Conventional and Microwave Histo-processing of Soft Tissue Specimens: A Comparative Study

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Abstract

Background: Microwave-accelerated tissue processing is believed to have brought a revolutionary improvement in the field of histopathology and histochemistry. This technique shortens the time for tissue processing from days to minutes, allowing even more rapid histopathologic diagnosis. The aim of the study is to compare the macroscopic and microscopic quality of the microwave histoprocessing with that of conventional method and to determine its impact on turnaround time. Materials and Methods: There was nine types of normal and pathological soft tissue specimens. Each type of tissue was divided into seven sets and were further, cut into a size of 0.5x0.5x0.5cms. All the Sixty three specimens were processed by conventional method only and by domestic microwave in combination with conventional methods according to six protocols. Results obtained showed that the macroscopic and microscopic features of microwave processed tissue were similar to conventionally processed tissue and the correlation coefficient 'r' value was 0.764. Microwave assisted tissue processing reduced the total time for preparing tissue blocks to about an hour without compromising the overall quality of the histologic sections. Conclusion: Microwave stimulated processing provides an attractive alternativeover traditional conventional processing.

Keywords: Microwave; histoprocessing; histologic quality; turnaround time.

INTRODUCTION

In order to preserve the structure of any tissue and impregnate them with a suitable media, they have to be adequately fixed and processed, so that thin sections can be made for staining and microscopic evaluation.[1] Examination of tissues under a microscope requires a slice of tissue that is thin enough to transmit light, and the preparation of such thin slices is called section cutting or microtomy. The soft 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.[2]

Tissue processing in histology is a physical process that involves chemical solutions reacting with biological specimens.[3] Conventional tissue processing is as old as 100 years and still remains the gold standard against which all new technologies and methods need to be assessed. Although routine use of formalin fixation, an overnight dehydration, paraffin infiltration, manual embedding, and sectioning have served well in producing relatively good-quality tissue sections, it is the major bottle neck in the workflow of histopathological laboratories.[4] It includes the aforementioned steps and is completed in 21-24hr. Advantages are its reliability and inexpensive nature. The disadvantages are that it is time consuming and the need to work with noxious chemicals.[5] However this too means delay in report generation for one day or longer leading to delay in planning or institution of the treatment which is crucial in critically ill patients. Thus to reduce this turnaround time, various technology has been introduced in the field of histoprocessing.[6]

Rapid processing of histopathologic material is becoming increasingly desirable to fulfill the needs of clinicians treating acutely ill patients.[7] As we moved into 21st century, the standard practice is now increasingly challenging because of the 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[8] tissue processing are the "future ray of hope".[9]

Microwave which invented by Percy Spencer in 1945 are becoming an integral part of our lives.[10] Although used in food processing, chemical, pharmaceutical and many other industries for many years, it was Kok and Boon from Netherland and Antony Leong from Australia who advocated microwave heating for fixation and processing of the tissue in the late 1980's.[4] 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.[5] Thus, a novel histoprocessing method for paraffin section was developed and fast processing was possible due to stimulated diffusion of the heated reagent.[4]

Microwave causes heating within a material by exciting molecules to rotate. The rotation produces energy in the form of heat. Heat reduces the viscosity of liquids, thereby increasing the rate of diffusion of reagents into and out of the tissue. Unlike conventional heating, the effect occurs simultaneously throughout the whole material being microwaved ('internal heating'). This resulted in substantial reduction in each of the basic steps of histoprocessing, thereby reducing turnaround times and permitting same day diagnosis for a variety of types of tissue biopsy specimens.[11]

Hence the present study was carried out to document the usefulness of microwave-assisted tissue processing and to compare the turnaround time and histologic quality with that of the conventional method.

MATERIALS AND METHOD

The soft tissue specimens were selected from the Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Bareilly, (UP). Normal and pathological soft tissue specimens were included and hard tissue specimens were excluded from the study. A total of sixty three normal and pathological soft tissue specimens from which seven each of salivary gland, muscle, lymph node, adipose tissue, stratified squamous epithelium & connective tissue, Odontogenic cyst, odontogenic myxoma, oral squamous cell carcinoma and fibroma were included in the study.

