

## Impregnation and embedding using bees wax and paraffin wax in oral tissue: a comparative study

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### Abstract

Paraffin wax is normally used in impregnation and embedding of tissue samples in biopsy. This study aims to compare paraffin wax that is used as routine embedding media and beeswax in impregnation and embedding of tissues. 10 biopsy specimens were impregnated and embedded in paraffin wax and 10 biopsy specimens were impregnated and embedded in beeswax. After manual processing all sections were stained with Haematoxylin and Eosin (H and E) to compare the effect of beeswax and paraffin wax based on the features of integrity of the section, uniformity of the stain, staining which includes nuclear details, cytoplasmic details and the background staining. The study findings proved that beeswax showed well impregnation and embedding of the tissues as well as preservation of the nuclear details, good cytoplasmic appearance, good tissue architecture and no bad effect on staining characteristics of the tissue. In addition, beeswax reduced the time needed for wax cooling. We conclude from our study that beeswax work well in preparation of tissue specimen when compared to paraffin. Thus, we recommended using beeswax in our laboratories as national available waxes.

**Keywords:** Bees wax, Paraffin, Embedding, Impregnation.

### Introduction

Processing is the process of removal of water from the tissues to allow for the penetration of wax, in which the tissue is finally embedded. Embedding of the tissue occurs after processing. When the processed cassettes are removed from their molten wax bath, the cassette are opened one by one and they are placed in L shaped discs. The specimens are organized in the mold so that the most representative facets of tissue are available for subsequent studies. The wax is then poured over the sample. The solid block of wax with the tissues can be stored in for many years to come. These blocks are retained by the laboratory for any future studies if required.

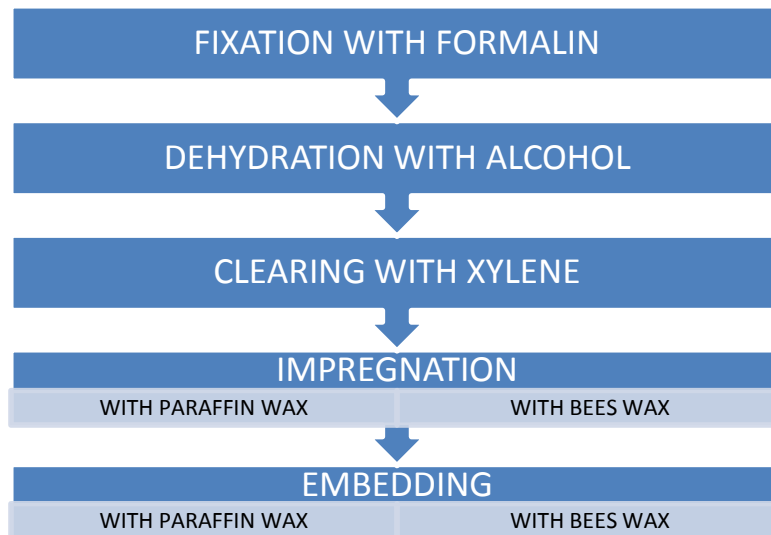
Paraffin wax is a white or colourless soft solid derivable from petroleum, coal or oil shale, that consists of a mixture of hydrocarbon molecules containing between twenty and forty carbon atoms.<sup>(1)</sup> It is solid at room temperature and begins to melt above approximately 37°C (99°F); its boiling point is >370°C (698°F).<sup>(2)</sup> Paraffin wax was first created in the 1850s and marked a major advancement in candle making technology. Its uses in the field of Medicine is its impregnation and embedding property of biopsy specimens for histopathological diagnosis. Beeswax (Cera alba) is a natural wax produced by honey bees of the genus *Apis*<sup>(3)</sup> and consists mainly of esters of fatty acids and various long-chain

alcohols.<sup>(4)</sup> Beeswax has a relatively low melting point range of 62°C to 64°C (144°F to 147°F).<sup>(5)</sup> If beeswax is heated above 85°C (185°F) discoloration occurs. Natural beeswax when cold it is brittle; at ordinary temperatures it is tenacious; its fracture is dry and granular. It softens when held in the hand, and melts at 62 to 66°C (143.6 to 150.8°F); it solidifies at 60.5 to 63°C (140.9 to 145.4°F).<sup>(6)</sup> This study is the first of its kind to compare bees wax and paraffin wax in the impregnation and embedding of tissue samples.

