Original Article

Microwave-assisted tissue processing, fixation and staining in tissues of different thicknesses: A comparative study

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Abstract

Aim and Objectives: The study aimed at assessment of microwave assisted tissue fixation, processing and staining and to determine if it can replace standard formalin fixed paraffin embedded processing in tissues of different thickness.

Materials and Methods: Specimens from buccal mucosa and gingiva was used in the study and were divided into three different thickness and was fixed, processed and stained according to conventional method and with a use of kitchen microwave oven respectively. The present study is the first of its kind where oral tissues was fixed, processed and stained with a kitchen microwave in three different thickness. The results obtained was statistically analyzed using IBM SPSS Statistics version 21.0 software.

Results: The new technique of fixation, tissue processing and staining using a microwave employed in the present study represented a major change from conventional method and achieved significant reduction in time taken.

Conclusion: The ease of application and speed of this technique significantly reduced turnaround time in diagnostic labs.

Keywords: Fixation, kitchen microwave, processing, staining, thickness

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INTRODUCTION

Routine use of formalin fixation, overnight dehydration, paraffin infiltration, manual embedding and sectioning has served well in producing relatively uniform, good quality tissue sections, but it is the major bottleneck in the workflow of histopathology laboratories. As we moved into the 21st century, the standard practice is now increasingly challenged because of its inability to meet the support required by current clinical demands. Because the routine manual histoprocessing remains laborious, time-consuming and requires toxic chemicals, alternative methods such as microwave tissue processing are the "future ray of hope."[1]

The microwave-assisted tissue processing is believed to have brought a revolutionary improvement in

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histopathology. The technique shortens the tissue processing time from hours to minutes. The technique is responsive to the patient and physician needs, improves the use of reagents while reducing or eliminating their toxicity, creates a personnel-friendly workflow and places the laboratory in a better position to meet the demands of the rapidly expanding field of molecular medicine.[2] Thus, in brief, microwave tissue processing achieves three aims of reducing cost of reagents, reducing time taken and eliminating noxious materials from the process.[3]

Microwave technology has advanced to the point where a patient can come to a hospital or clinic in the morning, see a physician for a checkup, get a biopsy from a surgeon and, if the biopsy is sent to the laboratory by noon, have the results by 3 PM that afternoon. Improvements in equipment design and a better understanding of tissue processing using microwave technology make the catchphrase "same-day turnaround" a reality. This quick turnaround not only reduces patient anxiety and reagent use but also increases efficiency.[4] It was Boon *et al*. from The Netherlands and Antony Leong from Australia who advocated microwave heating for fixation and processing of tissues in the late 1980s.[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 tissue blocks or sections even more effectively in contrast to conventional heating.[5-7]

The pattern of microwave heat distribution depends on many physical parameters, which may include the electromagnetic field, the specific absorption rate and structure of the processed material and the geometrical dimensions of the processing cavity. One of the major drawbacks of microwave heating in food industry is the existence of hot spots in several zones depending on product geometry.[8] While a number of authors have reviewed the techniques and results of microwave-facilitated tissue fixation and processing, we are unaware of any previous studies comparing the quality of microwave processing and routine processing from matched specimens of different tissue thicknesses procured from the workload of an ordinary surgical pathology laboratory, using a commercially available microwave oven.

The purpose of the present study was to document the usefulness of kitchen microwave-assisted tissue fixation and processing and to determine whether it can replace standard formalin fixation and paraffin-embedded overnight processing as the new routine technique in tissues of different thicknesses.

MATERIALS AND METHODS

Specimens for the study were obtained from biopsies received from the Department of Periodontology (periodontal and gingival specimens following surgeries) and also from the Department of Oral Surgery (excision specimens from *en bloc* resection of carcinoma cases) of KVG Dental College and Hospital, Sullia, over a span of 1½ years. Biopsies from buccal mucosa and gingiva were included.

Method of collection of data

The study group comprised 15 buccal mucosal biopsies and 15 gingival biopsies of 10 mm × 10 mm dimensions. Each group was divided into two. One group was labeled as an experimental group and another as a control group. The experimental (microwave fixation and processing) and control groups (conventional fixation and processing) were divided into three subgroups, as shown in Figure 1.

