving epitope recovery in formalin-fixed tissues for many markers within 10 min to 15 min.[9]

ADVANTAGES OF MICROWAVE[26]

For the histologist
- Improved workload distribution
- Process as required for a more even workload distribution
- Flexibility
- Process 55-110 cassettes
- Easy sectioning

For the pathologist
- Microwave-processed slides enable the pathologist to deliver same-day diagnosis of lesions
- Same-day diagnosis will enhance the pathologist's role in management of the cancer patient

For the lab administrator
- Improved work environment for laboratory personnel
- Reduced cost for reagents storage and disposal

For the clinician
- Within hours, oncologists and clinicians can advise patients on the base of definitive diagnosis
- Treatment can be initiated immediately

For the hospital administrator
- Reduced patient anxiety and stress by providing results within hours
- Dramatic improvements in efficiency and laboratory productivity

For the patient
- Elimination of needless stress while waiting for a diagnosis
- More timely start of needed treatment

SAFETY[26]

- Converting to the chemicals safe, i.e., less fumes, non-regulated disposal may include rotating smaller quantities more often, causing a net increase in chemical consumption.
- The safety benefits of removing undesired regulated waste in addition to calculating net volumes may offer immediate cost savings.

DISADVANTAGES OF MICROWAVE[26]

- Microwaving tissue in formalin releases large amounts of dangerous vapors
- Expensive
- Requires proper use of calibration and monitoring

GUIDELINES[27]

Always…
- Use a laboratory microwave for laboratory work
- Connect chamber-venting system to external fume-removal system
- Use manufacturer-approved containers
- Use vented or uncovered containers when not using vacuum [Figure 1]
- Handle containers with potholders
- Use a fume hood to work with hazardous reagents such as formalin

Periodically…
- Use a microwave leakage detector to check for leakage
- Inspect/clean chamber, door seal, and hinges
- Inspect/clean air intakes and filters (if so equipped); replace filters as necessary
- Check samples (staining, processing, unmasking, etc.) against controls

To be avoided…
- Never use a consumer-grade microwave for laboratory work
- Do not cover containers tightly

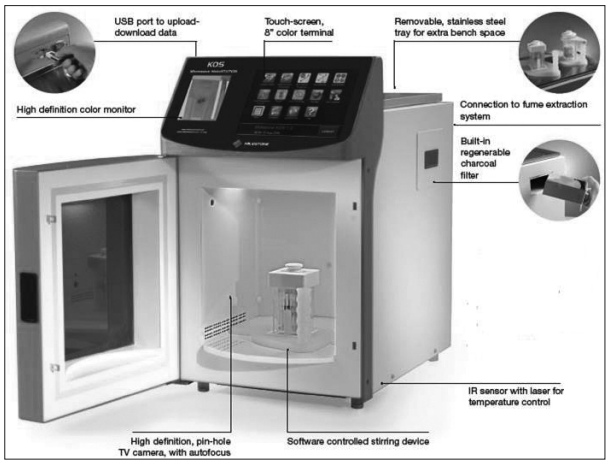

Figure 1: Model of microwave rapid histoprocessor

- Do not operate the microwave without a load
- Never use metal accessories inside the unit
- Avoid heating food items in a microwave oven used for laboratory procedures
- Never breathe warm reagent fumes, especially formalin

CONCLUSION

Rapid processing of histopathologic material is becoming increasingly desirable to fulfill the needs of clinicians treating acutely ill patients. Traditional techniques for rapid processing of paraffin-embedded tissues require 4 h to 5 h, delaying treatment for some critically ill patients and requiring additional shifts of technologists in the laboratory. microwaveprocessing further shortens this time, allowing even more rapid histopathologic diagnosis. It is encouraging to see the growth of this beneficial technology in our discipline. When used properly, it can decrease the turnaround time and reagent costs tremendously. Most tangible of all, perhaps, is the diminished wait by patients for their diagnosis which makes microwave technology a place in today's laboratory.

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