### Materials and Methods

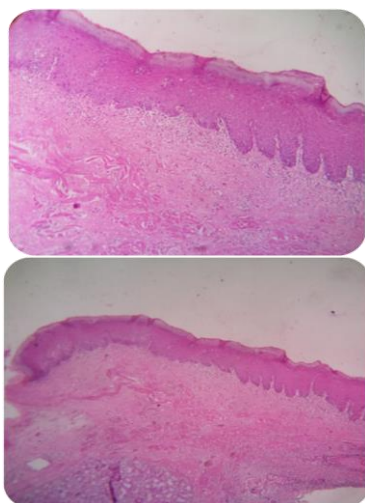
Study was carried out at the department of Oral Pathology, Saveetha Dental College. Random sampling was done based on the biopsy samples received. Sample size was calculated to be 20. The specimens were split into two and was labeled as A and B. Group A contains 10 specimen in which bees wax was added for impregnation and embedding. Group B included 10 specimens in which paraffin wax was added for impregnation and embedding. Then routine tissue processing and embedding was done except for the wax. Scoring criteria includes intensity of the section, staining features which included nuclear details, cytoplasmic details and background staining. Scoring was then calculated and chi square statistical analysis was done and the results are tabulated.

**Procedure**



**Results**

Among the samples, excellent integrity of the section was seen in group A (50.98%) compared to group B(49.02%)(Table 1). Nuclear details was more pronounced in Group B (47.9%) when compared to Group A(52.1%)(Table 2). Regarding the cytoplasmic staining Group A (51.06%) showed excellent results when compared to group B(48.94%)(Table 3). The background staining was good in group A (75%) when compared to group B (25%)(Table 4). Then in Group B(55.6%) and Group A(44.4%)(Table 5). Fig. A and B shows tissues impregnated with bees m-wax and paraffin wax respectively. Chi square statistical analysis was done and was found to be statistically significant.



**Fig. A-**tissues impregnated and embedded with bees wax and **Figure B-** tissues impregnated and embedded with paraffin wax

**Table 1: Integrity of the specimen**

Group A	Group B
47.9%	52.1%

**Table 2: Integrity of the specimen**

Group A	Group B
50.98%	49.02%

**Table 3: Cytoplasmic details**

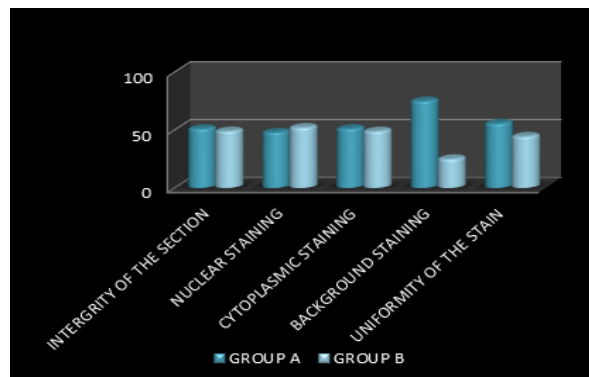
Group A	Group B
51.06%	48.94%

**Table 4: Background staining**

Group A	Group B
75%	25%

**Table 5: Uniformity of staining**

Group A	Group B
55.6%	44.4%



**Fig. 2:** Shows the graph between two groups

**Discussion**

The above results proved that bees wax showed good intensity of the section when compared to the

paraffin wax. Intensity of the section was good and there was no distortion in the sections when compared to paraffin wax. Bees wax impregnated slides showed minimal tissue destruction when compared to paraffin wax may be due to the density of the wax. Staining was excellent with crisp and clear morphological details. Nuclear details was good in paraffin wax when compared to bees wax. Cytoplasmic staining was excellent in bees wax. Background Bees wax showed excellent features when compared to paraffin wax. Overall results proved that bees wax has good impregnation and embedding properties.

Other advantages of bees wax was that it's was easier for the technicians in sectioning when compared to bees wax. Bees wax embedded samples didn't stick to the microtome blades and was easier for sectioning the tissue samples. So it is easier for the technicians and also provided excellent cellular details.

### **Conclusion**

To go organic is a theme of the present day; everyone is trying to explore it in their own field to combat the global warming. An attempt was made to explore the natural substance such as bees wax as a substitute for paraffin wax in embedding and impregnation of tissues. Apart from the nuclear details all other criteria showed excellent features when compared to paraffin wax. With an added benefit bees wax being eco-friendly, easily available, cost effective, nontoxic and noninflammable, it can also be used as an effective alternative.

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