Materials used for the study comprised the following, as shown in Tables 1 and 2.

Methodology

Five gingival surgery tissue specimens were processed as per microwave fixation and processing protocol to standardize the microwave fixation and processing in kitchen microwave used for the present study.

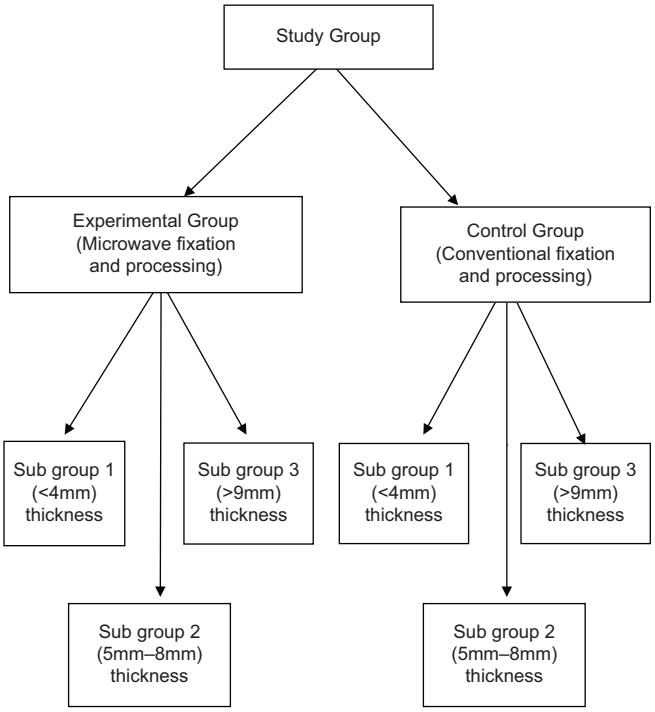

Figure 1: Distribution of study group

Each of the biopsy specimens was cut into approximately two equal halves, of which one bit was processed by conventional fixation and processing and another bit was processed by microwave fixation and processing. The tissues were processed by conventional method as per schedule mentioned in Table 3.

The tissue to be processed in microwave oven was wrapped in paper and placed in plastic cassettes and placed in bowl containing 100 ml of isopropyl alcohol. The bowl was then placed on the rotating table in microwave oven. The tissues were processed in microwave as per the schedule mentioned in Table 4.

Microwave was operated at the lowest output power level of 100 W. Both the conventionally processed and microwave-processed tissues following impregnation in paraffin wax were embedded in paraffin wax using "L" blocks. The wax-filled mold containing the tissue was then allowed to cool. The hardened wax block was removed from the mold. Using semiautomatic soft-tissue microtome [Figure 2], the blocks were first trimmed and then sectioned at 5 μm thickness. Further, all the blocks were serially sectioned to consume the entire thickness of tissue. Sections obtained from routine and microwave-processed tissues were then mounted and then stained using hematoxylin and eosin (H & E) stain, as mentioned in Tables 5 and 6 for conventional processing and microwave processing, respectively. Due to serial sectioning, a total of 150 paired slides processed by routine and microwave processing were obtained.

The mounted slides were assigned a number and coded for routine and for microwave-processed tissues. These coded paired slides were then evaluated by three observers for the following criteria:

1. Cellular clarity
2. Nuclear details
3. Cytoplasmic details
4. Color intensity
5. Epithelium and connective tissue interface.

