

Embedding Media in Histopathology: A Narrative Review of Paraffin Wax, and Natural Alternatives

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ABSTRACT

Tissue embedding is a critical step in histopathological processing because it provides the structural support required for thin sectioning, staining, and microscopic examination. For more than a century, paraffin wax has remained the standard embedding medium because of its excellent tissue infiltration, consistent ribbon formation, preservation of tissue morphology, long-term storage stability, and compatibility with routine histology, immunohistochemistry, and molecular diagnostic techniques. However, concerns regarding its petroleum-derived origin, environmental impact, and sustainability have stimulated interest in renewable natural waxes as alternatives. This narrative review critically evaluated paraffin wax and selected natural embedding media, including beeswax, soy wax, coconut wax, candelilla wax, carnauba wax, rice bran wax, palm wax, and bayberry wax. Relevant peer-reviewed literatures were synthesized to compare their physicochemical properties, embedding performance, advantages, limitations, and suitability for routine histopathological practice. Evidence showed that paraffin wax continues to provide superior overall performance and remains the benchmark for diagnostic tissue embedding. Among the natural alternatives, beeswax demonstrated the strongest direct evidence for tissue embedding, while soy wax showed potential in modified formulations. Evidence supporting coconut, candelilla, carnauba, rice bran, palm, and bayberry waxes remains largely indirect and based on physicochemical or industrial studies rather than validated histopathological applications. Key challenges include limited comparative studies, lack of standardized evaluation protocols, variability in natural wax composition, and inadequate validation with ancillary diagnostic techniques. Overall, natural waxes offer promising sustainable alternatives, but current evidence is insufficient to support their routine replacement of paraffin wax in diagnostic histopathology, highlighting the need for standardized experimental studies.

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INTRODUCTION

Histopathology is a crucial technique in disease diagnosis and biomedical research, providing insights into the microscopic structure and cellular changes linked to normal and pathological processes (Al-Sabaawy *et al.*, 2021). Appropriate tissue processing is an important part of the accuracy of the histopathological diagnosis and the sequence of steps has changed significantly since the nineteenth century. These steps involve fixation, dehydration, clearing, infiltration, embedding, sectioning, and staining, all of which help to maintain the tissue structure and ensure that the resulting microscopic sections are of high quality (Isaac *et al.*, 2023). Embedding is one of the most critical stages as it makes tissue rigid and firm enough for thin sectioning, and preserves the morphology and integrity of tissues for accurate microscopic evaluation (Al-Sabaawy *et al.*, 2021; Zhanmu *et al.*, 2020).

For a long time, paraffin wax has been known as the standard embedding medium used in routine histopathology because of its favourable physicochemical properties such as a controlled melting range of about 55–58°C, efficient tissue infiltration, excellent ribbon formation during microtomy and compatibility with a wide range of routine histological stains, immunohistochemistry and *in situ* hybridization techniques (Zhanmu *et al.*, 2020). In addition, formalin fixed paraffin embedded (FFPE) tissue is the most widely used standard for

archival storage and can be preserved at room temperature for long periods of time, enabling reliable long-term preservation, retrospective diagnostic evaluation and a variety of molecular investigations (Bai *et al.*, 2024; Likhithaswamy, 2022).

Despite these benefits, increasing efforts have been placed into finding more suitable embedding media. The impetus for this interest is largely due to the environmental issues surrounding the fossil fuel origin of paraffin wax and the solvent intensive tissue processing procedures that its use entails, and not because of any failure of paraffin wax to perform histopathologically. The traditional method of paraffin processing involves dehydration and subsequent clearing and deparaffinization with heat and xylene, which has been linked to health risks for workers, such as neurotoxicity, and to environmental pollution (Wang *et al.*, 2024). Therefore, environmentally friendly alternatives that can minimize the use of harmful reagents while ensuring acceptable histological quality have been of great interest (Marinho & Hänscheid, 2023).

It is important, however, to distinguish between research on sustainable tissue-processing reagents and studies investigating alternative embedding media. The literature that has been reported as 'natural alternatives' is more concerned about replacing xylene in tissue clearing and deparaffinization or about using specialized synthetic embedding materials like hydrogels and resins (Thajudeen *et al.*, 2022). Thus, the use

of natural waxes in routine histopathological embedding is still in its infancy. In this context, this narrative review critically reviews paraffin wax and eight natural waxes namely beeswax, soy wax, coconut wax, candelilla wax, carnauba wax, rice bran wax, palm wax and bayberry wax. The review summarizes the state of the art on their physicochemical properties, histopathology performance, advantages, limitations, and new applications; identifies research gaps and future directions of the development of sustainable embedding media in histopathology.

Review Methodology

This paper adopts a narrative review to critically review the literature on embedding media used in tissue preparation for histopathology with a focus on paraffin wax and some natural waxes that were used as alternatives. Key words like tissue embedding, paraffin wax, natural waxes, histopathology, tissue processing and related words were used to retrieve relevant literature from electronic databases like PubMed, Scopus, Web of Science, ScienceDirect and Google Scholar. Peer-reviewed journal articles, review papers and relevant technical reports published mainly in English were considered, and focused on both classical and recent studies on the physicochemical properties, histological performance, advantages, limitations and emerging applications of embedding media. The retrieved literatures were critically appraised and thematically synthesized to give an overall picture of the existing knowledge, research gaps and future directions of the application of paraffin wax and natural alternatives in histopathology.

