Original Article

Comparing the Quality of Castor Oil with DPX as a Mounting Medium

Uma P Kannan, Prathiba Ramani, Anuja Natesan, Herald J Sherlin, S Gheena, R Abilasha, Gifrina Jayaraj, K R Don, Archana Santhanam

Department of Oral and Maxillofacial Pathology, Saveetha Dental College and Hospital, Chennai, Tamil Nadu, India

ABSTRACT

Background: Pure Castor oil has been used in the medical field for its healing properties. Its non medicinal uses include it as a food additive, flavoring agent, mold inhibitor, ingredient in skin care products and cosmetics. Its used in manufacturing of lubricants, dyes,resins and paints.Castor oil is a clear colorless viscid oil and has excellent keeping quality and good refractive index. So it was tried as an alternative to DPX as mounting media. **Aim:** To compare and evaluate the efficacy of Castor oil with DPX as a mounting medium. **Materials and Methods:** Two sections from each block of 5 histopathologically confirmed cases of mucocele were taken from the archives of the department. Sections were stained with routine H&E protocol. All the processing parameters were the same and done by the same person except for the choice of mounting media.Evaluation was done by two observers who were blinded to the choice of mounting media. **Result:** The results were tabulated. Student unpaired t test was used for comparing the results.Even though the results were not statistically significant,castor oil showed better details of cellular architecture than DPX. **Conclusion:** Castor oil showed superior quality and characteristics as a mounting media on basis of clarity of cellular characteristics.But it did not adhere to the slide.It can be used as an excellent alternative if some adhesive agents can be added to it which will help in its adherence and hence help in the preservation of the slides from mechanical damage

Keywords: *Castor oil, DPX, mounting media, refractive index*

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Introduction

Mounting is the last step in the series of histological preparation of a slide. A mounting medium creates a permanent bond between the slide and the coverslip. This protects the cell film from damage, air drying effect, and stain fading. To properly visualize cellular characteristics, the refractive index (RI) of the glass, cellular material, coverslip, and mounting medium should closely match each other. Mounting media should ideally have a RI as close as possible to that of the fixed protein (tissue) (approximately 1.53). As light passes from one medium to another, it changes speed and bends. A mounting medium with an RI close to that of the fixed tissue will, therefore, render it transparent, with only the stained tissue elements visible. This is where the term "clearing" comes from-xylene, for instance, has an RI very close to that of fixed tissue; therefore, inducing a certain amount of transparency.[1] A mounting medium with an RI too far either side of 1.53 will provide poor clarity and contrast. The most commonly used is DPX, a mixture of distyrene (a polystyrene), a plasticizer (tricresyl phosphate), and xylene called DPX was introduced in 1939. It is being used as the mounting media usually because of its ability to preserve stains, and dry quickly. Its colorless and has replaced xylene balsam. It undergoes a considerate amount of shrinkage and has to be liberally applied.[2] Pure castor oil has been used in the medical field for its healing properties. Its used in the manufacturing of lubricants, dyes, resins, and paints. Castor oil is a clear colorless viscid oil and

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Address for correspondence:
Dr. Uma P Kannan, E-mail: umacherry@gmail.com

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has excellent keeping quality and good RI. Castor oil preserves stains well and provides greater quality of image.[3] Gutierrez states that "no mounting media are fully satisfactory" so there is no existence of an ideal mounting medium. Castor oil has an RI of 1.477–1.481 nearing that of glass. Studies have used castor oil as an immersion oil in microscopy. It can easily be removed with xylene in the case of air entrapment. Hence, it was used in our study as a mounting medium.

Materials and Methods

For comparing the two mounting media, five cases of formalin-fixed paraffin-embedded blocks of histopathologically confirmed cases of mucocele were taken from the archives of the department. Two sections of 3 μm thickness from each block were prepared using a soft tissue microtome. The sections were deparaffnized after incubation, with xylene for 20 min, then the routine protocol for Hematoxylin and Eosin staining[2] was carried out. The slides after staining were cleaned and ready for mounting. The sections were labeled into two groups A and B. Group A sections were mounted with DPX and Group B sections were mounted with castor oil. Two observers who were blinded to the choice of mounting media scored the slides. The scores were given for clarity of cellular details, the presence of air bubbles and adhesion of coverslip and glass slide. Score 0, 1, 2, 3 for no, mild, good, and very good clarity of cellular details, respectively. Score 0, 1 for presence and absence of bubbles. Score 0, 1, 2, 3, for no, mild, good, and very good adhesion.