These criteria were graded as follows:

- Excellent = 3
- Good = 2

Figure 2: Semi-automatic soft tissue microtome

Table 1: Microwave fixation and processing

Kitchen microwave oven (Model Onida Power Convection 20)
Isopropyl alcohol
Paraffin wax
Soft-tissue microtome
Water bath
Hematoxylin and eosin stains
Mounting media
Glass slides
Cover slips
Microwavable bowls of 150 ml capacity (4 no)
Plastic cassettes
Leukart's L blocks
Compound microscopes

Table 2: Conventional fixation and processing

Formalin
Glass jars of 500 ml (7 no's)
Isopropyl alcohol
Xylene
Paraffin wax
Soft-tissue microtome
Water bath
Hematoxylin and eosin stains
Mounting media
Glass slides
Cover slips
Stainless steel cassettes
Leukart's L blocks
Compound microscopes

Table 3: Conventional tissue fixation and processing method [Figure 2]

Steps	Reagent/processing fluid	Time
Dehydration	70% isopropyl alcohol	30 min
	80% isopropyl alcohol	30 min
	95% isopropyl alcohol	30 min
	Absolute alcohol-I	45 min
	Absolute alcohol-II	45 min
Clearing	Xylene-I	30 min
	Xylene-II	30 min
	Xylene-III	30 min
Impregnation	Paraffin wax-I	I h
	Paraffin wax-II	1 h
	Paraffin wax-III	1 h
Total time		450 min

Table 4: Microwave tissue fixation and processing method

Reagent/processing fluid	Time (min)
Isopropyl alcohol-I	20
Isopropyl alcohol-II	20
Molten paraffin wax-I	20
Molten paraffin wax-II	20

- Average = 1
- Poor = 0.

Data analysis

The results so obtained from three observers were statistically analyzed using IBM SPSS Statistics Version 21.0. for (Windows, Armonk, NY: IBM Corp) using the following nonparametric tests:

1. Wilcoxon's matched-pairs test
2. Mann–Whitney test.

RESULTS

In this study, 150 pairs of slides of which one was routinely fixed processed and the others were microwave fixed and processed were stained simultaneously with H & E. The results obtained are tabulated in Tables 7-9, respectively.

Color intensity and epithelium and connective tissue interface were statistically significant in tissues of <4 mm thickness, as shown in Table 7 and Figure 3. Color intensity was statistically significant in tissues of 5 –8 mm thickness, as shown in Table 8 and Figure 4.

Cellular clarity, color intensity and epithelium and connective tissue interface were statistically significant in tissues of >9 mm thickness, as shown in Table 9 and Figure 5.

Cellular clarity and epithelium and connective tissue interface were statistically significant, as shown in Table 10.

Table 5: Schedule for H & E staining by conventional method

Reagent	Time
Xylene-1	10 min
Xylene-II	10 min
95% isopropyl alcohol	1 dip
Absolute alcohol	1 dip
Water bath	5 min
Hematoxylin	7-10 min
Water bath	10 min
1% acid alcohol	1 dip
Water bath	10 min
Eosin	1 dip
95% isopropyl alcohol	1 dip
Absolute alcohol	1 dip
Xylene	10 min

Table 6: Schedule for hematoxylin and eosin staining by microwave method

Reagent	Time
Dewax in xylene, 2 changes 10 min each	20 min
Running water hydration	5 min
Hematoxylin	30 s
Bluing	2 min
Water wash	5 min
Eosin stain	30 s
Total time	33 min

Table 7: Microwave processing versus conventional processing of tissues <4 mm

Parameters	Conventional (mean)	Microwave (mean)	*P*
Cellular clarity	2.8	2.8	1.00
Nuclear details	2.90	2.93	0.64
Cytoplasmic details	2.23	2.33	0.39
Color intensity	2.56	3.00	0.00
Epithelium and connective tissue interface	2.13	2.80	0.00

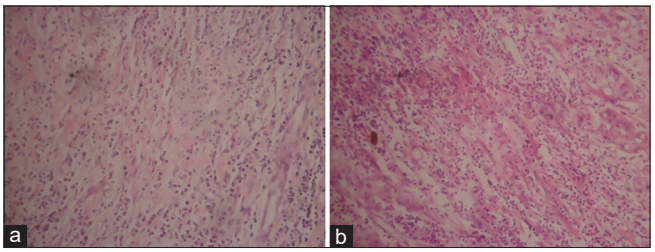

Figure 3: (a) Microwave processing versus conventional processing of tissues <4 mm. (b) Microwave processing versus conventional processing of tissues <4 mm

