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RESEARCH ARTICLE

Kitchen Microwave-Assisted Accelerated Method for Fixation and Processing of Oral Mucosal Biopsies: A Pilot Study

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ABSTRACT

Background and aim: Conventional protocols of formalin fixation and processing add time and cost to histoprocessing. This study aims to evaluate an indigenously developed protocol for microwave histoprocessing and compare it with the routine protocol for histoprocessing.

Materials and methods: A total of 40 human buccal mucosal specimens were processed according to the microwave and the routine protocol. Sections obtained were coded and evaluated for the following: Section quality, staining quality, artifacts, cellular details in epithelium, nuclear detail and diagnostic ability. Parameters were graded as suboptimal, optimal and good, and results were statistically analyzed.

Results: The number of good and optimal specimens in both the protocols were almost similar for all parameters assessed with better results for cellular detail, nuclear detail, staining and sectioning quality seen in microwave-fixed samples. Artefacts were lesser in routine processing protocol.

Conclusions: Microwave alcohol fixation protocol provides good and faster fixation compared to routine fixation.

Keywords: Microwave, Fixation, Processing, Isopropyl alcohol.

INTRODUCTION

Formalin fixation has for long been considered the gold standard in routine tissue processing. Good preservation of morphology, ease of use and ready availability are its strengths.[1] The three basic steps of dehydration, clearing and infiltration, subsequent to formalin fixation permit tissue samples to be embedded in a solid medium, such as paraffin wax. Routine fixation and processing techniques usually involve an overnight fixation in formalin followed by processing which takes at least 6 to 8 hours for sections to be ready for reporting.

Diffusion is a key factor in histoprocessing permitting chemicals to permeate into the tissue faster. Increased temperatures decrease the viscosity of the processing fluids and thereby facilitate diffusion. Hence, it is theoretically possible to hasten tissue fixation and processing through the use of heat.[2] Conventional heat, because of its nonuniform heating, is unsuitable for this purpose. Microwave oven generates heat from within (internal heating) and warm the object uniformly. This has resulted in a substantial reduction in the basic steps of histoprocessing thereby reducing turnaround time and permitting same day reporting.[3]

The potential application of microwave energy was first recognized by Mayers in 1970, who successfully fixed tissue with a microwave generator used in physiotherapy.[4] The technique of microwave-assisted tissue processing has been achieving great acceptance over the last decade and this has led to the production of commercially available microwaves specifically designed for tissue processing, however, the cost involved in these is high.[4,5]

Domestic microwaves are readily available, affordable and have been used earlier for tissue processing with good results. We have earlier found excellent results with microwave processing of oral biopsies.[6] Fixation of tissues using formalin followed by clearing using either chloroform or xylene carries a high degree of risk because of the toxicity of fumes involved. Alcohol, on the contrary, is safer and frequently used in microwave tissue fixation and processing.[1,4,6]

The present study uses a single chemical (isopropyl alcohol) and a domestic microwave oven for tissue fixation. The purpose of this study was to document the usefulness of a microwave-assisted accelerated method for tissue fixation and processing protocol developed indigenously in our laboratory and to compare it with the routine protocol.

BRIEF REVIEW OF LITERATURE

Microwave heating depends on oscillating or exciting polar or charged molecules. Microwaves force dipolar molecules of proteins to rotate through 180° at the rate of 2.45 billion cycles per second.[7-9] The molecular kinetics induced results in generation of instantaneous heat that is proportional to the energy flux and continues until radiation ceases.[10] The microwaves which stimulate polar molecules cause collision with adjacent molecules which causes part of the rotational energy to be transferred to them. This friction expresses as heat.[9] Unlike conventional heating, this effect occurs simultaneously

throughout the whole material being microwaved.[7] Application of microwave energy in histotechnology was first recognized by Mayers in 1970, who successfully fixed human and mouse tissue with a microwave generator used in physiotherapy.[3] At the same time, Stavinoha employed microwaves to stop enzymatic degradation by acetylcholinesterase in rodent brain. The most detailed early studies were published by Bernard in 1974, who successfully produced fixation of a wide variety of organs by microwave irradiation.[11,12]

