Microwave: Useful in kitchen / Pathology Lab?

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Abstract:

Conventional tissue processing of histologic specimens has been carried out in the same manner for many years. It is a time-consuming process involving batch production, resulting in a 1-day delay of the diagnosis. Microwave-assisted tissue processing enables a continuous high flow of histologic specimens through the processor with a processing time of as low as 1 h. In this article we review the working principle and various applications of domestic microwave in pathologic laboratory.

Kev words: Microwave, Tissue processing, Fixation ,Turn around time

Introduction

We have come a long way from the time the conventional tissue processing was proposed in the 19th century to frozen sections to automatic tissue processor to the successful application of microwaves in the field of histotechniques for fixation and then processing.[1, 2]

The microwave used for histotechniques works on the principle that electromagnetic field causes excitation of molecules which brings about its rotation. This produces energy in the form of heat from within the materials. This heat enhances the rate of diffusion of fluids in and out of the tissues blocks or sections even more effectively in contrast to conventional heating.[3,4,5]

Tissue processing for microscopic examination

Tissue processing for microscopic examination of tissues is based on the physical and chemical process[6] and is greatly influenced by:

- The type of reagents used for processing
- The viscosity of these reagents
- Diffusion of these reagents in and out of the tissue, which is in turn accelerated by
    - Agitation of fluids
    - Vacuum Heat. [7]

There were many investigations conducted in the past to select suitable processing fluids. An experimentally controlled study was conducted on a series of various dehydrating agents namely dioxan, isobutyl alcohol and ethyl alcohol with chloroform. Except for dehydration, the tissues were treated identically. They found that dioxan proved to be a better dehydrating agent. It was concluded that the shrinkage and hardening of tissue so often attributed to the hot paraffin was directly related to the method

of dehydration and preparation of tissue for immersion in the melted paraffin rather than to the effect of the paraffin.[8]

Another comparative study on some dehydrating and clearing agents was conducted in order to determine their advantages namely Dioxan, isobutyl alcohol, tertiary butyl alcohol, ethyl alcohol as dehydrating agents and chloroform, toluol, xylene, benzol, methyl benzoate, methyl salicylate and acetone as clearing agents were used in the study and they found that slow dioxan was the best method of dehydration and ethyl alcohol-chloroform mixture produced rapid dehydration and impregnation within 17 hours without distortion of tissues.[9]

A study conducted described modified paraffin wax for microtomy. This was based on the findings that that certain dyes like phenanthrene when added to wax-to facilitate photography appeared to have the ability to reduce compression. Phenanthrene- a colorless crystalline aromatic hydrocarbon obtained by distillation of coal-tar and was used as a substitute for oil red O. Adding phenanthrene to paraffin wax produced an infiltrating and embedding medium superior to unmodified wax with smoother texture, reduced compression and improved adhesion of wax. This was responsible for the absence of lifting, creasing and displacement of tissues.[10]Serious problem of compression of tissue was noted while sectioning tissues embedded in various embedding medium including paraffin wax ester wax, water soluble wax, resin, agar, gelatin, celloidin etc. In order to overcome this double embedding technique using paraffin wax with different resins was described. It was found that this double embedding technique was applicable for many tissues and very easy to use. The results obtained were excellent and was used routinely in their laboratory.[11]

Methods and technique for early diagnosis

As early diagnosis and initiation of emergency therapy is based largely on histopathological findings, there is often a request for urgent reports. Many methods and techniques have been explored in the past in this regard.

It includes

1. Frozen sections
2. Rapid tissue processing method,
3. Automatic tissue processor and
4. Microwave.

Frozen section technique

Frozen section technique produces sections of fresh tissues without the use of fixative, dehydrating solutions, clearing agents and embedding media. The principle advantage of frozen sections is the speed of preparation of tissue block and is a one step process using either liquefied nitrogen (-190^{0}C) or carbon dioxide (-70^{0}C).[7,12,13] Frozen sections were originally produced for histological techniques, later were used to demonstrate soluble substances and were adapted for rapid reporting of urgent biopsy specimen like intra-operative histopathologic evaluation. But the disadvantage of frozen sections was the ultimate poor quality

of light microscopic images because of the damage to the sections that made histopathological interpretation difficult. It was also difficult to prepare and cut certain type of tissues like fatty tissue.[12,13]