Table 8: Microwave processing versus conventional processing of tissues <5-8 mm

Parameters	Conventional (mean)	Microwave (mean)	*P*
Cellular clarity	2.76	2.80	1.00
Nuclear details	2.32	2.80	0.64
Cytoplasmic details	2.52	2.70	0.66
Color intensity	2.72	3.00	0.00
Epithelium and connective tissue interface	2.82	2.80	0.80

Table 9: Microwave processing versus conventional processing of tissues >9 mm

Parameters	Conventional (mean)	Microwave (mean)	*P*
Cellular clarity	2.51	1.11	0.00
Nuclear details	1.17	1.17	1.00
Cytoplasmic details	1.42	1.21	0.74
Color intensity	1.40	1.08	0.02
Epithelium and connective tissue interface	1.88	1.08	0.00

Table 10: Microwave tissue processing versus conventional tissue processing

Parameters	Conventional (mean)	Microwave (mean)	*P*
Cellular clarity	2.66	2.01	0.00
Nuclear details	1.90	2.06	0.10
Cytoplasmic details	1.95	1.93	0.79
Color intensity	2.07	2.17	0.68
Epithelium and connective tissue interface	2.24	2.00	0.02

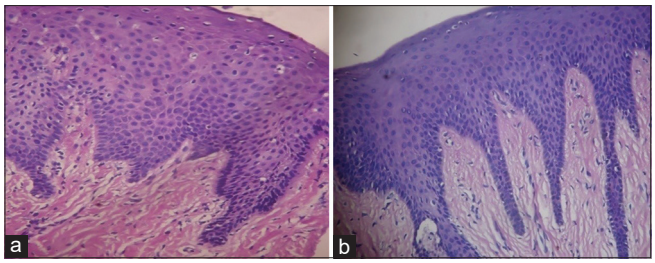

Figure 4: (a) Microwave processing versus conventional processing of tissues <5 mm–8 mm. (b) Microwave processing versus conventional processing of tissues <5 mm–8 mm

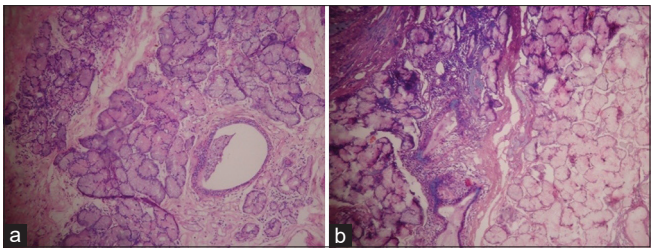

Figure 5: (a) Microwave processing versus conventional processing of tissues >9 mm. (b) Microwave processing versus conventional processing of tissues >9 mm

A comparison of turnaround time of microwave tissue fixation, processing and staining with conventional tissue fixation, processing and staining is shown in Table 11.

Table 11: Turnaround time of microwave versus conventional processing and staining

Method	Time taken
Microwave	113 min
Conventional	515 min + overnight fixation with formalin

DISCUSSION

The new technique of fixation and tissue processing using a microwave employed in the present study represents a major change from conventional fixation and tissue processing. The ease of application and speed of this technique has significantly reduced turnaround time in diagnostic laboratories for the past three decades. Initially, application of microwave techniques into histotechnology was not accepted but nowadays is growing in its popularity and versatility.

Serial sectioning was done in the present study, consuming the entire tissue to ensure the analysis of histomorphologic features throughout the tissue thickness. The two different protocols of tissue processing showed a similar kind of results considering the various histologic parameters in tissues of different thicknesses with the microwave-processed tissues of <4 mm and 5–8 mm showing better color intensity. Interface of epithelium and connective tissue has been a special area of interest for a pathologist in ruling out invasion and in many other immune-mediated disorders.[9] When the integrity of epithelial tissues was considered, the present study revealed better results in microwave-processed tissues of <4 mm thickness. However, the cellular clarity, color intensity and epithelium and connective tissue interface were better in tissues of >9 mm thickness processed in conventional method. This may be attributed to the formation of "hot spots" in microwave oven in case of tissues with greater thickness. Inhomogeneous energy dissipation means selective heating of different parts of the material is possible and may lead to temperature gradients in the microwave oven. The presence of these zones with a higher temperature than others is termed as hot spots.[8] As a result, uniform heat distribution is not possible. In the present study, this factor might have led to nonuniform processing in tissues of greater thickness.