Procedure: All the tissue specimens were cut into a standard size of 0.5×0.5×0.5 cm and each specimen was measured before and after tissue processing using a graph & metric scale in order to identify the shrinkage. Fixation of all the tissue specimens was carried out with 10% buffered formalin.([8]) The 200ml of chemical reagents were used for processing of all the tissue specimens. The tissue specimens was processed using only conventional, only microwave and the combination of conventional and microwave processing

techniques. Later the formalin fixed paraffin embedded tissue block were prepared and microtomy was carried out followed by hematoxylin & eosin staining.[12] (Figure 1). The slides were then histopathologically evaluated for quality of tissue sections, tissue architecture, staining quality, nucleus and cytoplasmic differentiation and for overall quality of diagnosis.[3] (Figure 3-10). A review of turnaround times for tissues processed under different protocols were also evaluated.

The Conventional / Manual Tissue Processing: The tissue specimens were processed by conventional method according to the criteria of Bancroft JD.[12] All the processing was done at room temperature, except for the impregnation and embedding, which were carried out at 56°C followed by section cutting and H & E staining. (Table 1)

Microwave Tissue Processing: A domestic microwave (INALSA: EG8021TP-AN) which had all programs fully loaded to run at appropriate time and predetermined power was used. In order to absorb the excess heat generated by the microwave, a second beaker containing a water load of 200ml was used in all the procedures next to the beaker with the tissue where the temperature was in the range of 45°C-58°C. According to Klump et al.,[13] and Prasad GK et al.,[14] fixation in microwave was carried out with 10% buffered formalin for 15 min at power of 30%. Dehydration was carried out with 100% Iso propyl alcohol for 15 min at power of 50%. Xylene was used as clearing agent for 15 min at power of 50%. The paraffin impregnation took 15 min at power of 50%. The tissue specimens were then embedded in paraffin, cut with rotary microtome of 4-6μm thick and stained with haematoxylin and eosin.[12] According to Mathai AM, et al.,[11] a combination of conventional and microwave tissue processing was adapted using various protocols. (Table 2)

Histopathological Evaluation: All the slides were microscopically evaluated and scored using a customized evaluation form under the microscopic parameters listed below:

- **Quality of Tissue Sections:** According to the modified criteria of Prasad GK, et al.,[4] the cellular morphology was classified as **Interpretable** (score 2) on the basis of greater eosinophilia of cytoplasm producing enhancement of the nuclear cytoplasmic contrast, good stroma, whether secretory products are appreciable, absence of red blood cell lysis, and whether differentiation can be made between inflammatory cells.[15] If there was granularity of cytoplasm,[15] focal condensation of stroma, cellular outline blurred,[16] mucin was not seen, red blood cells lysed (focal or generalized),[15] and no differentiation could be made between inflammatory cells then it was classified as **Intermediate** (score 1). If none of the features were present, then it was convinced as **uninterpretable** tissue section (score 0).
- **Tissue Architecture:** All the tissue sections was assessed using modified criteria by Babu M, et al.,[7] and Patil S, et al.,[1] for the parameters like cellular clarity, cytoplasmic details, nuclear details, color intensity and interface of epithelium & connective tissue under the grading of **Not maintained** (score 0), **Intermediate** (score 1) and **Well maintained** (score 2) tissue architecture.
- **Quality of Staining:** According to the modified criteria of Prasad GK, et al.,[4] staining of tissues was evaluated as poor, non-uniform, and uniform. **Poor** (score 0) indicates that the tissue failed to take up stain adequately, stained unevenly or had artifacts in processing or staining. **Non-uniform** (score 1) indicates that details were not visualized up to the mark, but slide was suitable to give diagnosis. **Uniform** (score 2) means good contrast between the nucleus and cytoplasm, and visibility of details along with brilliance of staining.
- **Nucleus and Cytoplasmic Differentiation:** According to the modified criteria of Prasad GK, et al.,[4] slides were evaluated on the basis of chromatin condensation, prominent nuclear membrane, and crisp staining of the nucleus and mitotic activity, if appreciable. It was graded as **Good** (score 3) if all features were appreciated, as **Average** (score 2) if smudging and pyknosis of nuclei were seen,(17) as **Poor** (score 1) in case of indistinct nuclei and as **Not**

seen (score 0) when the nuclei are not appreciated.

