Original Article

Impregnation and Embedding using Bees Wax and Paraffin Wax in Oral Tissue Samples: A Comparative Study

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Abstract

Aim: The aim of this study is to compare paraffin wax that is used as routine embedding media and beeswax in impregnation and embedding of oral tissues. **Materials and Methods:** Ten biopsy specimens were impregnated and embedded in paraffin wax, ten biopsy specimens were impregnated and embedded in beeswax. After manual processing, all sections were stained with Hematoxylin and Eosin to compare the effect of beeswax and paraffin wax based on the features of the integrity of the section, uniformity of the stain, staining which includes nuclear details, cytoplasmic details, and background staining. **Results:** Beeswax showed well impregnation and embedding of the tissues as well as the 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. **Conclusion:** Beeswax can be used as an alternative to paraffin wax. Thus, we recommended using beeswax in our laboratories as they are widely available.

Keywords: *Beeswax, embedding, impregnation, paraffin*

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Introduction

The predominant method for producing tissue sections is by cutting Paraffin wax embedded tissue on a microtome.[1] Tissue contains water molecules and will not allow the embedding media to enter. Dehydration substitutes the water in the tissue with alcohol substitute. Clearing is to make the tissue miscible with wax in which the tissue is finally embedded.[1] Embedding of the tissue is done to produce tissue blocks for microtomy.[2] The solid block of wax with the tissues can be stored in for many years to come.[1] These blocks are retained by the laboratory for any future studies if required. Paraffin wax is a white or colorless soft solid derived from petroleum, coal or oil shale, that includes a mixture of hydrocarbon molecules containing between 20 and 40 carbon atoms.[3] It is solid at room temperature and begins to melt above approximately 37°C (99°F) its boiling point is >370°C (698°F).[4] Paraffin wax was first created in the 1850s, and marked a major advancement in candle making technology.[5] Other uses of paraffin in the field of medicine is its impregnated and embedding property of biopsy specimens[6] for histopathological diagnosis. Beeswax (Cera Alba) is a natural wax produced by honey bees of the genus Apis. The wax is formed into "scales" by eight wax-producing glands in the abdominal segments of worker bees, who discard it in or at the hive.[7] Chemically, beeswax consists mainly of esters of fatty acids and various long-chain alcohols. Beeswax has a relatively low melting point range of 62°C–64°C (144°F–147°F). If beeswax is heated above 85°C (185°F) discoloration occurs. The flash point of beeswax is 204.4°C (400°F).[8] Density at 15°C is 958 kg/m^3–970 kg/m^3.[8] Natural beeswax. When cold it is brittle at ordinary temperatures, it is tenaciousits fracture is dry and granular. It softens when held in the hand, and melts at 62°C–66°C (143.6°F–150.8°F);[9] it solidifies at 60.5°C–63°C (140.9°F–145.4°F).[9] This study is the first of its kind to compare beeswax and paraffin wax based on the impregnation and embedding of tissue samples.

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Materials and Methods

The study was carried out at the Department of Oral Pathology, Saveetha Dental College, Chennai, Tamil Nadu, India. A random sampling of excision tissue samples was done based on the biopsy samples received. The sample size was calculated to be 20. The specimens were split into two and was labeled as A and B. Group A included 10 specimens that were impregnated and embedded with beeswax. Group B included 10 specimens that were impregnated and embedded with paraffin wax. The specimens underwent the same fixation and tissue processing cycle but for the wax impregnation and embedding was done. After microtomy, sections were stained with Hematoxylin and Eosin following the conventional protocol. The sections were then evaluated for the ease of sectioning, integrity of the section, quality of staining, clarity of nuclear and cytoplasmic details and the presence of background staining. The slides were evaluated by two independent observers, and the results were compared using Chi-square statistical analysis.

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 were 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 more in Group A (75%) when compared to Group B (25%) [Table 4]. The uniformity of the stain was better in Group B (55.6%) compared to Group A (44.4%) [Table 5].

Discussion

Impregnation is the process of complete removal of clearing reagents by substitution of paraffin[10] or any such similar media such as beeswax. After complete impregnation with a suitable medium, solid block of suitable medium containing impregnated tissue is obtained by a process called embedding.[10] The most commonly used media for impregnation and embedding is a paraffin wax.[11] This study is the first of its kind to use beeswax in both impregnation and embedding and to compare the staining characteristics and integrity of the section with paraffin wax.

