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Research Article

COMPARISON OF CYANOACRYLATES AND DPX FOR MOUNTING A HISTOLOGICAL SECTION

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ABSTRACT

Context: Histological sections before being examined under microscope, need to be mounted under a cover-slip in order to preserve the sections and most importantly, to obtain a clear image. The substance which serves this purpose is called a mountant or mounting media. In practice, Dibutyl phthalate xylene (DPX) is the widely used mounting media. Although considered ideal, DPX has several drawbacks. This triggered us to explore alternatives for DPX such as Bio adhesive and Fevikwik that can overcome these drawbacks

Aims: To compare Fevikwik and Surgical glue with DPX.
Settings and Design:

Methods and Material: A total of 24 sections were taken from archival collection of the Department of Oral Pathology and General pathology. All the sections were mounted using Dpx, Fevikwik and Bio adhesive**.** Comparison was drawn between these three mountants based on quality index

Statistical analysis used: One way analysis of variance

Results: Quality index of Fevikwik and Surgical glue were significantly equal to that of DPX quality index. 'p' value < 0.005 was considered as significant.

Conclusions: Hence, Cyanoacrylates **(**Fevikwik and Surgical glue) can be used as alternatives to DPX.

INTRODUCTION

The biopsied tissue in a pathology laboratory undergoes a series of steps before examining microscopically. The steps involved are grossing, fixation, processing, embedding, sectioning, staining & mounting[1]. Every step is crucial because of the specific material & method employed in each step. Selection of appropriate materials always depends on their availability and hazardous effects[2]. Mounting being the last step is important to obtain a clear image when viewed under microscope and also to preserve the sections for long time[1,3,4]. Mounting media lies between the tissue section & the coverslip, hence it is mandatory to choose the ideal one[2]. Using the incorrect mounting medium may cause signal loss and optical aberrations. A substance with highest refractive index, close to that of glass (i. E, 1.5) would be the best mounting medium. DPX is the most commonly used mounting media[4]. It contains a neutral plastic resin dissolved in xylene and dibutyl phthalate as a plasticiser.[5]Although considered ideal, DPX has several drawbacks like flammability, prolonged setting time, health hazards which include its teratogenecity.[6-13]

Cyanoacrylates (CA) as adhesives have been widely used for many years for general household use. They also have clinical applications in dentistry and medicine, especially as adhesives and sealing materials. Biocompatible CA's also known as Bio adhesives (Tissue adhesives) are widely used in the surgical field. Numerous researchers have elaborated the use of CA's in all fields of dentistry except Oral Pathology[14.] Despite their increasing use since its inception in all fields, no attempt has been made to try their potential in mounting histological sections. Hence, the aim of the study was to find whether Fevikwik and Surgical glue which have the advantages of easy availability and less hazardous effects, can meet the requirements of ideal mountants and be alternatives to DPX. We intend to accomplish the same by comparing physical properties, handling characteristics and histomorphological features of Fevikwik, Surgical glue and DPX

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Subjects and Methods: A study with a sample size of 24 was conducted in the Department of Oral Pathology. 24 samples comprised of

- 15 sections from 5 blocks of normal gingival tissue and stained with hematoxylin and eosin (H&E), (5 each were mounted with DPX , Fevikwik and Surgical glue mountant)
- 3 sections were from normal prostate gland, which were stained immunohistochemically for prostate specific antigen(PSA) (1 each was mounted with DPX , Fevikwik and Surgical glue mountant)
- 3 cytological smears stained with Papanicolaou stain (Pap) (1 each was mounted with DPX , Fevikwik and Surgical glue mountant)
- 3 tooth ground sections. (1 each was mounted with DPX , Fevikwik and Surgical glue mountant)

Physical properties of Fevikwik and Surgical glue were compared with that of DPX. The ideal physical properties of mounting media were taken as standard for this purpose. (Refer Table 1). Physical properties considered were, transparency, viscosity, refractive index (R.I) and toxicity.

Table 1 Ideal requisites of mounting media

• Refractive index should be as close as possible to that of glass i.e.1.5.
• Be freely miscible with xylene or toluene
• Not cause stain to diffuse or fade.
• Not crack or appear granular on setting.
• Should be dry to a non-sticky consistency and harden relatively quickly.
• Should not shrink back from edge of cover-glass.
• Should be free flowing and free bubbles.