While considering all the tissues in total, irrespective of the tissue thickness, there were no significant differences when considering nuclear details, cytoplasmic details and color intensity; however, cellular clarity and epithelium and connective tissue interface were superior in tissues processed in conventional method than microwave processing. This may be attributed to the incorporation of tissues >9 mm thickness in the evaluation. In a study done by Babu *et al*., cellular clarity, cytoplasmic details, nuclear details and color intensity were slightly better in microwave method than in routine method.[10] Similar studies by various authors showed no significant differences in the histologic quality when they compared these two protocols.[9,11-13]

From the perspective of the end product, microwave irradiation substantially shortens the time from specimen reception to diagnosis. In the present study, the slides were ready in 113 min with microwave tissue fixation and processing as compared to 515 min in addition to the overnight fixation in routine processing and staining. This allowed same-day tissue processing and diagnosis of small biopsy specimens without compromising the overall quality of histologic sections. This is in agreement with Rohr *et al*.[3] where histological slides were produced in 2–3 h using microwave irradiation. In a study done by Babu *et al*.,[10] the turn around time for microwave tissue fixation and processing and staining was 75 min. In a study done by Henwood A,[14] the tat for microwave tissue fixation and processing was 80 min. Relatively longer duration for microwave tissue processing in the present study can be attributed to the

low power option chosen to ensure minimum tissue damage and prevent boiling of chemicals. In a study done by Patil S *et al*.,[15] microwave processing was found suitable, and drastic reduction in tat was noted which led to early reporting of slides.

The kitchen microwave oven used in the present study had a maximum output of 800 W, but it was operated at the lowest output of 100 W throughout the study. Although literature from various studies suggest that the microwave can be operated at higher output levels, i.e., 200–2000 W, in turn, reducing the time of processing from 1 to 2 h to as less as 5 min,[3] the lowest output of 100 W was preferred since evaporation and boiling of chemicals were noted when microwave was operated at higher power.

Metals and metallic utensils are contraindicated for use in microwave oven due to total internal reflection of microwaves, leading to sparkling,[5] hence plastic cassettes in place of metal cassettes during tissue processing were used. These cassettes are relatively cheap and can be reused.

Those working in the laboratory pursue a reduction or preferably elimination of toxic reagents from histopathology. Microwave procedures are conducive to that aim because the volume of reagents used and the toxic exposure are significantly lower. In particular, replacing xylene with mineral oil or isopropyl alcohol for clearing and excluding formaldehyde for fixation from processing through microwave methods is a welcome improvement in histopathology. Ethyl alcohol is a good dehydrating agent, and isopropyl alcohol dehydrates and has been found to be a good intermedium or clearing agent. The current study supports the same and has demonstrated comparable results, implying that microwave fixation with alcohol provides a good and faster fixation which is comparable to routine fixation. Isopropanol is less toxic than xylene and is cheaper than both chloroform and xylene. Isopropanol is not very popular as a clearing agent in conventional histoprocessing because of its slow diffusion. In microwave technique, the problem of slow diffusion can be overcome by microwave heating. Even Kok and Boon in their study found that during microwave impregnation, xylene could not be boiled out because of its high boiling point and low microwaveability and thus retarded the diffusion of paraffin, whereas highly microwavable isopropanol could be easily boiled out. Like others, paraffin wax for impregnation has been used in the present study as well.[11]

In spite of several advantages over conventional histoprocessing methods, the present study revealed a few limitations of microwave tissue processing. In kitchen microwave oven used for the histoprocessing, the temperature and exact power control were not standardized and vacuum processing was not available. Although the histologic quality of tissues >9 mm was inadequate, the present study achieved reduction in time taken, eliminating noxious reagents and comparable microscopic features in tissues of <4 mm and 5–8 mm. The present study is the first of its kind where oral tissues were fixed processed and stained with a kitchen microwave in three different thicknesses.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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