ORIGINAL RESEARCH

Comparison of three different methods of tissue processing

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ABSTRACT
Biopsied tissue is cut into thin slices and stained suitably for microscopical examination. Enabling the tissue for sectioning by paraffin embedding is known as tissue processing. The three most commonly employed means of tissue processing are routine manual method, rapid manual method and the microwave method.
In this study, sections obtained from the same site of the same tissue were processed by these three methods and stained by hematoxylin and eosin. These sections were then microscopically evaluated by various parameters to compare the three methods.
The results that were obtained, after subjecting to statistical analysis, showed no significant differences between the three different processes in terms of quality of staining, clarity of nucleo-cytoplasmic differentiation in various cells and the presence of artifacts. Tissue shrinkage was less in microwave-processed tissue as compared to the other methods. Microwave tissue processing was also found to be more cost-effective than other methods.
Key words: Microwave method, rapid method, routine method, tissue processing

INTRODUCTION

The examination of tissues with a microscope usually requires a slice of tissue, which is thin enough to transmit light and the preparation of such thin slices is called section cutting or microtomy. In most cases, the tissues must undergo preparatory treatment before being sectioned, which involves impregnation in a suitable embedding medium to provide support and a suitable consistency for microtomy. This preparatory treatment is known as tissue processing. The stages of tissue processing include dehydration, clearing, impregnation and embedding, each of a designated duration to ensure completion of the procedure. The three most commonly employed means of tissue processing are routine manual method, rapid manual method and the microwave method. Each of these methods is unique with their own advantages and disadvantages.

Routine manual tissue processing has been the most commonly employed method for the past 100 years. It includes the aforementioned steps and is completed in 21-24h. It's advantages are its reliability and its inexpensive nature. The disadvantages are that it is time consuming and the need to work with noxious chemicals like xylene and formalin.

Rapid manual tissue processing is of a shorter duration than the above method, requiring 3-4h. It includes the same steps as in routine method, but for shorter durations. The advantages are that it consumes only 20% of time as compared to routine method and the disadvantage is that noxious chemicals like xylene and formalin need to be used and there is a greater degree of tissue distortion and shrinkage.

Microwave method is a recent tissue processing technique, first used by Boon and Kok in 1985.[1] In this process, the penetrative properties of the microwave and the conversion of this incident energy into heat, is made use of, the advantages include shorter processing times, eliminating noxious chemicals like xylene and lesser degree of denaturation of nucleic acids.[2] The disadvantages are the high costs involved.

Considering these facts, the three different methods of tissue processing were compared in terms of the effect on staining, cytoplasmic-nuclear details, tissue shrinkage and cost-effectiveness.

MATERIALS AND METHODS

Ten different formalin fixed tissue samples were procured from the archives of Department of Oral Pathology, Meenakshi Ammal Dental College, Chennai. Each sample was sectioned into three pieces, such that one of the pieces was sent for routine manual tissue processing, another for rapid manual

tissue processing and the third for microwave tissue processing. The steps followed in the routine and rapid method were in accordance with those outlined by Culling.[3] For the microwave method the tissues were processed using a domestic microwave oven (IFB India 20 PG 1S). The tissues to be processed were placed in microwave oven safe glass containers and the oven was operated at 40% power with a water load of 200 ml. The steps followed are outlined in Table 1.

Statistical analysis

The dimensions of the tissues were recorded before dehydration and paraffin embedding. The percentage shrinkage of the tissues after processing were calculated. Tissue sections obtained by the three methods were stained simultaneously with Harris' hematoxylin and eosin. The slides were independently evaluated by four qualified oral pathologists for quality of staining and nuclear-cytoplasmic details. The cost-effectiveness of the three methods was also evaluated. The values obtained were subjected to statistical analysis by Kruskal-Wallis test using SPSS (version 9.0) software.

RESULTS

The three different methods of tissue processing showed no statistically significant difference as far as quality of staining, clarity of nucleo-cytoplasmic differentiation of epithelial tissue, fibrous tissue and glandular tissues were concerned [Tables 2-5]. Comparison of tissue shrinkage showed a statistically significant lesser shrinkage in microwave-processed tissues (27.32 ± 8.63) than that among routine and rapid methods of tissue processing (39.96 ± 9.80 and 46.87 ± 18.94) [Table 6]. The cost per tissue was less for microwave processing (Rs. 11.60) compared to routine and rapid methods of processing (Rs. 32.88 each) [Table 7].

DISCUSSION

Tissue contains water molecules and will not allow the embedding media to enter. The process of dehydration is needed to replace the water in the tissue by alcohol or a substitute; clearing comprises of the exchange of alcohol by a reagent miscible with paraffin or its substitute; and impregnating is the process in which the clearing agent is replaced by paraffin or its substitute. The physicochemical basis of tissue processing lies in the diffusion of reagents into the substance of the tissue to be processed. Microwaves are non-ionizing radiations with electromagnetic properties. Their frequencies range from 300 MHz to 300 GHz and wavelengths from 1 mm to 1 m. All domestic microwaves operate at 2.45 GHz, corresponding to a wavelength in vacuum of 12.2 cm.[1] Microwave excitation of molecules is a process in which applied energy penetrates into the tissues to a greater depth as compared to the other manual methods of tissue processing. Dipolar molecules, which are present in the field are forced to oscillate and this leads to an increase in thermal agitation.[1] The

Table 1: Microwave tissue processing

Step	Time (minutes)
Absolute alcohol	15
Absolute alcohol	15
Molten paraffin wax*	15
Molten paraffin wax*	15

*Paraffin wax was used in molten state as paraffin is a poor conductor of thermal energy