Rapid tissue processing method

A rapid method of tissue processing was described based on the casual observation that tissues taken from certain dehydrating agents to hot wax under vacuum were satisfactorily impregnated in relatively short period of time even without the use of an intermediate wax solvent. This lead to impregnation of tissues within 5-15min. It was also noted that the quality of the sections of tissues obtained by this technique was superior to that produced by the conventional method and hence was suitable for processing biopsy material rapidly. Such rapid reporting made possible by modification of routine processing technique permits processing of specimen and production of H & E stained slides in approximately few hours. But it was associated with the disadvantage that it was limited only to small biopsy specimen.[14]

It was also noted that acidified dimethoxypropane could be used as a combined dehydrating and clearing agent, permitting tissues to be led to paraffin for less than an hour after fixation is completed. Dimethoxypropane reacts chemically with water thereby ensuring that the tissues become completely dehydrated. Histology and histochemistry of tissues after the dimethoxypropane procedure is equal to that after conventional dehydration and clearing.[15]

Automatic tissue processor

Half a century ago automatic histoprocessor was invented which revolutionized histoprocessing by reducing processing time of all biopsy specimens from several days to 16-18hrs except for very large tissues. Constant agitation of tissues in this machine is responsible for superior results obtained. There are two advantages of automatic tissue processor i.e., the tissues are mechanically transferred from reagent to reagent both in the day as well as in the night and continuous agitation of the reagents reduces the time required in each reagent. This method eliminates possibility of human errors and forgetfulness. The machine may offer other advantages like vacuum and heat enclosed in the automatic processor. Heat and vacuum speeds up the whole procedure to just 2-3 hrs. The tissues may be routinely processed overnight, so that it is ready for embedding in the morning. These autoprocessor are not generally used in institutions and lab where the number of tissues to be processed is small. Power failure affects the processing schedule. Very large bits of tissues cannot be processed efficiently in autoprocessor.[7]

Microwave assisted tissue processing

The latest addition in the list of techniques introduced for rapid processing of tissues is microwaves, which has revolutionized histotechniques.

History

The term microwave seems to have first appeared in writing in the first issue of Alta Frequenza.[4] The original magnetron- the main functional unit of microwave was invented at the GE Research Laboratory in

1916. The microwave oven was invented in 1945 for which US patent award was awarded in 1950.[3] Microwave technique was first applied in the histopathology for fixation of tissues in 1970 by Mayers[1] and for processing of tissues in 1985 by Kok & Boon.[2]

Microwave assisted tissue processing

Microwaves are non-ionizing electromagnetic waves with frequencies ranging from 300 MHz to 300 GHz corresponding to the wavelength of 1m to 1mm respectively and all kitchen microwave oven operate at 2.45GHz with corresponding wavelength of 12.2 cm.[3,4,5]

Literature from Physics and chemistry suggest that the viscosity of liquid decreases at constant pressure and absolute temperature thereby increasing diffusion and heat are known to increase diffusion. Heat has known to increase diffusion[3,4,5,6,7] and so initially conventional heating was employed into histoprocessing in order to achieve increased diffusion thereby reducing the processing time. But this led to uneven distribution of heat energy, which resulted in hardening of outer layer whereas the central part remained unprocessed, and therefore soft.[3]

Microwaves works on the same line by causing 'rotation of water molecules[3,4,5] wherein one molecule of water has one big atom of oxygen to which, two little hydrogen atoms are attached

Water molecules have a positively charged side and a negatively charged side[4], so, when negative charges are brought near electromagnetic field, there is repulsion as they are like charges, causing molecules to rotate as they are asymmetrical . The charges in the electric field are subject to a force from same direction or at 180^0 from electric field depending on the sign of the charge.[5] This is true even for dipole of water molecules. Polar molecules are subject to torque in electric field itself. Energy for this is provided by the field. 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 surrounding. As the molecules slow down in its rotation it causes frictional forces, which, produces heat energy. Unlike conventional heating, heating in microwave is from within (internal heating) and its effect occurs throughout the material being irradiated.[3,4,5]

Application of microwave in fixation

The main aim of fixation is to prevent or atleast arrest autolysis of tissues and thereby maintain the tissues close to their living state. This can be achieved by cross-linking of proteins, which make proteins insoluble.[6,7]

Microwave technology was first applied into the field of histopathology in a study of histological fixation of fresh specimen using microwave heat. There was no loss of macroscopic detail and the staining was uniform throughout. The shrinkage noted was slight and customary artifacts were slight or absent. Cellular and

nuclear details were well preserved but there was unexplained disappearance of erythrocytes and collagen fibers to lesser extent.[16]