Table 1: The inter observer scores

Average of inter observer scores			
Mounting media	**Clarity of cellular details**	**Adhesion**	**Presence of air bubbles**
1. DPX	2	1	1
Castor oil	3	0	1
2. DPX	2	1	1
Castor oil	3	0	1
3. DPX	2	1	1
Castor oil	3	0	1
4. DPX	2	1	1
Castor oil	1	0	1
5. DPX	2	1	1
Castor oil	3	0	1
Average for DPX	2	1	1
Average for castor oil	2.6	0	1

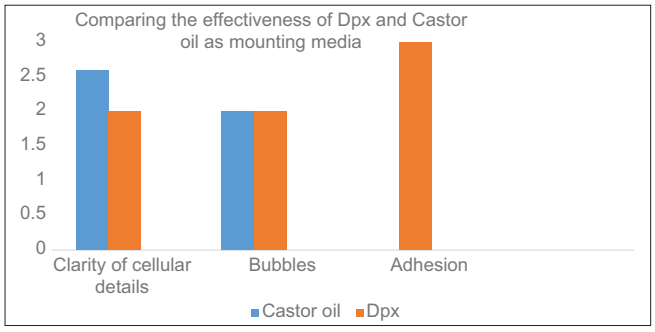

Figure 1: The graphical representation

Results

According to the average scores given by the two observers, castor oil had the maximum clarity of cellular details with score 2.6 and 2 for DPX. There were no bubbles with both the media, so the average score was 1 for both. Based on adhesion DPX had maximum adhesion with score 3 and 0 for castor oil. The results are represented in Table 1 and Figure 1.

Student unpaired *t*-test was used to compare the significance, where the value of $P = 0.172$ for clarity of cellular details and was not statistically significant.

Discussion

The mounting medium is the solution in which the specimen is embedded, generally under glass. It may be liquid, gum or resinous substance, soluble in water, alcohol, or other solvents and be sealed from the external atmosphere by nonsoluble ringing media.[4]

The main purpose of mounting media is to physically protect the specimen; the mounting medium bonds specimen, slide, and coverslip together with a clear durable film. The medium is important for the image formation as it affects the specimen's rendition.[2,5]

Properties of a mounting media

RI should be as close as possible to that of glass, i.e., 1.5. It should be colorless, transparent, dry and must harden relatively quick. It should not shrink or cause the stain to diffuse or fade. It should be able to completely permeate and fill tissue interstices. It should have no adverse effect on tissue components. It should be resistant to contamination (particularly microorganism growth). It should protect the section from physical damage and

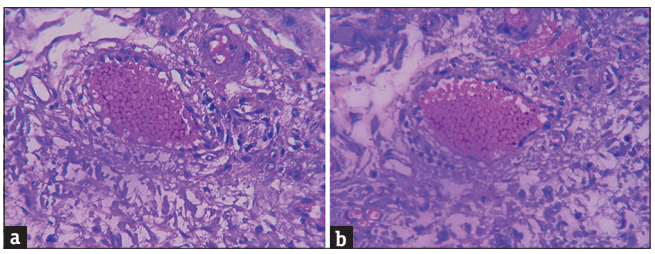

Figure 2: (a) Photomicrograph of slide with castor oil as mounting medium. (b) Photomicrograph of slide with DPX as mounting medium

chemical activity (oxidation and changes in pH). It should be completely miscible with dehydrant or clearing agent. It should set without crystallizing, cracking, or shrinking. Finally, once set, the mountant should remain stable (in terms of the features listed above).[5,6]

Studies show castor oil has been used as an immersion oil for microscopy due to its viscid nature and RI. Castor oil has a RI of 1.477–1.481 and a density of 0.953–0.964 and DPX has RI of 1.5240. In this study, we have tried it as a mounting medium. The advantages of castor oil were almost equal to and superior in some aspects. RI is a measure of the reduction in the velocity of light in a medium.

When light enters a dense medium, it bends toward normal.[1] Due to its RI, the clarity was superior when castor oil was used. Clarity of cellular architecture, like the nuclear-cytoplasmic details were far superior. Four slides had maximum score 3 with very good clarity and one slide alone had clarity of 1. Hence, averaged it was 2.6. For DPX, clarity was good for all five slides and the average score was 2. The details were easily appreciated but not as good as castor oil. The difference can be seen in Figure 2a and b. There were no bubbles on both the set of slides so maximum score of 1 was given on an average. This maybe because of the viscid nature that held the coverslip together and prevented entrapment of air bubbles. Based on adhesion, the slides with castor oil as mounting media showed only cohesiveness and no adhesion of the cover slip to the slide so was scored as 0.The coverslip slided when pushed with fingers or when in physical contact with an object. If not there was no movement of the coverslip and the slides with DPX as mounting media showed maximum adhesion with score castor oil has the advantage that it can be easily removed with xylene in cases of air entrapment.

Conclusion

This is the first study where castor oil has been used as a mounting media. This study was done to find an easily available, cheap, and useful alternative to commonly used DPX. Except adhesion, castor oil showed superior quality in appreciating the cellular characteristics. It can be used as an excellent alternative if some adhesive agents can be added to it which will help in the preservation of the slides from mechanical damage.

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Conflicts of interest

There are no conflicts of interest.

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