Table 2: Effect of tissue processing on quality of staining

Method	Observer 1	Observer 2	Observer 3	Observer 4
Routine	15.50	12.75	18.50	13.35
Rapid	18.50	17.45	15.50	17.00
Microwave	12.50	16.30	12.50	16.15
	$P = 0.159$	$P = 0.379$	$P = 0.213$	$P = 0.553$

Mean rank obtained by Kruskall-Wallis test

Table 3: Epithelial tissue

Method	Observer 1	Observer 2	Observer 3	Observer 4
Routine	14.30	14.45	16.10	12.30
Rapid	16.60	14.85	13.35	17.10
Microwave	15.60	17.20	16.85	17.10
	$P = 0.807$	$P = 0.728$	$P = 0.638$	$P = 0.327$

Mean rank obtained by Kruskall-Wallis est

Table 4: Fibrous tissue

Method	Observer 1	Observer 2	Observer 3	Observer 4
Routine	13.65	13.25	18.25	15.30
Rapid	17.15	18.95	19.00	17.00
Microwave	15.70	14.30	9.5	14.20
	$P = 0.581$	$P = 0.244$	$P = 0.012$*	$P = 0.647$

Mean rank obtained by Kruskall-Wallis test

Table 5: Glandular tissue

Method	Observer 1	Observer 2	Observer 3	Observer 4
Routine	16.50	15.15	18.80	15.80
Rapid	13.50	12.60	10.05	11.80
Microwave	16.50	18.75	17.65	18.90
	$P = 0.607$	$P = 0.253$	$P = 0.032$*	$P = 0.156$

Mean rank obtained by Kruskall-Wallis test

Table 6: Comparison of shrinkage of tissues among the three methods of processing

Method	% of shrinkage (mean ± SD)	*P* value
Rapid	46.87 ± 18.94	
Routine	39.96 ± 9.80	0.009*
Microwave	27.32 ± 8.63	

*Significant; One way ANOVA

Table 7: Comparison of cost effectiveness among the three methods of processing

Method	Total cost (Rs.)	Number of tissues	Cost/tissue (Rs.)
Routine	328.80	10	32.88
Rapid	328.80	10	32.88
Microwave	116.00	10	11.60

kinetic energy thus generated is converted into heat energy. As in other forms of tissue processing, here too, diffusion is the key factor. The formula which governs the rate of diffusion is $<x^2> = 2Dt$, where x stands for net distance covered by a particle in solution in a certain direction; t is the time period during which diffusion occurs; D is the diffusion constant for the substance; < > stands for the average value. The basic effect of microwave irradiation is stimulation of diffusion and enhancement of reaction rates with internal heating being the key element in the process.[4] Heating by conventional means can also be used for tissue processing, but the results obtained are markedly inferior to that in microwave processing. The suggested reason is that in conventional method of heating, the heat might not be uniformly distributed throughout the tissue.[5]

The three different methods of tissue processing studied, did not show any significant variation in the quality of staining (hematoxylin and eosin). This was in consonance with the findings of Boon *et al.*[5] and Chaudhari *et al.*[6] Morales *et al.*[2] found the tissue architecture, stroma, secretory products, cell and nuclear morphology were same between conventionally processed and microwave processed tissue, except that the microwave processed tissue showed brighter staining with eosin and a stronger reaction with haematoxylin, as compared to the manually processed sections.

The effect of the three methods of tissue processing on cytoplasmic and nuclear details as assessed in terms of epithelial, fibrous and glandular tissues, showed no statistically significant variation. Boon *et al.*[5] found that in microwave processed tissues the epithelium was of better quality and the stroma showed more focal condensation.

In the present study, shrinkage of the tissues was evaluated by metric measurement of the size of the tissues prior to dehydration and embedding in paraffin wax. Tissues processed by the microwave method show statistically significantly less shrinkage than tissues processed by the manual techniques. Kok *et al.*[1] compared the nuclear diameter of various types of cells, which were processed by the conventional and the microwave method. They found no difference in the amount of tissue shrinkage in the conventional and microwave method.

In the present study, the cost-effectiveness of the three processes was calculated as the cost of various reagents consumed per tissue processed. Microwave process was found to be the most cost-effective with the routine and rapid method showing equal values for the parameter. When a rapid diagnosis is required, it is the microwave process, which is capable of doing so, with the entire time lag between excision of the tissue to the submission of the histopathology report not exceeding two hours. The cryostat is generally used in such cases. The disadvantage is that, there is loss of nuclear details under higher power.[7] In addition to this, the profitability of any diagnostic laboratory would be increased by the use of this technique as a large batch of samples can be handled in a single day and it will also be a boon for the technical personnel whose work practices and lifestyles would change for the better and this is something which defies statistical analysis. Microwaves, because of their wide range of applications (electron microscopy, antigen preservation) can be used in diagnostic laboratories as a means of cost containment.[8] Apart from the distinct advantages that microwave tissue processing confers by its routine usage in diagnostic laboratories, there is also the advantage of obviating the usage of noxious chemicals like formalin and xylene. Formalin is an irritant to the conjunctiva and the nasal mucosa. It has also been implicated in the aetiology of cancer of oropharynx and the respiratory tract.[2] By eliminating xylene from the process, microwave tissue processing achieves three aims of reduced cost, reduced time taken and eliminating a noxious material from the process.

CONCLUSION

Microwave tissue processing is a cost-effective method of tissue processing that has no adverse effects on the quality of staining and cellular details with lesser amount of tissue shrinkage as compared to the conventional methods of tissue processing. However, further studies using an automated microwave oven with adjustable cycle time and precise temperature control, and a larger sample size in a clinical setting are required to draw definitive conclusions about the advantages of microwave tissue processing over conventional methods.

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