Iesurum et al studied an ethanol-based fixative with microwave stimulated processing in postmortem material. Their results indicated good preservation of morphologic details, histochemical and antigenic properties of the tissue which were comparable with formalin fixed material. The rapid stabilization of the tissues by microwave irradiation was very useful in forensic pathology. The microwave irradiation prevented autolysis and putrefaction and reduced the artefacts. Ethanol was found to be a good preserving agent of nucleic acid and protein material, and may be used for proteomic analysis. It also provided a safer working environment.[13] Various investigators analyzed the use of domestic microwave for urgent histo-processing and the concurrent use of ethanol and isopropyl alcohol, and found that ethanol was a good dehydrant and isopropyl alcohol was effective as both dehydrant and clearing agent, the slow rate of diffusion being overcome by microwave heating. The histology resembled the routinely processed sections, thus requiring no special experience for the pathologist. A very good quality special staining and immunohistochemical staining could be performed. The limitations found were sampling errors, tissues containing fat responded poorly to microwave processing, absence of standardized temperature, power control and nonavailability of vacuum processing.[14-16]

Munkaholm et al evaluated the effect of the automated microwave-assisted tissue processor on the histomorphologic quality and the turnaround time (TAT) for histopathology reports and concluded that not only the TATs for histopathology reports improved but also for most tissue types the microwave-assisted processing method yielded histologic material comparable in quality to tissue processed by the conventional method. Furthermore, the new technique diminished the use of formalin and xylene in the procedure, thus reducing the potential risk to health.[17]

MATERIALS AND METHODS

Prior ethical clearance for the study was obtained from the college ethical clearance committee. The total study group consisted of 60 samples. The first 20 samples were from the buccal mucosa of goats. A biopsy punch was specially designed to obtain specimen of 5 × 5 mm and 10 × 10 mm. Five specimens, each of 5 × 5 mm and 10 × 10 mm, were processed to the microwave protocol and the routine protocol. These specimens were used to standardize the microwave processing technique.

Remaining 40 human buccal mucosal biopsies formed the core study group. The specimens were measured and divided into two equal halves and processed according to the microwave protocol and the routine protocol as listed below. The microwave fixation was done using a Samsung 12 lt domestic microwave (Fig. 1).

Fig. 1: Samsung kitchen microwave, 12 l

Protocol for microwave fixation: Tissues measuring 5 mm or less

- 100% Isopropyl alcohol: 10 minutes
- Molten paraffin wax: 10 minutes
- Maximum of three specimens processed at a time
- Microwave setting: 100 Watt
- Total time taken: 20 minutes.

Protocol for microwave fixation: Tissues measuring greater than 5mm

- 100% Isopropyl alcohol: 2 × 20 minutes
- Molten paraffin wax: 2 × 20 minutes
- Maximum of three specimens processed at a time
- Microwave setting: 100 Watt
- Total time taken: 80 minutes.

Routine protocol used in our laboratory

- Overnight fixation in 10% formalin
- One hour each in graded alcohol (70%, 80%, 90% and absolute × 2 changes) for dehydration
- Three hours in xylene (2 changes) for clearing
- Four hours in paraffin wax (2 changes)
- Total time taken 24 hours.

Around 5µ sections were obtained from all the samples. All sections were stained with hematoxylin and eosin stain. The hematoxylin staining time for the microwave fixed samples was standardized to 40 seconds for optimum staining as against 10 minutes for the routine fixation.

The samples were then coded and evaluated by three oral pathologists who were blinded to the nature of fixation and processing. All the sections were evaluated for the parameters including section quality, staining quality, artifacts, cellular details in epithelium, nuclear detail and diagnostic ability. Parameters were graded as suboptimal, optimal and good by three independent observers blinded to the nature of fixation

and processing. The results obtained were subjected to a Chi-square test. All the statistical methods were carried out through the SPSS for Windows (version 16.0).

RESULTS

When we compared the two fixation protocols, similar results were found between the microwave alcohol and routine protocols for all parameters examined. A total of 24 (60%) samples of the microwave alcohol and 23 (57.5%) samples of the routine fixed samples were found to be diagnostic (Table 1). The number of specimens considered poor for diagnosis were also similar for both the protocols.

Larger numbers of samples were graded as good/optimal in the microwave fixed tissue as compared to routine fixation in all parameters evaluated other than in the occurrence of artifacts.

The section quality was better in 25 (62.5%) of the microwave fixed specimen when compared to 22 (55%) of the specimen fixed using the routine protocol (Table 2). Better cellular outlines in 24 (70%) and nuclear details in 30 (75%) of the specimens were seen in the microwave processed samples (Tables 3 and 4).