- **Overall Quality of Diagnosis:** The scoring was done according to the modified criteria described by Boon et al.,[18] and Ayala et al.,[19] as **Poor** (score 0) if the tissue was not clearly demonstrated (Not good for microscopy), **Average** (score 1) if tissues not very well demonstrated, but can be used for microscopy and as **Good** (score 2) if the tissue was clearly demonstrated.

RESULTS

The comparison was carried out on different parameters like tissue shrinkage, histopathological evaluation and turnaround time.

Tissue Shrinkage: The Pre- and post processing soft tissue specimen shrinkage ranged from minimum of 0.0mm to a maximum of 0.2mm among all the study groups except in protocol IV which was 0.1mm. The mean and standard deviation values of tissue shrinkage in pre-processed tissues was 0.5±0.0 in all the study groups, whereas post-processed tissue in protocol I showed 0.467±0.071, in protocol II was 0.400±0.087, in protocol III was 0.422±0.083, in protocol IV was 0.478±0.044, in protocol V was 0.467±0.071, in protocol VI was 0.456±0.088, and in conventional group was 0.444±0.073. Upon statistical evaluation Protocol II, III and conventional group showed a statistical significance with the p value of 0.008, 0.023 & 0.050 respectively, whereas protocol I, IV, V and VI showed a non-significance results with p value being 0.195, 0.169, 0.195 and 0.169 respectively. (Figure 2)(Table 3; Graph 1)

Histopathological Evalution: (Figure: 2 & 3) The microscopic assessment between Conventional and Microwave Tissue Processing under Protocol I showed a non-significant p value of 0.159 for quality of tissue, 0.331 for tissue architecture, 0.094 for quality of staining, 0.360 for nucleus and cytoplasmic differentiation and 0.427 for overall quality of diagnosis. Whereas under Protocol II a non-significant p value of 0.331 for quality of tissue, 0.056 for tissue architecture, 0.396 for quality of staining, 0.360 for nucleus and cytoplasmic differentiation and 0.556 for overall quality of diagnosis was noted. (Table 4)

The microscopic assessment between Conventional and Microwave Tissue Processing under Protocol III showed a non-significant p value of 0.535 for quality of tissue & tissue architecture, 0.113 for quality of staining, 0.427 for nucleus and cytoplasmic differentiation and 0.696 for overall quality of diagnosis. Whereas under Protocol IV, a non-significant p value of 0.331 for quality of tissue, 0.159 for tissue architecture, 0.499 for quality of staining, 0.193 for nucleus and cytoplasmic differentiation and 0.311 for overall quality of diagnosis was noted. (Table 4)

The microscopic assessment between Conventional and Microwave Tissue Processing under Protocol V showed a non-significant p value of 0.136 for quality of tissue & tissue architecture, 0.936 for quality of staining, 0.185 for nucleus and cytoplasmic differentiation and 0.427 for overall quality of diagnosis. Whereas under Protocol VI showed a non-significant p value of 0.539 for quality of tissue, 0.609 for tissue architecture, 0.455 for quality of staining, 0.100 for nucleus and cytoplasmic differentiation and overall quality of diagnosis. (Table 4)

Correlation Coefficient for protocols I, II, III, IV, V, & VI of microwave with conventional tissue processing was 0.542, 0.389, 0.732, -0.142, 0.123 & 0.764 respectively. (Table 5)

Turnover Time: The turnover time for various protocols of I, II, III, IV, V, VI and conventional tissue processing were 7hrs 15mins, 13hrs 30mins, 18hrs 15mins, 1hr 45mins 12hr 45mins, 1hr and 19hrs respectively. (Table 6)

DISCUSSION

For decades, instrumentation used in tissue processing remained relatively unchanged. A recent addition in the list of techniques involved for rapid processing of tissues is the use of microwaves, which has revolutionized histotechniques.[10] Microwaves are the electromagnetic wave that can penetrate various types of materials. Their penetration depth is dependent on the electric conductivity of the medium. Upon penetration into tissues, the energy is absorbed by the molecules.[20] The usage of microwave provides with a shorter processing time, and lesser degree of denaturation of nucleic acids. Also, domestic microwave are readily available, affordable and have provided

appreciable results in previous studies.[2, 4, 7, 10, 18, 21-23] The present study was carried out to compare and evaluate, if various protocols using domestic microwave was useful and better alternative to conventional tissue processing.