Paraffin wax is the most commonly used medium in embedding and impregnation of tissues over many

Table 1: Integrity of the section

Group A (%)	Group B (%)
50.98	40.02

Table 2: Nuclear details

Group A (%)	Group B (%)
47.9	52.1

Table 3: Cytoplasmic staining

Group A (%)	Group B (%)
51.06	48.94

Table 4: Background staining

Group A (%)	Group B (%)
75	25

Table 5: Uniformity of the stain

Group A (%)	Group B (%)
55.6	44.4

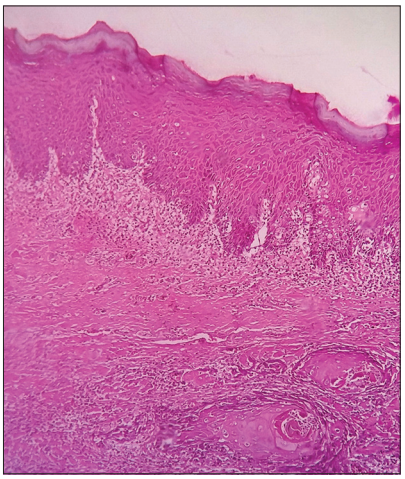

Figure 1: Impregnation and embedding using bees wax

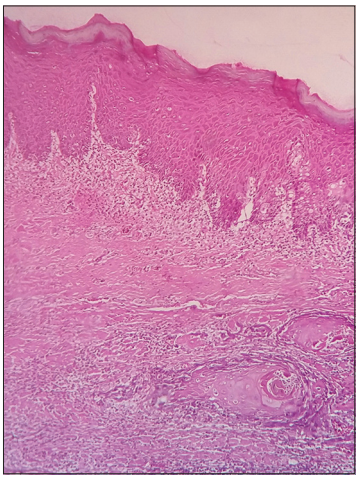

Figure 2: Impregnation and embedding using paraffin wax

years.[11] It is fairly inert mixture of hydrocarbons produced by the crackling of petroleum. These waxes are mixtures of purified paraffin wax and various additives that may include resins such as styrene or polyethylene the wax is liquid at 60°C and can be infiltrated into tissue at this temperature and then allowed to cool to 20°C where it solidifies to a consistency that allows sections to be consistently cut. The embedded tissue with the paraffin wax is sectioned at a thickness of 2 μm, to form ribbons as the sections are cut on the microtome and they retain sufficient elasticity[12] to flatten fully during flotation on a warm water bath.

The results from our study proved that beeswax showed good integrity of the section (50.98%) when compared to the paraffin wax. The integrity of the section was good and there was no distortion in the sections when compared to paraffin wax because of the crystal size of the beeswax and increased cohesion. Furthermore, bees wax impregnated slides showed minimal tissue destruction when compared to paraffin wax due to the density of the beeswax.[9]

The staining of tissue sections embedded in beeswax was excellent [Figure 1] and showed crisp and clear morphological details compared to the paraffin wax. Nuclear details was good in paraffin wax (52.1%) [Figure 2] when compared to bees wax. Cytoplasmic staining (51.06%) was excellent in beeswax. Furthermore, the staining was more uniform in sections impregnated and embedded in beeswax (55.6%) compared to the paraffin wax. However, beeswax showed relatively more background staining compared to the paraffin wax. This might be overcome by establishing protocols that are customized for beeswax impregnation and embedding. Overall results proved that beeswax has good impregnation and embedding properties when compared with paraffin wax.

Another advantage of beeswax was that its was easier for the technicians in sectioning when compared to beeswax. Beeswax embedded samples did not stick to the microtome blades and was easier for sectioning the tissue samples. This is because it increases adhesion, reduces brittleness and makes the formation of ribbons during sectioning easier.

Conclusion

To go organic is a theme of the present day. Everyone is trying to explore it in their field to combat the ill effects of petroleum based products. An attempt was made to explore the natural substance such as beeswax 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 beeswax being eco-friendly, easily available, cost-effective, nontoxic and noninflammable, it can also be used as an effective alternative. The study needs to be expanded further and an appropriate protocol to be established so that beeswax can substitute paraffin wax in our Pathology Laboratories.

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Conflicts of interest

There are no conflicts of interest.

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