The staining quality was found to be good or optimal in 24 (60%) when compared to the routine processing which showed 22 (55%) samples with good or optimal staining (Table 5).

The microwave fixed samples showed slightly more eosinophilia of the cellular cytoplasm and accentuated clumping of chromatin. Artifacts (sections folds, heat artifacts) were also more in number in the microwave-processed samples 16 (40%) while the routine protocol showed 13(32.5%) of cases with artifacts (Table 6).

No statistically significant differences were observed in any of the parameters assessed (Tables 1 to 6).

DISCUSSION

Newer techniques in histopathology and advances in science have enriched the practice of pathology during the last few decades. Morphologic expression of disease, however, has remained the mainstay of diagnostic pathology. The handling of tissue samples from surgical removal to slide preparation has largely remained impervious to scientific advances. In particular, formalin fixation followed by conventional processing has been the standard for almost 100 years.

Substantial shortcomings associated with this technique include:

- Delay in providing diagnosis (at least 1 day)
- Reagent toxicity
- Degradation of nucleic acids.[18]

Modifications to routine processing have allowed the processing of small biopsy specimen in approximately 5 hours but this involves the use of costly equipment and is relatively labor intensive.[5] Alternatives to this, such as cryostat, are also largely prohibitive because of the cost involved.

Microwave-assisted fixation and processing represents a paradigm shift by simplifying and reducing the sequential steps involved in conventional processing, i.e. fixation, dehydration, clearing and impregnation. This technique permits a more rapid completion of fixation, dehydration involves a single step instead of multiple graded solutions of alcohol and paraffin impregnation occurs at a higher temperature speeding the process. The residual alcohol is boiled out by the microwave energy during

Table 1: Diagnostic ability of tissues fixed using microwave alcohol and routine fixation

Microwave alcohol (n = 40)	*Routine (n = 40)*
Good: 4 (10%)	Good: 3 (7.5%)
Optimal: 20 (50%)	Optimal: 20 (50 %%)
Suboptimal: 16 (40%)	Suboptimal: 17 (42.5%)
X^2 (Microwave alcohol) = 4.951; $p < 0.084$ (NS)	X^2 (Routine) = 6.725; $p < 0.035$ (S)
X^2 (Overall) = 0.240; $p < 0.887$ (NS)	

NS—non significant; S—significant

Table 2: Comparison of section quality between microwave alcohol and routine fixation

Microwave alcohol (n = 40)	*Routine (n = 40)*
Good: 4 (10%)	Good: 3 (7.5%)
Optimal: 21 (52.5%)	Optimal: 19 (47.5%)
Suboptimal: 15 (37.5%)	Suboptimal: 18 (45%)
X^2 (Microwave alcohol) = 5.201; $p < 0.074$ (NS)	X^2 (Routine) = 6.667; $p < 0.036$ (S)
X^2(Overall) = 0.693; $p < 0.707$ (NS)	

NS—non significant; S—significant

Table 3: Assessment of cellular outline in microwave alcohol and routine fixation

Microwave alcohol (n = 40)	*Routine (n = 40)*
Good: 4 (10%)	Good: 3 (7.5%)
Optimal: 24 (60%)	Optimal: 20 (50 %%)
Suboptimal: 12 (30%)	Suboptimal: 17 (42.5%)
X^2 (Microwave alcohol) = 6.108; $p < 0.047$ (S)	X^2 (Routine) = 7.196; $p < 0.027$ (S)
X^2(Overall) = 4.351; $p < 0.114$ (NS)	

NS—non significant; S—significant

Table 4: Assessment of nuclear details in microwave alcohol and routine fixation

Microwave alcohol (n = 40)	*Routine (n = 40)*
Good: 4 (10%)	Good: 3 (7.5%)
Optimal: 26 (65%)	Optimal: 23 (57.5 %%)
Suboptimal: 10 (25%)	Suboptimal: 14 (35%)
X^2 (Microwave alcohol) = 6.785; $p < 0.034$ (S)	X^2 (Routine) = 7.196; $p < 0.027$ (S)
X^2(Overall) = 5.573; $p < 0.062$ (NS)	

NS—non significant; S—significant

Table 5: Comparison of staining quality between microwave alcohol and routine fixation