Principles of Tissue Embedding in Histopathology

In the histopathological processing sequence (fixation, dehydration, clearing, infiltration, embedding, sectioning and staining), tissue embedding is a very important step. Each stage sets the stage for the next and mistakes made at any stage can have a negative impact on the quality of the final microscopic section. The primary role of fixation is to prevent autolysis and putrefaction, maintain tissue architecture and stabilize cellular components, but inadequate fixation or over-exposure to fixatives can cause tissue shrinkage, swelling, hardening and other processing artefacts. Dehydration then removes the water from the tissue via a series of graded alcohol solutions, followed by a clearing agent which removes the alcohol and allows the specimen to be infiltrated with a hydrophobic embedding medium. The clearing agent is gradually replaced by the molten embedding medium which fills the tissue spaces and gives internal support when it cools (Al-Sabaawy *et al.*, 2021; Isaac *et al.*, 2023; Taqi *et al.*, 2018). Embedding is a process of turning the infiltrated tissue into a solid block which can be suitable for reproducible sectioning. A specimen is carefully oriented in a mould according to the required plane of examination, surrounded by the embedding medium and allowed to solidify. It is important because a well processed specimen might yield diagnostically poor sections if embedded incorrectly. The hardened block should be strong enough to allow sections only a few micrometres thick to be produced without compression, tearing, cracking, fragmentation, folding or chatter. Therefore, the ability to embed depends not only upon the compatibility of the embedding medium with preceding processing reagents, but also upon its ability to infiltrate the tissues properly, solidify uniformly and remain dimensionally stable during microtomy (Al-Sabaawy *et al.*, 2021; Nilcham *et al.*, 2025).

Physical and mechanical characteristics of embedding medium are important factors in the quality of sections. The medium can be too soft and deform during microtoming,

compress tissues or fail to provide continuous ribbons, or too hard and brittle and crack, separate from the tissue or create chatter and tearing. The balance of hardness and flexibility is influenced by the melting range, viscosity, crystallinity, cohesiveness, plasticity, cooling rate and dimensional stability. These are also influenced by laboratory factors such as block temperature, sharpness of blades, speed of sectioning and type of tissue to be cut. Too high a paraffin temperature can cause shrinkage or hardening of the tissue, while too low a paraffin temperature can cause unsupported areas to fall apart during the sectioning process. To achieve this, it is important to control the temperature so as to keep the embedding medium in a fluid state and at the same time minimize unnecessary heat exposure of the tissue (Al-Sabaawy *et al.*, 2021; Taqi *et al.*, 2018).

Differences in the physical properties of the embedding medium are also demonstrated by the differences among the various tissue types. Copper *et al.* (2018) have shown that the quality of histological preparations was affected by the combination of fixation, decalcification, embedding formulation and sectioning procedures, which means that processing protocols need to be optimized for specific types of specimens. In the same way, Zhanmu *et al.* (2020) showed that for paraffin embedding of whole brains and other large biological specimens, longer processing times and size- and tissue-specific protocols are needed for success. These results show that an embedding medium's performance must not be judged apart from characteristics of the tissue, size of the specimen and processing conditions.

The best embedding media should penetrate tissues evenly and set in a reproducible manner to produce thin, continuous, and perfect sections. It should maintain both the structure of the tissue and the detail of the cells and should reduce shrinkage, distortion and separation from the matrix surrounding the tissue. It should also help to make efficient ribbon and be compatible with the standard haematoxylin and eosin staining and other special histochemical stains and ancillary diagnostic and research methods. Formalin fixed paraffin embedded tissues continue to be the gold standard for routine histological and immunohistochemical analysis and are becoming more and more popular for advanced molecular analyses of archived tissues such as spatial and transcriptomic analysis (Al-Sabaawy *et al.*, 2021; Bai *et al.*, 2024). However, pre-analytical variables such as fixation methods and tissue processing conditions could still affect the quality of these applications. Stability at room temperature is also of critical importance for long-term storage, which allows for retrospective diagnosis, teaching and research of tissue blocks. There is evidence that paraffin blocks in general can be stored for a long period of time without compromising the quality of morphological preservation, though the quality of fixation, the storage conditions and the age of the specimen may affect subsequent analyses (Likhithaswamy, 2022).

Practical aspects also need to be taken into consideration when evaluating the appropriateness of an embedding medium. An ideal medium should be safe to handle, readily available, cost-effective and compatible with existing laboratory equipment, and should not involve a complex processing procedure that results in a significant increase in turnaround time, technical workload or risk of processing error. In today's high throughput laboratories, compatibility with automated tissue-processing and embedding systems is becoming more critical, especially when handling small biopsy specimens and cytological samples that can be easily displaced or lost. Automated paraffin cell-block preparation has been shown to increase standardization without compromising reagent penetration, cellular morphology or

staining quality; however, special support matrices may be needed with very small specimens (Taffon *et al.*, 2024). Similarly, embedding accessories like the Histo-LOOP have been developed to facilitate the orientation of tissues and standardise embedding of punch biopsies, tubular tissues and multiple small tissue specimens in a single paraffin block (Nilcham *et al.*, 2025).

No one embedding medium is adaptable for all tissues or analytical use. In some cases, the three-dimensional structure is lost by conventional paraffin processing due to the loss of hydration in microbial communities and other structurally complex specimens, which is why hydrophilic resin systems are suitable in these cases (Hasegawa *et al.*, 2017). Comparative studies have also indicated that glycol methacrylate resin is superior in terms of intracellular resolution while paraffin is better in visualizing mineralized tissues and is the medium of choice for routine diagnostic practice (İnan *et al.*, 2025). Likewise, very small skin biopsies, organoids and cytological specimens may require agar, gelatinous matrices or specialised holders as pre-embedding supports prior to final paraffin processing (Ridolfi *et al.*, 2019; Taffon *et al.*, 2024), and undecalcified bone requires specialized resin-based embedding techniques to preserve the mineralized structure. The use of any embedding medium should therefore be judged on how well it infiltrates and sections, how well it preserves the morphological and molecular integrity of the specimen, the nature of the specimen to be embedded and the application to be made, for diagnostic or research purposes.