In the present study, the tissue shrinkage noted in both conventional as well as in microwave technique was almost similar. The results were in concordance with that of Kok, et al.,[20] and Panja P, et al.[5] However, contrasting results were observed by Kayser K, et al.,[24] who identified tissue processed by microwave showed excess shrinkage as compared to conventional processing. This could be due to the heat generated by microwave oven[24] or the concentration gradient between the fluids inside & outside the tissue, diffusion current crossing the cell membrane during the fluid exchange increases the possibility of tissue shrinkage.[12]

In the present study, upon microscopic assessment, when quality of tissues was statistically compared between conventional and microwave processing methods, it showed a non-significant difference. It was similar to the studies conducted by Morales et al.,(2002 & 2004)[22, 25] and Mathai AK, et al.[11] Thus microwave processed tissue sections had similar nuclear cytoplasmic contrast, with good erythrocyte integrity and lymphocyte appearance as that of conventional method. The contrasting results were observed in the study performed by Patil S, et al.,[1] and Babu T, et al.[7] The microwaves stimulate the polar molecules causing collision with the adjacent molecules which causes part of the rotational energy to be transferred through them producing heat. This effect occurs simultaneously throughout the whole material being microwaved.[26]

In the present study, upon microscopic assessment, when tissue architecture was statistically compared between conventional and microwave processing methods it showed a non-significant difference. It was similar to the studies conducted by Kango GS, et al.,[4] Boon et al.,[18] Morales et al.,(2002 & 2004)[22, 25] and Chaudhari et al.,[16] who found that stroma, secretory products, as well as cellular and nuclear morphology were identical between conventionally and microwave processed tissue. As the mechanism of microwave heating depends on oscillating or exciting polar or charged molecules in the tissue. Alternating electromagnetic fields are produced which cause polar molecules of proteins to rotate through 180° at 2.45 billion cycles per second. This result in generation of instantaneous heat that is proportional to the energy flux and continues until the radiation ceases.[26]

The quality of staining in the microwave and conventionally processed tissue did not show any significant variation in the present study. This was in consonance with the findings of Boon et al.,[18] Chaudhari et al.,[16] Moraleset al.,(2002 & 2004)[22, 25] Panja et al.,[5] Zenobia et al.,[27] Galvez et al.,[28] Suri et al.,[29] Leong et al.,[30], Rohr et al.,[23] and Kok et al.,(1988 & 1990).[20, 31] This can be attributed to uniform distribution of heat, chemical reagents and effective dehydration seen in the microwave technique.[7]

In the present study, the nuclear and cytoplasmic differentiation was similarly observed in tissues when processed by microwave and with conventional processed tissue. Contrastingly, Patil S et al.,[1] Boon et al.,[18] Panja P et al.,[5] and Babu, TM et al.,[7] in their study found better quality of cellular and nuclear details when processed by microwave technique. In this process, the penetrative properties of the microwave converts the incident energy into heat, there by creating a uniform environment for the chemical reagents to perform the work, and enhances the results, observed in microwave tissue processing.[7]

In the present study, the overall quality of diagnosis for tissue processed with conventional and microwave method under protocol VI were indistinguishable. This was similar to the findings of Mathai AK et al.,[11] whereas contrasting results were observed by Babu, TM et al.,[7] in their study, where overall quality of microwave-processed tissue appeared slightly better than routinely processed and routinely stained slides.

In the present study, when the microwave tissue processing method by various protocols were intercompared, it was observed that in Protocol VI, where all the steps was carried out only in microwave was best among them. Similar findings were observed by Sivadas P et al.,[32] and Mathai AM et al.[11] In this process, the penetrative properties of the microwave and the conversion of

this incident energy into heat is used. The increased rate of processing is ascribed to the increased rate of diffusion. Diffusion is a key factor in histoprocessing, permitting chemicals to infuse into the tissue faster. Increased temperatures decrease the viscosity of the processing fluids and thereby facilitate diffusion.[2, 22]

The turnover time estimated was minimum in microwave protocol VI method of just one hour as compared to maximum of 19hours in conventional processing. Variuos studies like Babu et al.,[7] Panja et al.,[5] Mathai AM et al.,[11] Shashidhara et al.,[21] and Kongoet al.,[4] have also used a similar methodology with consistent results. For routine purposes, it is often desirable to obtain the paraffin sections in a few hours, but this is not possible with the conventional method. With the help of microwave it is theoretically possible to speed up tissue processing through the use of heat and it was possible to run several short cycles of about 1hr each, during the working day so that stained sections were available on the same day as the specimens were received.[25]

We believe that rapid microwave assisted tissue processing is an optimal method for substantially reducing the turnaround time and permitting the histopathology laboratory to provide same day diagnosis for a variety of tissue biopsy specimens.