All sections mounted with Cyanoacrylates (Fevikwik& Surgical glue) and DPX were observed by three pathologists in a blinded manner and comparison was drawn among all three mountants by following grading criteria given in table 2.

Table 2 Criteria for grading

Histological criteria for grading	Each histomorphologic criteria was rated on a scale of 1-3
Background	2 - clear 1 - unclear
• Overall staining	1- poor
• Cell morphology	2-satisfactory
• Nuclear details	3-good

Quality of the image was assessed using the above criteria. Maximum possible score for a single section is 11, by involving all four parameters, likewise, maximum possible score for each group is calculated by multiplying the number of samples by 11 (i.e, 11x8= 88).

Quality Index = Actual score obtained/ Maximum score possible.

Quality index of both the Cyanoacrylates were compared with the standard DPX

RESULTS

Physical properties of all three mountants are mentioned in Table 3. All three materials were transparent with varying refractive indices. Fevikwik's refractive index was found to be the highest (1.65) and DPX was the least with 1.52. Refractive index of Fevikwik was measured with the help of ellipsometer which gave the result of 1.65, but R.I couldnot be found for Surgical glue. According to the literature Surgical glue has been used as a surgical tissue adhesive in craniofacial plastic and reconstructive surgery, gynaecology, esophagogastric intestinal surgery, and dentistry on living tissues, because of its least toxicity, better healing and better cosmetic results [15][16].

Table 3 Comparison of properties of 3 mountants

Properties	DPX	Fevikwik	Surgical glue
Constituents	Dibutylphthalate xylene	Any homologue of CA	N butyl and 2 octyl homologues of CA
Transparency	Transparent	Transparent	Transparent
Viscosity	viscous	Watery	Watery
Refractive index	1.52	1.65	-
Toxicity	Hazardous	?	least toxic

Comparison of handling characteristics was based on setting time, miscibility, air bubbles during mounting procedure, demounting time and demounting solution and re-staining. **Table 4** shows the properties, related to handling characteristics, of all the three mountants. Fevikwik had the least setting time, followed by Surgical glue and DPX took the longest setting time. Surgical glue's miscibility with xylene is doubtful. During mounting procedure few air bubbles were encountered with Surgical glue and very few air bubbles with Fevikwik. It is to be noted that demounting and re-staining is possible for C.A's, with acetone (not with xylene). The demounting solution used for both the cyanoacrylates was acetone. Re-staining is possible with both the cyanoacrylates.

Table 4 Comparison of handling characteristics of three mountants

Properties	DPX	Fevikwik	Surgical glue
Setting time	48 hours	1 minute	5 minutes
Miscibility with xylene	Miscible	Miscible	Miscible
Air bubbles	No	Very few	Few
Demounting solution	Xylene	Acetone	Acetone
Demounting time	20 hours	2 days	20 hours
Re-staining	Possible	Possible	Possible

Histological Analysis: As we mentioned in materials and methods, histological analysis was donebased on the following parameters, Background, overall staining, cytoplasmic and nuclear features (considering DPX histological picture as the standard) and scored by 3 Pathologists. Table 5 shows the scores and quality index of all the three mountants. Quality indices of both cyanoacrylates (0.94 & 0.97) were significantly equal to DPX quality index (1.0).

Table 5 Comparison of quality index scores of DPX, Fevikwik and Surgical glue

Mountant	Actual score obtained	Quality index
DPX	88	88/88 = 1.0
Fevikwik	85	83/88 = 0.97
Surgical glue	83	83/88 = 0.94

One way analyses of variance were used to test the difference between groups. (Refer table 6). The formula used for calculating mean values is

F = Ms between groups / MS Within groups (MS= Mean sum of square)

Table 6 Descriptive statistics for DPX, Fevikwik and Surgical glue

Mountant	N(samples)	Mean	Standard deviation	Median	Min.	Max.	'F' value	'p' value
DPX	8	11.00	0.000	11.00	11	11		
Fevikwik	8	10.63	0.518	11.00	10	11	2.891	0.078
Surgical glue	8	10.38	0.744	10.50	9	11		

In the above test the "p" value of less than 0.05 was accepted as indicating statistical significance. The obtained 'p' value shows no significant difference between all the three mountants.