Paraffin Wax as the Conventional Embedding Medium

Paraffin wax is a petroleum-derived mixture of saturated hydrocarbons that has been specially formulated for use in histopathology. Most laboratory grade paraffin waxes have a controlled melting range of about 55-58°C and this is one of the reasons why they are suitable for routine tissue processing (Al-Sabaawy *et al.*, 2021; Isaac *et al.*, 2023). Due to its good physico-chemical properties, paraffin has emerged as the standard embedding material in diagnostic histopathology. The processing sequence starts with the formalin fixation, which stabilizes the tissue components and preserves the tissue architecture, and then the tissue is dehydrated by using various grades of alcohol. This is usually done by replacing the alcohol with a clearing agent, such as xylene, which makes the tissue compatible with molten paraffin. The molten wax slowly replaces the clearing agent and infiltrates the tissue, which helps to support it internally until it is properly oriented in an embedding mould and allowed to cool to form a paraffin block suitable for microtomy (Al-Sabaawy *et al.*, 2021; Zhanmu *et al.*, 2020). The solidified block is then sectioned on a rotary microtome to make thin reproducible sections for microscopic examination (Al-Sabaawy *et al.*, 2021; Isaac *et al.*, 2023). During this process, the temperature of paraffin should be kept just above its melting point to avoid excessive heating which causes more harden, brittleness and sectioning artefacts (Al-Sabaawy *et al.*, 2021; Taqi *et al.*, 2018).

Practical Strengths and limitations as the Benchmark Medium

Despite recent advances in other embedding media, paraffin wax remains the standard embedding medium used in routine histopathology due to its good compromise between tissue infiltration, sectioning quality, the capacity to stain well, cost effectiveness and long term preservation. After solidification, it offers firm, yet flexible support for tissues enabling the production of smooth block faces, continuous ribbons and reproducible serial sections for routine diagnosis (Al-

Sabaawy *et al.*, 2021; Isaac *et al.*, 2023). These properties help in the preparation of thin sections without altering the overall tissue architecture, and hence paraffin can be used for diagnostic and research purposes (Al-Sabaawy *et al.*, 2021). Another benefit of paraffin is that it can be used with standard histological methods and for sophisticated diagnostic applications. Formalin fixed paraffin embedded (FFPE) tissues are suitable for routine haematoxylin and eosin staining, a wide variety of special histochemical staining, immunohistochemistry and *in situ* hybridization. They are also becoming more and more important in molecular pathology, where they can be used to support DNA and RNA analyses, quantitative polymerase chain reaction, next-generation sequencing, spatial transcriptomics, and other retrospective molecular investigations, provided the correct pre-analytical procedures are followed (Al-Sabaawy *et al.*, 2021; Bai *et al.*, 2024). The creation of large FFPE tissue banks has also strengthened the field of translational research by the availability of clinically annotated specimens over long time periods. Furthermore, paraffin blocks have been found to provide good long term morphological preservation when stored under proper conditions and these have been useful for retrospective diagnosis, education and biomedical research (Likhithaswamy, 2022).

Comparative studies, also, support the status of paraffin as the standard embedding medium. While resin-based media might be more advantageous for specialized applications, paraffin offers better preservation of overall tissue architecture and is more convenient for routine use in the laboratory (İnan *et al.*, 2025). Similarly, while frozen tissue preparations offer better retention of some molecular components for specific analyses, paraffin offers better structural preservation, handling, and long-term storage compared to frozen tissues (Bai *et al.*, 2024). All these benefits combined are the reasons why paraffin is still considered as the standard for which all potential natural embedding media are compared. Therefore, the effectiveness of any proposed alternative should be assessed, in addition to its environmental impact, for its effectiveness in tissue infiltration, ribbon formation, sectioning quality, staining compatibility, tissue morphology, block stability, archival capabilities and ease of incorporation into the routine laboratory.

However, paraffin wax has its drawbacks which have spurred the quest for other embedding media. The classic paraffin processing can cause shrinkage, distortion and retraction artefacts in the tissues, primarily due to dehydration, clearing, and thermal exposure during paraffin processing (Taqi *et al.*, 2018; Mazzoni & Quagio-Grassiotto, 2021). These procedures can also remove lipids and other soluble tissue components, resulting in the removal of some extracellular matrix components and in subtle changes in tissue morphology (Mazzoni & Quagio-Grassiotto, 2021). In addition, high processing temperatures can cause harden and brittleness of tissues, affecting the quality of sections and making tissues more prone to tearing or compression during microtomy (Al-Sabaawy *et al.*, 2021).

Another acknowledged constraint is the preservation of molecules. FFPE tissues are still useful for many molecular applications, but formalin fixation and paraffin processing result in chemical cross-linking and fragmentation of nucleic acids. Long-term storage can also lead to a progressive decrease in the quality of the RNA which may impact the results of some downstream molecular analyses (Bai *et al.*, 2024; Likhithaswamy, 2022). In addition, xylene is extensively used in paraffin processing for clearing and deparaffinization which is a routine procedure. Because of the association of exposure to xylene with occupational hazards

such as neurotoxicity, respiratory irritation, skin and mucosal irritation, and environmental pollution, safer and more environmentally friendly alternatives are encouraged (Wang *et al.*, 2024; Thamilselvan *et al.*, 2021; Thajudeen *et al.*, 2022).

Tissue-Specific Challenges

Some tissue types and special applications further exemplify the drawbacks of paraffin embedding. For fragile specimens like lungs, processing conditions need to be carefully optimized to maintain the fine alveolar structure and reduce any distortion during the embedding and sectioning process (Zhanmu *et al.*, 2020). Likewise, paraffin is not the ideal embedding medium for undecalcified mineralized tissues, as glycol methacrylate-based resins allow for better preservation of cellular and mineralized structures (Inan *et al.*, 2025). For large specimens, such as whole brains, there are also additional challenges, as it may take a longer processing time and need to be adjusted to the size of the specimen to ensure uniform infiltration of the tissue with paraffin without compromising the morphology of the tissue (Zhanmu *et al.*, 2020).