Thereafter numerous studies were conducted using microwave irradiation as a form of heat for histological fixation. In one study they found that the optimum temperature of fixation was different for each tissue.[17] A similar study for light and electron microscopy was conducted; here the tissues were irradiated in normal saline at 48 ^{0}C, which caused fixation of tissues with a quality comparable with that produced by conventional fixation using 10% formalin. The heat used did not have deleterious effects on special stains and produced less shrinkage artifact than conventional formalin fixation. Immunocytochemical staining performed to demonstrate more stable cytoplasmic antigens revealed no significant difference between microwave fixations and formalin fixation. Tissues samples for electron microscopy were immersed in 2.5% glutaraldehyde and fixed for 90s by irradiation to 50 ^{0}C. This showed excellent preservation of ultrastructural morphology.[18] A slight change was found in a study, which investigated and compared microwave fixation with formalin fixation for routine techniques, histochemistry, immunohistochemistry and electron microscopy. It was found that the tissues for electron microscopy showed some tissue damage whereas red cells were largely destroyed. But otherwise the tissues fixed by microwaves produced good histology and may be stained with the commonly used techniques. Although there may be some variation in color balance they can be used for pathological investigation of tissues.[19]
A comparative morphometric study was performed to evaluate this form of fixation of surgical specimen placed in various solutions against the conventional fixation in buffered formalin and they found that there was no statistical significantdifference in area and minimum diameter of nuclei between the different groups.[20]

Investigators who performed rapid fixation of large biopsy specimen using microwave stated that formalin can be completely eliminated from the autoprocessor based on the results of their study which showed no difference in the quality of sections.[21] Fixation of sputum sample was carried out using a commercial oven in less than 35minutes and the microscopical results were of superior quality. This method was successfully applied to all specimens with large amounts of mucous.[22]
Microwave fixation was also extended and combined with cryostat section. This technique was adopted to overcome the disadvantage of poor quality, which interfered with the histopathological diagnosis. This technique showed that the cryostat-microwave sections were excellent. The quality of light microscopic images was comparable to that of the best conventional paraffin technique. With this method the positive features of freezing and fixation with chemicals were maintained and the time consumed was less.[12] Similar studies were conducted by irradiation of cryostat sections and the results were comparable with that of conventional sections.[13,23]
The specimens fixed by fast and ultra fast method were evaluated by light microscopy and it displayed excellent morphologic preservation along with preservation of fatty tissue compared to the standard fixation. Fast method also preserved diagnostically useful membrane and intracellular antigens for detection by immunohistochemical technique.[24]

Clarification of the cause of vagaries of formaldehyde fixation in the microwave oven was described using five different formaldehyde containing fixatives which were evaluated using five different protocols. The beneficial effect of microwave irradiation was attributed to the acceleration of the reaction of formaldehyde in the tissues. The formation of free formaldehyde by the dehydration of methylene glycol present in thetissues was enhanced when irradiated and the described technique led to uniform microscopical results.[25]

A three step method for fixation of brain using microwave irradiation was reported where the first two steps hardened and solidified brain tissues and step 3 completed formalin fixation.[26] This enabled production of microscopic slides of human brain tissues within one day. The efficacy and precision of the method was compared with slides of conventionally processed brain tissue that had been fixed in formalin for six weeks. The quality of microscopic section was excellent with good preservation of brain tissue and equaled that of conventionally processed slides. As an attempt to improve ultrastructure, microwave irradiation was applied during aldehyde fixation in various steps of electron microscopic procedure. The results showed a more life like ultrastructural preservation. Peroxidase like activity in erythrocytes, acid phosphatase activity in resident macrophages and peroxidase activity in monocytes granules were apparently not influenced by microwave irradiation.[27]

A comparison of in vivo phosphorylation state of several phosphoproteins by focused microwave irradiation was conducted. Based on the results focused irradiation sacrifices may be required to achieve biologically relevant data for the in vivo phosphorylation state of many phosphoproteins.[28]

Application of microwaves in processing of tissues

Microwave technique was first applied for processing of tissues to prepare tissues blocks for paraffin sections within 30minutes. This method was based on the microwave stimulated diffusion reducing the dehydrating, clearing and impregnating time. The histotechnical microwave results showed excellent light microscopic results and were indistinguishable from those of the well-performed classical method. The nuclear size of several cell types hardly differed in both methods.[2] A novel method of preparing tissue blocks for paraffin sections within 30-60 minutes was proposed in the early 1985. More than 2 years additional experience of testing various microwaves ovens led to the new protocols reported by these authors. The microscopic results of these methods were excellent even for fatty tissues. The dense fibrous tissue was more coagulated in microwave method than in the conventional techniques however, this did not have any effect on the diagnostic pathology.[29]