Microwave alcohol (n = 40)	*Routine (n = 40)*
Good: 4 (10%)	Good: 2 (5%)
Optimal: 20 (50%)	Optimal: 20 (50%)
Suboptimal: 16 (40%)	Suboptimal: 18 (45%)
X^2 (Microwave alcohol) = 4.951; $p < 0.084$ (NS)	X^2 (Routine) = 9.030; $p < 0.011$ (S)
X^2 (Overall) = 1.154; $p < 0.562$ (NS)	

NS—non significant; S—significant

Table 6: Comparison of occurrence of artifacts between microwave alcohol and routine fixation

Microwave alcohol (n = 40)	*Routine (n = 40)*
Good: 4 (10%)	Good: 3 (7.5%)
Optimal: 20 (50%)	Optimal: 24 (60%)
Suboptimal: 16 (40%)	Suboptimal: 13 (32.5%)
X^2 (Microwave alcohol) = 4.951; $p < 0.084$ (NS)	X^2 (Routine) = 8.179; $p < 0.017$ (S)
X^2 (Overall) = 1.099; $p < 0.577$ (NS)	

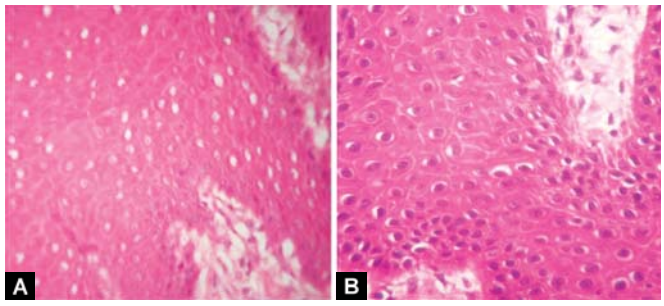
NS—non significant; S–significant

impregnation thereby eliminating the need for a separate clearing procedure.[5]

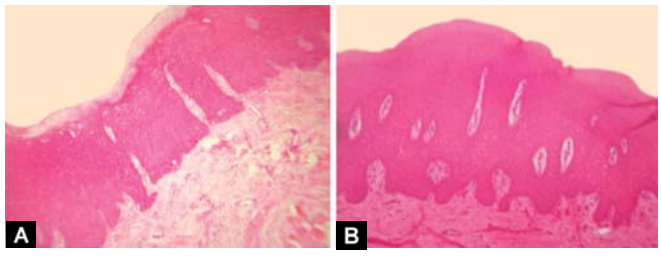
Microwave fixation has several advantages over routine methods from the perspective of laboratory personnel, such as elimination of harmful chemicals like xylene and formalin. The most important benefit from the perspective of the pathologist and patient is the shortened time of diagnosis. This means more time for decision making, repeat sections, special stains, immunohistochemical investigations and, if necessary, a repeat biopsy. This effectively reduces patient anxiety and increases patient compliance.

Our study demonstrated that significant differences do not exist in the overall quality or diagnostic ability of the samples fixed using microwave-based protocol when compared to the conventional protocol. The numbers of good and optimal specimen in both the protocols were almost similar for all parameters assessed, implying that the microwave alcohol fixation protocol provides good and faster fixation which is comparable to routine fixation (Figs 2A and B). The Chi-square test validates this observation.

Similar results have also been found by various other authors.[1,2,4,5] More prominent eosinophilia (Figs 3A and B) and chromatin clumping are features that were noted previously in microwave processing techniques.[1] Digressing from a didactic point of view the greatest benefit of using the microwave alcohol protocol is in the turnaround time. Using this protocol, samples < 5 mm were ready in 20 minutes and tissues between 5 and 10 mm were ready in 80 minutes saving around 90% of time taken for routine processing schedule and decreased reporting times. An added benefit is that this procedure requires a commonly available and affordable kitchen



Figs 2A and B: Routine fixation (A); microwave fixation, note the better nuclear and cellular detail (B)



Figs 3A and B: Routine fixation (A); microwave fixation, Note the increased eosinophilia (B)

microwave and uses only isopropyl alcohol which is used in every histopathogical laboratory. Hence, expensive equipment required for other rapid fixation techniques is rendered redundant. We believe that the protocol developed by us is robust and can be used for the routine fixation and processing of oral mucosal biopsies. However, studies on more number of samples and a more critical analytical protocol including cost benefit analysis compatibility with different types of tissues, special stains, immunohistochemistry and PCR will reflect better on its reliability.

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