Even small biopsies, cytological specimens and other small fragile pieces of tissue must be handled carefully and oriented precisely prior to embedding so that tissue loss is minimised and diagnostic yield maximized. For this purpose, specialised pre-embedding supports, matrices and orientation devices have been developed to improve the recovery of the specimen and to standardize the procedure of paraffin embedding small specimens (Ridolfi *et al.*, 2019; Taffon *et al.*, 2024; Nilcham *et al.*, 2025). However, paraffin wax still is the most reliable embedding medium for daily histopathology with its known drawbacks. It remains the benchmark processing protocol, is widely accepted in the laboratory, demonstrates good diagnostic performance, is compatible with conventional and molecular methods, and has proven long-term archival stability, which continue to make it a good choice as a reference standard for which new natural embedding media are evaluated (Al-Sabaawy *et al.*, 2021; Bai *et al.*, 2024; Likhithaswamy, 2022).

Natural Alternatives to Paraffin Wax

As the importance of environmental sustainability of laboratory practices has grown, the use of natural waxes as alternatives to paraffin wax derived from petroleum has been sought. This interest is not just due to the dependence on fossil-based materials but also due to the health and environmental hazards of xylene and other chemicals involved in traditional paraffin processing (Dewi *et al.*, 2021). However, although there is an increasing interest in using natural waxes as histopathological embedding media, there is a lack of evidence to support the use of natural waxes. The difference should be made between investigations of the natural waxes as embedding media for tissue and investigations of plant-derived waxes or oils for industrial, pharmaceutical, food or materials-science applications. The latter give valuable data on properties such as melting behaviour, hardness, crystallinity and mechanical performance, but do not evaluate critical histopathological parameters such as tissue infiltration, ribbon formation, sectioning quality, staining characteristics or preservation of tissue morphology. Therefore, the subsequent discussion is structured according to the strength of the available evidence and not because it is assumed that all natural waxes have similar potential as embedding media.

Beeswax

Beeswax is one of the natural waxes that has the most direct evidence for its possible use as an embedding medium for histopathology. Beeswax is an animal-derived wax which is primarily made up of long-chain esters, hydrocarbons and fatty acids, and has desirable biodegradability and physicochemical properties (Bucio *et al.*, 2021). It has also greater flexibility and ductility than paraffin wax, which may decrease the brittleness, but could affect block hardness during sectioning (Bucio *et al.*, 2021). Beeswax is one of the few natural waxes to have been tested directly for embedding tissues. Antony *et al.*, (2017) showed that it could infiltrate and embed oral tissue specimens, with a good level of nuclear and cytoplasmic detail and acceptable haematoxylin and eosin staining. This indicates potential for use of beeswax for routine histological examination; however, some optimization and validation under routine laboratory conditions are still needed.

Other evidence from materials-science research indicates that the properties of beeswax can be changed by blending it with other natural waxes. For instance, beeswax–soy wax composites possess controllable mechanical properties and degradation behaviors, allowing the formulation to control their physical properties (Atreya *et al.*, 2022). Comparative studies have also shown that beeswax is more flexible than paraffin wax and less prone to cracking when stored, which indicates its good mechanical properties (Bucio *et al.*, 2021). These observations, however, are from materials engineering and are therefore only indirectly relevant to embedding applications and are based in histopathology. In sum, the available evidence suggests that, while beeswax is the most promising natural alternative at present, they are a small number of studies involving limited tissue types and relatively small experimental validation.

Soy Wax

Among the most promising plant-based material for developing sustainable embedding media is soy wax. It is produced by hydrogenating soybean oil and has the benefits of being renewable, biodegradable and not relying on petroleum-based materials (Romero *et al.*, 2021). However, in general, soy wax has less hardness and a lower melting range compared to the conventional paraffin, which can negatively influence the sectioning performance if it is used alone as an embedding medium.

Current support for soy wax is derived from indirect evidence. Materials-science research has demonstrated that combining soy wax with beeswax and other natural waxes can greatly alter its mechanical properties, such as hardness and elasticity and degradation behaviour, making it suitable for specific applications (Atreya *et al.*, 2022). In a similar way, bio-paraffins obtained from soybean oil have presented physicochemical properties similar to those of the conventional mineral waxes, which indicates their potential as an environmentally friendly alternative (Romero *et al.*, 2021). But, until now, no direct studies have been done to evaluate tissue infiltration, block formation, ribbon quality, staining performance and long term preservation with soy based embedding media. Therefore, it seems that soy wax is a good emerging candidate at the moment, whose potential has not been properly established yet by dedicated histopathological investigations.

Coconut Wax

The use of coconut wax as a histopathological embedding medium is still largely based on indirect evidence. It should be noted that there is a difference between coconut wax and

coconut oil; most of the published studies have tested the use of coconut oil as an alternative to xylene for tissue clearing and deparaffinization, rather than coconut wax being tested as an embedding medium (Al-Sabaawy *et al.*, 2021; Thajudeen *et al.*, 2022). Therefore, the results of the studies cannot be taken as evidence to support the use of coconut wax as an embedding media for tissue embedding since the function of the clearing agents and embedding media are quite different. Previous research has shown that the use of coconut oil is less effective than some other deparaffinizing agents in terms of staining quality and the usual performance of the laboratory (Thajudeen *et al.*, 2022). Till date, no published study has directly evaluated coconut wax for purposes of tissue infiltration, block formation, ribbon production, sectioning quality or staining compatibility or long-term block preservation. While its physico-chemical characteristics indicate that it could be useful for composite wax embedding, there is still not enough evidence to support its use as a routine embedding medium.

Candelilla Wax

Another renewable plant-derived wax that has a number of physicochemical properties that could be useful in embedding applications is candelilla wax. It has relatively high hardness, good hydrophobicity, favourable thermal stability and its melting range is broadly similar to that of paraffin wax (Nuñez-García *et al.*, 2022). It has been chemically analyzed and found to contain a high percentage of long-chain hydrocarbons, and has structural properties that make it useful in industrial and pharmaceutical applications (Nuñez-García *et al.*, 2022).