The merits of microwave histoprocessing have surpassed the routine conventional protocol in many ways like: Being less labor intensive and facilitating rapid diagnosis. Tissue processing using a microwave is cheaper and using the domestic microwave instead of a highly expensive commercially available microwave further reduces the cost. By this innovative method, pathologists can now offer an early final diagnosis which eventually results in a more efficient and better management of patients. Since the only equipment required for this method in histopathology is a microwave oven, the technique is considered highly suitable for hospital laboratories as well as research laboratories where histological materials are routinely processed.

We strongly believe in a famous quote “A stitch in time saves nine” and hence we have made an attempt toward faster, reliable, cost-effective diagnosis and timely institution of treatment for better health care.

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Processing steps	Reagents	Time
Fixation	10% buffered formalin	Over night 720 mins (12hrs)
Dehydration	Isopropyl Alcohol 70%	60 mins (1hr)
	Isopropyl Alcohol 80%	60 mins (1hr)
	Isopropyl Alcohol 90%	60 mins (1hr)
	Isopropyl Alcohol 100%	60 mins (1hr)
Clearing	Xylene	60 mins (1hr)
	Xylene	60 mins (1hr)
Impregnation	Paraffin wax	30 mins(1/2hr)
	Paraffin wax	30 mins (1/2hr)
Total duration	1140 minutes (19 hours)	

Table 1: The conventional method of tissue processing

Steps	Protocol I	Protocol II	Protocol III	Protocol IV	Protocol V	Protocol VI
Fixation	Microwave	Conventional	Conventional	Microwave	Conventional	Microwave
	15 min	Over night	Over night	15min	Over night	15min
Dehydration	Conventional	Microwave	Conventional	Microwave	Microwave	Microwave
	Iso propyl alcohol 70%, 80%, 90%, & 100%	Iso propyl alcohol 100%	Iso propyl alcohol 70%, 80%, 90% & 100%	Iso propyl alcohol 100%	Iso propyl alcohol 100%	Iso propyl alcohol 100%
	1 hour each	15mins	1 hour each	15mins	15mins	15mins
Clearing	Conventional	Microwave	Conventional	Microwave	Microwave	Microwave
	Xylene I & II	Xylene	Xylene I & II	Xylene	Xylene	Xylene
	1 hour each	15mins	1 hour each	15mins	15mins	15mins
Wax Impregnation	Conventional	Conventional	Microwave	Conventional	Microwave	Microwave
	Paraffin I & II	Paraffin I & II	Paraffin	Paraffin I & II	Paraffin	Paraffin
	30min each	30min each	15min	30min each	15min	15min
Duration	7hr 15min	13hr 30min	18hr 15min	1hr 45min	12hr 45min	1 hour

Table 2: The Microwave tissue processing protocols

Study Groups	Pre-Processing	Post- Processing	p Value
	Mean±Std. Deviation	Mean±Std. Deviation	
Protocol I	0.50±0.0	0.467±0.071	0.195
Protocol II	0.50±0.0	0.400±0.087	0.008
Protocol III	0.50±0.0	0.422±0.083	0.023
Protocol IV	0.50±0.0	0.478±0.044	0.169
Protocol V	0.50±0.0	0.467±0.071	0.195
Protocol VI	0.50±0.0	0.456±0.088	0.169
Conventional	0.50±0.0	0.444±0.073	0.050

Table 3: The mean±standard deviation shrinkage values of pre- & Post-processed tissues

Microscopic Assessment	p Value of Conventional Vs Different Protocols					
	I	II	III	IV	V	VI
Quality of Tissue	0.159	0.331	0.535	0.331	0.136	0.539
Tissue Architecture	0.331	0.056	0.535	0.159	0.136	0.609
Quality of Staining	0.094	0.396	0.113	0.499	0.936	0.455
Nucleus and Cytoplasmic Differention	0.360	0.360	0.427	0.193	0.185	0.1000
Overall Quality of Diagnosis	0.427	0.556	0.696	0.311	0.427	0.1000