These existing microwave irradiation protocols were adapted and employed in processing and immuno-electron microscopy of both plastic embedded and frozen sections and they found that the use of microwave irradiation greatly shortened the fixation, processing and immunolabelling times without compromising the quality of ultrastructural preservation and the specificity of the labeling.[30]

A comparative study on the microwave technique and conventional technique was performed for which a kitchen microwave was used for fixation and for histoprocessing. They employed five different protocols; each protocol had different reagents and with different time of irradiation. The experiment was divided into

different phases depending on the use of different protocols and different powers of microwave. They found comparable histoprocessing results with kitchen microwave oven as against the conventional technique.[31]
Later studies conducted evaluated the quality of histological section and advantages of microwave processing and compared with the routine tissue processing. It was found that the microwave processing considerably shortens the preparation time for permanent histological section without demonstrable decrease in section quality or readability. The overall quality of microscopic tissues of the traditional processing and the microwave-processing method was similar and it was not possible to distinguish between the two techniques.[32]

Following this, a rapid new method of tissue processing using a new continuous, high- throughput technique of 1 hour tissue processing was described. Here common histological reagents were combined (excluding formalin and xylene) and irradiated with microwave energy to develop this rapid processing method. The quality of histomorphology, histochemistry, immunohistochemistry and RNA content of processed tissue was comparable with that of adjacent tissue sections processed by conventional processing technique. The new technique preserved RNA better than the conventional method. It reduced the processing time to about 1 hour from the fresh or prefixed tissues and also eliminated the need of formalin and xylene and reduced the volume of other chemical.[33]

Subsequently experiments were conducted to compare objectively the quality of ultrastructural preservation of mouse retinas processed using conventional and microwave-assisted techniques. They found that the overall ultrastructural preservation of the retina was similar for the conventional and microwave assisted technique. The artifacts were significantly reduced with microwave irradiation during fixation and processing and there was superior ultrastructural preservation with substantial reduction in the time required for sample preparation.[34]

Validation of histological quality and its positive impact on the turn around time of surgical pathology reports by the investigators on their new technique was documented and published. In this they studied the effect of a fully automated microwave assisted rapid tissue processor on histologic examination and the turnaround time for surgical pathology reports. They found that the rapid tissue processing reproducibly yielded histological material comparable in quality to conventional tissue processing. Moreover, use of rapid tissue processing enhanced safety by eliminating formalin and xylene from the procedure.[35]

Application of microwave in staining

Obtaining good histological images for successful interpretation is largely governed by good sample preparation and staining.[6,7] Staining of tissue sections and cell preparation is based on diffusion of dye into the tissue and its binding to the substrate.[6] Microwave irradiation has been beneficial for both. Microwave irradiation can be applied for accelerating routine, special, metallic as well as immunofluorescent stains.[5]
A modified and speeded up the staining of ultra thin sections for electron microscopy was performed using microwave irradiation. The slides so stained had more contrast, less artifact in the form of precipitate and more uniform overall staining. They found that the overall image quality of the transmission electron

micrographs generated by quick stained microwave enhanced sections was better than the routine stained sections. This technique reduced the time by approximately 39 minutes per specimen, without sacrificing the quality thereby increased the laboratory efficiency with a decrease in cost as well.[36] Similarly a new approach to the Ziehl-Neelsen stain using microwave oven for the stage of heating in carbolic magenta was conducted. The smears flooded with carbolic magenta were exposed to microwave irradiation for 30 seconds at full power of 640 Watts. The staining of sections of tissue by microwave method with carbolic magenta or magenta was comparable with the results obtained by the conventional Ziehl-Neelsen method, but 1% saffron was not suitable for histological sections as the dye was not retained when the sections were treated with acid alcohol.[37]

A modification of staining technique for Perl's, Alcian blue, Fontana-Masson and Romanowsky-Giemsa stain using microwave stimulation was described. It was found that this stain modification proved to be highly successful in daily practice for individual cases in which fast diagnosis is required. With this method the total time for staining is a matter of few minutes at the most. Results obtained were excellent and consistent and the method was economically advantageous as only small quantities of staining solutions were used.[38]

Conclusion

To conclude microwave assisted tissue processing yields histologic material of similar or superior quality to that provided by time-honored conventional processing. It has many advantages, including expediency, safety, potential for preservation of molecular integrity of specimens that might be used in subsequent studies, and improvement in the workflowof the laboratory, permitting the preparation of diagnostic material during the day at family-friendly hours.[33]

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