Materials-science research has also shown that candelilla wax can be used to create relatively hard gel structures and that the mechanical properties of the wax can be modified by blending it with long-chain ester waxes and other additives (Shimizu *et al.*, 2022). Moreover, a comparative study demonstrated that candelilla wax is harder than beeswax, but it is also more brittle and can undergo microcracks under some circumstances, displaying a similar behaviour to paraffin wax (Bucio *et al.*, 2021). These results suggest that candelilla wax may be useful for adding useful mechanical properties to composite formulations, but do not directly show its suitability for histopathological embedding. To date, no systematic studies have been published that have assessed its performance in terms of tissue infiltration, ribbon formation, sectioning quality, staining characteristics and diagnostic utility. It therefore has only theoretical applications as an embedding medium which needs to be experimentally validated.

Carnauba Wax, Rice Bran Wax, Palm Wax and Bayberry Wax

Other potential embedding media for sustainable embedding are carnauba wax, rice bran wax, palm wax and bayberry wax. These waxes, derived from plants, have interesting physicochemical properties that make them appealing from the materials engineering point of view, such as relatively high melting points, favourable crystallinity, variable hardness and compatibility with wax-blending technologies (Nuñez-García *et al.*, 2022; Shimizu *et al.*, 2022). Rice bran wax, for instance, has been studied as part of mixed wax formulations to adjust gel hardness, but it has low hardness compared with candelilla wax and could need to be optimized in the formulation (Shimizu *et al.*, 2022).

Yet, to date, no specific proof exists showing that carnauba wax, rice bran wax, palm wax or bayberry wax can be used as embedding media for routine histopathology. The studies

available are mostly from food technology, pharmaceutical sciences, cosmetics and materials engineering and not from diagnostic pathology. Thus, their physicochemical properties should be considered as markers of potential for future research and not as markers of histopathological suitability.

Critical Synthesis

A significant amount of variation exists in the number of investigations which have been conducted for the various natural waxes. The sole natural waxes that have meaningful direct histopathological evidence are beeswax, which preliminary studies have shown to have acceptable tissue infiltration, preservation of morphology and satisfactory routine staining. Among the plant-based candidates, soy wax seems to be the most promising one, but the supporting experimental work to date is largely from the area of materials-science and physics rather than embedding studies. Coconut wax and candelilla wax have desirable material characteristics that could benefit the formulation of composites for embedding in the future, although there is no direct evidence of their histopathological performance. Similarly, carnauba wax, rice bran wax, palm wax and bayberry wax are theoretical candidates whose potential is based on their physicochemical properties and not necessarily on their experimental use for tissue embedding.

Well-designed comparative studies with the same protocols should be performed to compare natural waxes with paraffin in terms of tissue infiltration, ribbon formation, sectioning quality, staining compatibility, preservation of tissue morphology, block stability, long-term archival performance and compatibility with modern molecular pathology techniques. In the absence of such evidence, paraffin wax should continue to be used as the standard embedding medium.

Comparative Performance of Paraffin Wax and Natural Alternatives

Paraffin Wax as the Reference Standard

The quality and amount of evidence should be considered when judging the performance of paraffin wax and natural alternatives, while favourable physicochemical properties should not be used as the sole criterion. Since paraffin's effectiveness has been well established for a variety of tissue types, staining procedures and diagnostic applications, it is still the standard embedding medium. It consistently offers efficient tissue infiltration, good ribbon formation, reproducible thin sections, and excellent compatibility with traditional haematoxylin and eosin staining and special histochemical stains and other established histological techniques (Zhanmu *et al.*, 2020; Lemaire *et al.*, 2007). Furthermore, formalin-fixed paraffin-embedded (FFPE) tissues facilitate immunohistochemistry, *in situ* hybridization and a variety of molecular pathology techniques such as proteomic and mass spectrometry-based analyses (Lemaire *et al.*, 2007; Ding *et al.*, 2023).

Another significant benefit of paraffin is that it remains stable for a long time. The FFPE tissue blocks may be kept at room temperature for a long time and are excellent resources for retrospective diagnosis, translational research and molecular investigations (Likhithaswamy, 2022). Additionally, comparative studies have shown that paraffin generally preserves the overall tissue architecture better than a few other embedding systems, and that for specialized application, glycol methacrylate may be better for the intracellular resolution (İnan *et al.*, 2025). Taken together, this large volume of evidence is enough to make paraffin wax the reference medium for all natural embedding media.

Beeswax: The Best Natural Alternative

Beeswax is the best natural wax for a comparison with paraffin at present. In the past, it was part of the first histological embedding mixtures, and various mixtures of stearin and white beeswax were used to give mechanical support to the tissue during sectioning before the introduction of paraffin embedding (van der Lem *et al.*, 2021). The results of a physicochemical study show that beeswax is rather ductile and flexible, while paraffin is rather brittle. It has a lower melting temperature, which could lower the thermal exposure of tissues during processing, but it also results in softer embedding blocks that may need to be optimized for consistent microtomy (Bucio *et al.*, 2021).

Unlike most other natural waxes, beeswax has been tested as an embedding medium for tissues. It can successfully penetrate and embed tissues and retain tissue structure, and it has good nuclear and cytoplasmic morphology and satisfactory haematoxylin and eosin staining (Vini & Antony, 2017). Further material science study has demonstrated that beeswax has good moisture barrier properties and is more resistant to cracking than paraffin, and therefore, its mechanical flexibility is better (Bucio *et al.*, 2021). However, the latter results come from non-histological use, so offer only indirect evidence for tissue embedding. Overall, the evidence to date is still inadequate to conclude that beeswax is equivalent to paraffin for the various types of tissues and conditions that are commonly encountered during routine diagnostic practice.