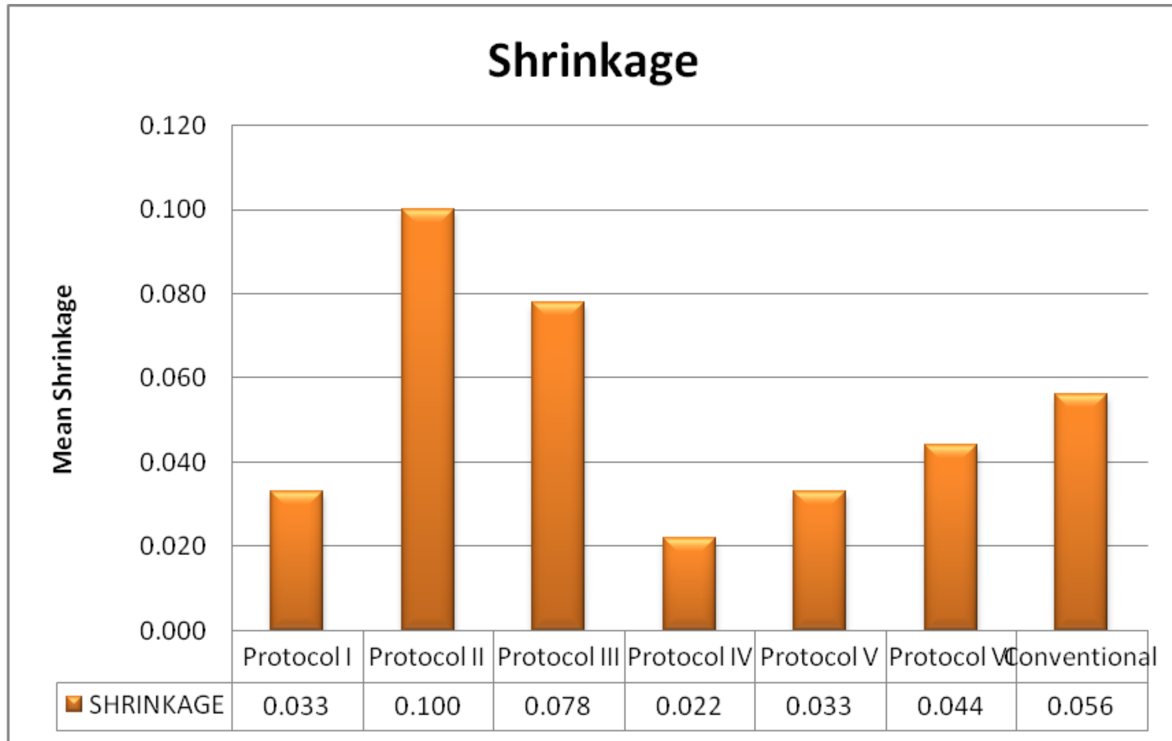
Table 4: Statistical analysis of microscopic assessment of tissue processed by conventional when compared to various protocols processed by microwave

Study Groups	Correlation Coefficient 'r'
Protocol I	0.542
Protocol II	0.389
Protocol III	0.732
Protocol IV	-0.142
Protocol V	0.123
Protocol VI	0.764

Table 5: Correlation Coefficient for various protocols of microwave with conventional tissue processing

Study Groups	Turnover Time
Protocol I	7 hr 15 Min
Protocol II	13 hr 30 Min
Protocol III	18 hr 15 Min
Protocol IV	1 hr 45 Min
Protocol V	12 hr 45 Min
Protocol VI	1 hr
Conventional	19 hr

Table 6: The turnover time for various protocols of microwave and conventional tissue processing



Graph 1: The mean±standard deviation values of tissue shrinkage among the study groups

Figure Legends

Figure 1: The pre and post processed tissue specimens being evaluated for its shrinkage

Figure 2: The photomicrograph showing the normal salivary glands under microwave different protocol tissue processing (H&E X10)

Figure 3: The photomicrograph showing the squamous cell carcinoma under microwave different protocol tissue processing (H&E X10)