Figure 1 shows the histological features of H & E stained sections that are mounted with 3 mountants. H& E stained sections mounted with Cyanoacrylates showed image which was as good as the DPX. **Figure 2:** shows the histological features of the IHC stained sections. Immunohistochemical stained sections showed positivity for PSA **Figure 3:** shows the cytological features of the smears, which again showed image quality equal to DPX mounted sections **Figure 4:** Shows the ground sections mounted using Cyanoacrylates. Ground sections mounted using Cyanoacrylates showed clear image of enamel, dentin and cementum.

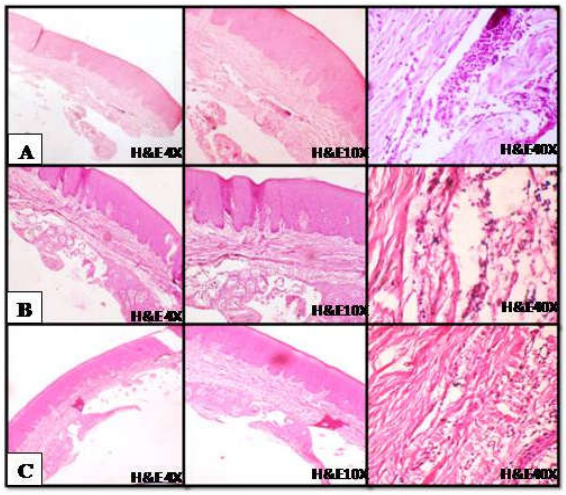

Figure 1 Histological features of sections mounted with three mountants A) Dpx B) Fevikwik C) Surgical glue

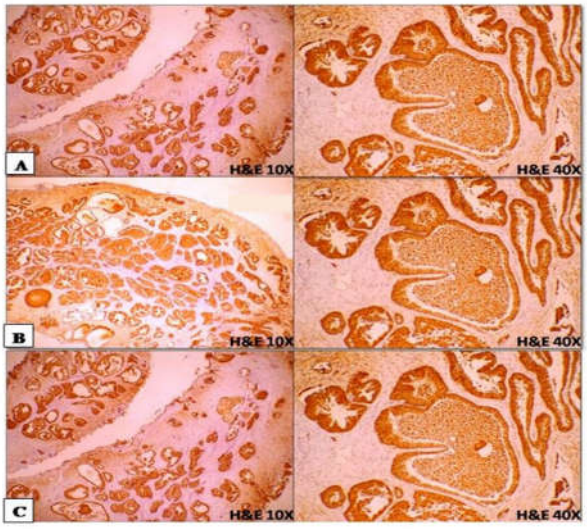

Figure 2 Histological features of IHC stained sections A. DPX B. Fevikwik C. Surgical glue

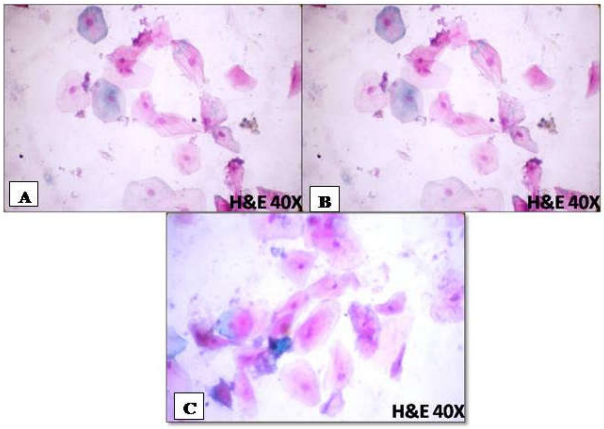

Figure 3 Cytological smears mounted with 3 mountants A. DPX B.Fevikwik C. Surgical glue

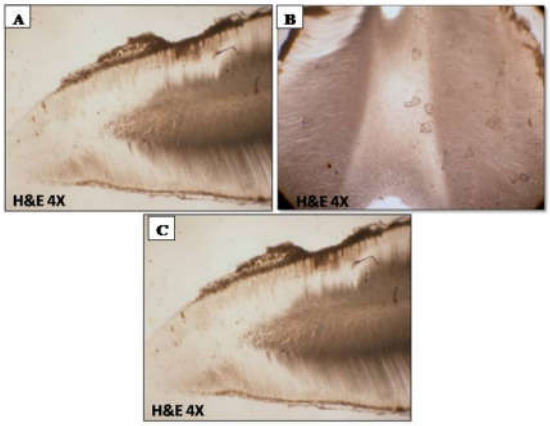

Figure 4 Ground sections mounted with 2 mountants A.DPX B. Fevikwik C. Surgical glue