Soy Wax: A Promising Emerging Candidate

Another embedding material that has also been utilized and has great potential is soy wax, which is a renewable substance, but so far, there are only few studies that support the use of this material, mainly performed on the physicochemical level (Romero *et al.*, 2021; Atreya *et al.*, 2022). The waxes obtained from the hydrogenation of soybean oil have shown good melting properties, crystallization and textural characteristics which indicate potential application as renewable alternatives to conventional mineral waxes. Modification of soybean-derived waxes has also been demonstrated to decrease the crystal size, change the crystalline structure and improve the plasticity, making them more suitable for structured wax applications (Romero *et al.*, 2021).

Likewise, when soy wax is mixed with beeswax and other natural waxes, it has been reported that the mechanical properties of the mixture can be varied according to the formulation to change the hardness and flexibility of the mixture (Atreya *et al.*, 2022). The results indicate that soy wax may be better suited as a part of an engineered composite waxes than as an unmodified embedding medium. However, there are limited direct evidence from tissue infiltration, ribbon formation, sectioning, staining and long-term archival stability.

Coconut Wax and Candelilla Wax: Predominantly Indirect Evidence

Much evidence cited for the use of either coconut wax or candelilla wax as embedding media is indirect. Specifically, it is crucial to differentiate coconut wax from coconut oil since most of the published studies have examined the feasibility of using coconut oil to replace xylene in tissue clearing or deparaffinizing processes, but not coconut wax as an embedding medium (Kharomah & Nailufar, 2024; Varshini *et al.*, 2026). Coconut oil has been shown to stain well after deparaffinisation, but the staining results have been found to be less consistent when compared to the results of

well-known laboratory reagents, and the results obtained after deparaffinisation cannot be applied to tissue embedding (Varshini *et al.*, 2026). So far there is no convincing evidence showing that coconut wax can offer sufficient tissue infiltration, block formation, ribbon production, sectioning quality or long-term archival stability.

Candelilla wax also has some physicochemical properties that make it potentially suitable for future embedding applications, such as its relatively high hardness, good thermal stability and a melting range similar to that of paraffin (Nuñez-García *et al.*, 2022). It is also possible to change the mechanical properties by blending with long-chain ester waxes like behenyl behenate, which significantly enhances the gel hardness (Shimizu *et al.*, 2022). Candelilla wax is also more brittle than beeswax and can crack under certain conditions, which also has been seen in paraffin in non-histological applications (Bucio *et al.*, 2021). These properties indicate that candelilla wax might provide beneficial structural properties to composite formulations, but no direct studies have been conducted to determine its utility for tissue infiltration, sectioning, staining or routine diagnostic interpretation.

Carnauba Wax, Rice Bran Wax, Palm Wax and Bayberry Wax: Theoretical Candidates

Carnauba wax, rice bran wax, palm wax and bayberry wax are still theoretical options, with their potential derived mainly from their physicochemical properties. From a materials engineering standpoint, these botanical waxes have different degrees of hardness, crystallinity, melting behaviour and compatibility with composite wax formulations, which makes them interesting (Nuñez-García *et al.*, 2022; Shimizu *et al.*, 2022). In general, rice bran wax was found to be softer in gel hardness than candelilla wax and may need to be blended or optimized in formulations for use in tissue sectioning (Shimizu *et al.*, 2022).

However, at present, there is no clear histopathological evidence to show that carnauba wax, rice bran wax, palm wax or bayberry wax can be used as embedding mediums alone. A lack of research comparing the ability to infiltrate, form blocks, produce ribbons, section and stain with paraffin makes it difficult to compare with paraffin. Therefore, good melting properties, hardness or crystallinity does not mean that it is suitable for histopathological embedding unless it can be demonstrated that these properties are correlated with reproducible tissue-processing behavior (Taqi *et al.*, 2018).

Environmental Considerations

Natural waxes can play a role in the environmental sustainability of histopathology since they are mainly renewable, biodegradable and not derived from petroleum sources (Atreya *et al.*, 2022; Romero *et al.*, 2021). Their adoption may thus lead to a reduction in dependence on fossil raw materials and a reduction in the environmental footprint of conventional paraffin production. But, the substitution of paraffin would not remove many of the occupational and environmental issues related to the regular tissue processing. In the conventional workflows, when natural embedding waxes are used, the tissue clearing and deparaffinization may still rely on xylene and other organic solvents (Thamilselvan *et al.*, 2021).

Therefore, the ecological advantages of natural embedding media need to be taken in the perspective of sustainable histopathology. Beyond choosing an embedding medium, there are other complementary objectives such as reducing hazardous solvent use, improving energy efficiency and minimising laboratory waste. A natural wax can therefore not

be considered an environmental solution per se when toxic processing agents or energy consuming laboratory procedures are necessary for its use.

Comparative Synthesis

From the overall evidence collected, there is a significant gap between the scientific validation of paraffin wax and that of the natural alternatives. Decades of evidence in various tissues, staining methods, archival uses and molecular pathology techniques support Paraffin. It has consistently proved effective in tissue infiltration, sectioning, ribbon formation, preservation of tissue morphology, long-term storage and integration in the routine laboratory (Zhanmu *et al.*, 2020; Lemaire *et al.*, 2007; Likhithaswamy, 2022).

Beeswax is currently the most credible natural wax to be developed further since it has the highest level of direct experimental evidence. However, current studies are still not sufficient to prove equivalence with paraffin for routine diagnostics. Soy wax shows promising potential but is mostly

based on formulation and physicochemical studies and not on direct histopathological assessment (Atreya *et al.*, 2022; Romero *et al.*, 2021). The use of coconut wax and candelilla wax is still largely based on indirect evidence, and carnauba wax, rice bran wax, palm wax and bayberry wax are still theoretical candidates, whose embedding performance is only derived from material properties (Nuñez-García *et al.*, 2022; Shimizu *et al.*, 2022).

At present, evidence thus indicates that paraffin wax should be kept as the reference embedding medium while beeswax is the most promising natural embedding medium for future studies. Additional comparative studies should expand beyond just the physicochemical measurements and incorporate the following standardized evaluations: tissue infiltration, ribbon formation, section quality, staining compatibility, nuclear and cytoplasmic detail, block stability, long-term archival performance and compatibility with standard diagnostic and molecular pathology techniques.

Table 1: Comparison of Conventional Paraffin Wax and Natural Wax Candidates

Feature	Paraffin	Beeswax	Soy Wax	Coconut Wax	Candelilla Wax	Carnauba Wax
Origin	Petroleum	Animal	Plant	Plant	Plant	Plant
Renewable	No	Yes	Yes	Yes	Yes	Yes
Biodegradable	Low	High	High	High	High	High
Direct histopathological embedding studies	Extensive	Limited	Very limited	None identified	None identified	None identified
Histological performance	Established	Preliminary evidence	Not established	Not established	Not established	Not established
Molecular pathology validation	Established	Not reported	Not reported	Not reported	Not reported	Not reported
Current level of evidence	Very high	Moderate	Low	Very low	Very low	Very low
Readiness for routine diagnostic use	Established	Experimental	Investigational	Theoretical	Theoretical	Theoretical

Source: adapted and synthesized from Bucio *et al.*, 2021; Atreya *et al.*, 2022; Nuñez-García *et al.*, 2022; Romero *et al.*, 2021; Shimizu *et al.*, 2022; Al-Sabaawy *et al.*, 2021; Bai *et al.*, 2024; Likhithaswamy, 2022)

Challenges Limiting the Adoption of Natural Embedding Media

The main problem with the use of natural embedding media is not that there are no natural waxes that can be used, but rather that there is not enough solid histopathological evidence to support the use of natural waxes. However, direct investigations into the use of natural waxes for tissue embedding are limited, and much of the literature available comes from physicochemical, food-coating and materials-science studies, and not from diagnostic histopathology (Aranda-Ledesma *et al.*, 2022)

For example, the physicochemical properties of beeswax, candelilla wax have been studied mostly in the context of their use in the industry and for coatings, but not for tissue processing (Aranda-Ledesma *et al.*, 2022). Similarly, the chemistry, structure and thermal properties of candelilla wax have been well described but not accompanied by performance evaluation as a tissue infiltration, embedding or microtomy medium (Nuñez-García *et al.*, 2022). Therefore, it is not easy to transfer favourable material properties to reliable evidence for routine application in the histopathological field.

An additional challenge is the absence of well-designed comparative studies to assess the natural waxes against paraffin under standardized tissue-processing conditions. The histological quality can be affected by a number of factors such as tissue type, fixation, processing time, embedding

temperature, section thickness, staining procedures and criteria for assessment. Conventional paraffin workflows have already shown that differences in fixation and processing conditions have a profound impact on the quality of sections, staining characteristics and tissue morphology, and therefore it is important to control these variables when evaluating alternative embedding media (Al-Sabaawy *et al.*, 2021; Copper *et al.*, 2018). However, a standard system for the evaluation of natural embedding waxes is not available. As a result, variations in study design, assessment methods and assessment criteria remain a challenge for reproducibility and meaningful comparisons across studies.

The other problem associated with the use of natural waxes is that they are variable in nature. Some of their physical and chemical characteristics can differ depending on their biological source, geographical origin, extraction method and refining process and chemical composition. For instance, candelilla wax harvested from various production areas and by different extraction techniques has shown measurable differences in colour, hardness, brittleness, acid value, saponification value, ester value, peroxide value and iodine absorption number (Nuñez-García *et al.*, 2022). In the same way, beeswax composition and thermal properties could vary depending on the geographical location and environmental factors (Bucio *et al.*, 2021). The differences in these properties can affect melting range, viscosity, crystallinity, hardness and thermal stability and, consequently, the

infiltration of tissues, consistency and sectioning characteristics of blocks. This variability makes the establishment of standard manufacturing specifications and quality-control procedures needed for routine diagnostic application difficult.

Another major technical difficulty is obtaining an optimal balance of efficient tissue infiltration and mechanical support for microtomy. An ideal embedding medium should be fluid enough to penetrate dehydrated tissues and hard enough to be thin sectioned and to form continuous ribbon. Some natural waxes may be too hard, but not infiltrating, while others may be very infiltrating but too soft or flexible to use for consistent microtomy. Bucio *et al.* (2021) demonstrated that beeswax is more ductile than paraffin and candelilla waxes, which have comparatively brittle behaviour. The use of behenyl behenate significantly enhances gel hardness of the candelilla wax, but the formulations depend on composition and have not yet been tested for histopathological sectioning (Shimizu *et al.*, 2022). For these reasons, it is probable that many natural waxes would need to be blended, chemically modified or optimized in some way to attain infiltration and mechanical properties suitable for routine tissue embedding (Romero *et al.*, 2021).

Another significant limitation is the feasibility of practical integration into existing laboratory workflows. In today's modern histopathology laboratory, the need for standardized, high throughput and automated processing systems is growing to enhance efficiency and consistency. The handling of specimens and the standardization of the process have been improved by automated cell-block preparation, and specialized embedding devices have been developed to facilitate the embedding of the tissue, and this improves tissue orientation and block preparation (Taffon *et al.*, 2024; Nilcham *et al.*, 2025). Likewise, the use of pre-embedding methods like agar support for small biopsy specimens demonstrates the need for new materials to fit into existing workflows without sacrificing specimen orientation, sectioning quality or workflow efficiency (Ridolfi *et al.*, 2019). Any natural embedding media used routinely, therefore, should be compatible with automated tissue processors, embedding stations, heated embedding reservoirs, moulds, cassettes, microtomes and standard staining procedures. Variations in melting range, viscosity, solidification characteristics, adhesiveness or block hardness may require changes in processing procedures and equipment settings, thus making the process technically more complex and costly to operate.