Table 7 Advantages and disadvantages of cyanoacrylate

Type of mountant	Advantages	Disadvantages
Fevikwik	• Faster setting time • Easily available compared to DPX • Re-staining possible • Cost effective	• Technique sensitive • Higher refractive index than DPX • Longer demounting time
Surgical glue	• Demounting time equal to DPX • Non toxic • Easily available compared to DPX • Re-staining possible	• Technique sensitive • Air bubbles • Longer setting time • Expensive

DISCUSSION

Mountant is a substance, usually resinous, used for mounting a coverslip on histologic suspensions. The purpose of mounting is for long term preservation of slides and to maintain a high refractive index necessary for microscopic analysis. Basically mounting medias are classified into aqueous and resinous.[4]Dpx is the most commonly used resinous media with several advantages.[3]There are several natural mountants such as glycerine and also many recipes available to make own

mounting medium[5]. The natural ones may be cheaper than commercial products, but shows variable refractive indices. Choosing a right mounting media involves certain factors to be considered, such as, toxicity, refractive index, compatibility with specimen, pigment stability, shrinkage, durability, cost and ease of use[2]. Although DPX satisfies all the requirements for an ideal mountant, it also has some disadvantages such as setting time and hazardous effects due to presence of xylene and dibutyl phthalate as its principal constituents. This drawback is given by OSHA and also by manufacturers themselves in the safety data sheet of DPX. The hazardous effects of DPX includes eye, skin and respiratory tract irritation, teratogenic, aspiration hazard if swallowed, can also enter lungs and cause damage, may be harmful if absorbed through skin, may also cause central nervous system depression.[6-13].

CA'S were first described in 1949, have numerous homologues like Methyl (MCA), ethyl (ECA), isobutyl-, isohexyl and octyl-CA exist. Methyl and ethyl forms are used in Fevikwik whereas the octyl form is used in Surgical glue. Formation of cyanoacrylates involves the following reaction

Compound formed by the above reaction is used as a glue, it works by rapid polymerization (5-60 seconds) that takes place by hydroxyl group on the surface to be glued.(Water can act as a catalyst to activate this anionic polymerization). Proteinaceous tissue contains many base residues, the potential for both good wetting of protein makes CA's extremely adhesive to biologic tissue. CA's retain their adhesive qualities even in the presence of moisture and also has bacteriostatic and haemostatic properties.[14,17,18] In our study, DPX mounted sections were compared with CA'S (Fevikwik& Surgical glue) in an attempt to find an alternative to DPX for temporary or permanent purposes. We found that, all three mountants were transparent in colour with varying viscosities and refractive indices. More viscous the mountant, less free flowing it is and more time it takes for setting. Fevikwik is watery and more free flowing followed by Surgical glue and DPX. Refractive index of Fevikwik is more compared to DPX. Surgical glue refractive index could not be found due to some technical errors in the signals of ellipsometry method. Few air bubbles were observed during Surgical glue mounting procedure, as the procedure is technique sensitive (requires skills). But these air bubbles didnot obstruct the microscopic analysis as they were formed majorly in the periphery of the slide. During histological analysis, all sections mounted with cyanoacrylates showed good quality image, and there was no statistical significant difference between DPX and Cyanoacrylates. We noted that, the slides mounted with these CA's can be demounted and re-stained with sufficient ease. (We would like to emphasize on this regard, because literature says otherwise)[19]. Hence Fevikwik and Surgical glue can be used as alternatives to DPX in certain situations like, unavailability of DPX & when rapid setting time is required. Limitations of the study are smaller sample size, shorter study duration (8 months). Further advantages and disadvantages of Cyanoacrylates individually are mentioned in table 7.

CONCLUSION

Hence CA'S can be used as alternatives to DPX. However further studies with higher sample size and long term follow up have to be done to prove CA's as alternatives to DPX.

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