Routine adoption should also not be considered based on the preservation of morphology alone, after routine haematoxylin and eosin staining. In addition to the traditional light microscopy examination, modern diagnostic pathology heavily depends on the use of immunohistochemistry, special stains, *in situ* hybridization and a variety of molecular techniques. An alternative embedding medium can sufficiently preserve tissue architecture, but may have a negative impact on antigen retrieval, antibody binding, probe hybridization or extraction of nucleic acids and proteins. The extensive use of archival formalin-fixed paraffin-embedded tissues in molecular and spatial analyses reflects the analytical compatibility that is anticipated of any replacement embedding medium (Bai *et al.*, 2024). For natural waxes to be acceptable for routine diagnostic use, they thus need to show consistent performance in these ancillary and molecular applications.

Other implementation challenges are the lack of commercially available histology grade waxes, lack of internationally accepted quality standards and lack of

knowledge of long-term archival performance. The paraffin-embedded tissue blocks have proven to be effective in maintaining tissue morphology for long periods and allowing retrospective histological and molecular studies (Bass *et al.*, 2014). Natural embedding media have yet to be proved to be stable over comparable time scales. However, limited validation in different types of tissues, lack of information on the preservation of antigens and subsequent molecular applications, as well as a lack of regulatory guidance and multicentre validation studies continue to hinder their widespread adoption. All of these drawbacks suggest that there is no natural wax available so far that can be considered as a fully valid alternative to paraffin for general routine use in the field of histopathology.

CONCLUSION

The use of paraffin wax as a standard embedding medium for histology has remained unchanged due to its ability to ensure good infiltration of tissues, ribbon formation, preservation of tissue morphology, archival stability and compatibility with routine staining procedures, immunohistochemistry and modern molecular diagnostic techniques. It has been widely validated for a variety of tissues and laboratory conditions and remains the standard for measuring the performance of other embedding media.

Growing concern with the environment associated with petroleum derived paraffin has led to an increase in the desire to use renewable, biodegradable embedding media. Of the natural waxes examined, beeswax has the best direct evidence in relation to histopathological embedding, and is the most promising to be investigated further. Soy wax shows interesting promise especially in engineered formulations, while coconut wax, candelilla wax, carnauba wax, rice bran wax and palm wax and bayberry wax are supported mainly with physicochemical or materials-science evidence and not with direct histopathological evidence. At present, therefore, no natural waxes can be recommended as a routine replacement for paraffin.

This review shows that, generally, the suitability of an embedding medium for diagnostic histopathology cannot be proven based on physicochemical properties alone. Good validation must include proof of efficient tissue penetration, consistent sectioning, maintenance of tissue structure and the ability to withstand routine laboratory conditions and be compatible with other diagnostic methods. Natural waxes have great potential as an alternative sustainable material, but more comparative studies of them are needed and need to be standardized before they are used routinely in the histopathological laboratory. Until evidence to the contrary is available paraffin wax is the most acceptable and scientific embedding medium for diagnostic and research applications.

Emerging Innovations and Future Directions

The increase in the number of bio-based embedding media is a reflection of the broader trend towards sustainable practices in histopathology laboratories. The idea of using paraffin wax as a pure solution has not been abandoned but rather has evolved to the development of engineered composite waxes and hybrid formulations that use the beneficial properties of various natural materials. The mechanical and degradation properties of beeswax-soy wax blends are also limitedly miscible and can be altered by modifying the individual components in different proportions (Atreya *et al.*, 2022). In the same way, formulations with interesterified hydrogenated soybean oil have also shown good melting behaviour, crystallization properties and enhanced plasticity, which means that the use of this renewable oil could become an

alternative to the use of conventional mineral waxes (Romero *et al.*, 2021). In addition, the addition of plant-derived wax esters (behenyl behenate) to candelilla wax has been demonstrated to significantly improve gel hardness, but these formulations have not yet been tested for regular embedding in histopathology (Shimizu *et al.*, 2022). These developments, taken together, indicate that optimized composite waxes with comparable performance to paraffin can be developed in the future, with better environmental sustainability.

The future progress will require more serious comparative histopathological studies and not only continued dependence on the physicochemical characterization. To improve the reproducibility and to allow meaningful comparisons between the results of various investigations, the use of standardized tissue types, fixation methods, processing schedules, embedding temperatures, section thicknesses, staining procedures and objective evaluation criteria is recommended (Taqi *et al.*, 2018). Assessment shall not only be based on basic material properties, but also on tissue infiltration, block integrity, ribbon formation, section quality, artefact production, preservation of tissue morphology, compatibility with routine and special stains, immunohistochemical performance, molecular integrity and long-term archival stability. A more extensive and unified evaluation method would be helpful in generating more evidence for the suitability of natural waxes as embedding media.

Future studies should also be directed towards optimizing wax formulations, developing histology grade purification methods, and batch standardization and compatibility with automated tissue-processing systems. The physicochemical properties of many natural waxes depend on their biological source, geographical origin and extraction process, so standardization will be necessary to ensure good laboratory performance (Nuñez-García *et al.*, 2022). Furthermore, environmental life cycle assessments should be performed in future investigations to assess if the materials provide true sustainability benefits during production, use in laboratories and disposal.

There is a need to increase the literature on beeswax because it has the best direct evidence for it being used as a natural embedding medium. While the potential for coconut wax and candelilla wax in tissue embedding is not excluded, they need to be carefully tested through experiments before conclusion on their suitability for further development of a formulation. Soy wax is still a good choice for further formulation development. For the same reason, carnauba wax, rice bran wax, palm wax and bayberry wax should only be considered for regular histopathological use after fundamental feasibility studies. Finally, advances in this area will require close collaboration between histopathologists, materials scientists, polymer chemists and biomedical engineers. Thus, the future of sustainable embedding of tissue is more likely to be the development of scientifically validated composite natural waxes, rather than the complete removal of paraffin wax by any single natural material.

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