

Cyanoacrylate: An Alternative to DPX Mounting Media for Tooth Ground Sections

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ABSTRACT

Background: Tooth being a hard tissue can be histologically examined either by ground section or decalcification. Histological sections, which need to be examined for any length of time or to be stored must be mounted under a cover-slip. There is various type of mounting agent available commercially with their own advantages and disadvantages. Out of all the available mounting media, DPX is most commonly used. However, handling of DPX requires a dispenser and it also requires a significant period of time to set. Cyanoacrylate glue, which is widely available for many years for general household purposes and are very easy to handle and require very less time to set. Very few studies have been done to compare the efficacy of cyanoacrylate and DPX as mounting media for ground sections. The aim of our study was to evaluate the efficacy of cyanoacrylate adhesive (Fevikwik™) as a mounting medium for ground sections of teeth and to compare these ground sections with those mounted with DPX.

Keywords: Cyanoacrylate, DPX, Ground sections, Mounting media.

INTRODUCTION

Hard tissues can be studied either by the process of decalcification or by cutting and/or grinding them into thin sections. After decalcification, the hard tissues become soft and the subsequent tissue processing procedures are similar to that of any other soft tissue.¹ However, in case of teeth, since enamel has the maximum amount of mineral content (93.6–98.5%), but only 0.05–0.8% of organic content and the rest water,² No enamel is left for observation on completion of decalcification. Though enamel has been studied by partial decalcification methods,³ One of the ways to study tooth enamel and other hard tissues is to prepare thin sections by grinding or using a hard tissue microtome.^{4,5} These sections are cleaned, dried and

placed on a glass slide and a coverslip is placed over it by using a mounting medium. The commonly used mounting media for ground sections of teeth are Canada balsam and Di-n-butyl phthalate in xylene (DPX)⁶

DPX is the most commonly used mounting media⁴. It contains a neutral plastic resin dissolved in xylene and dibutyl phthalate as a plasticizer. ⁷Although considered ideal, DPX has several drawbacks like flammability, prolonged setting time, health hazards which include its teratogenicity.^{8,9,10}

Cyanoacrylate glue is a clear, acrylic resin consisting of methyl alpha-cyanoacrylate and is also known as 'instant glue' because of its rapid setting ability. The glue bond is formed by an almost instant

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polymerization process from cyanoacrylate monomers in the presence of moisture. As the reaction proceeds, the monomers continue to bond, forming a long, strong chain polymer¹¹ which creates a very strong bond between the desired structures, such as coverslips and glass slides. Its translucency and fast bonding capability provide some advantages over other media for mounting microscope slides.

MATERIALS AND METHOD

40 teeth extracted from patients visiting Hazaribagh Dental College, with orthodontic or periodontic problems, were used for preparing ground sections. A 37C fine/coarse combination stone was used for making ground section of tooth with uniform thickness. Each section was then split into two halves, as much as possible in equal proportions. The two halves of the section from the same tooth were then mounted on the same slide, one half with DPX and the other with cyanoacrylate adhesive [Fevikwik™]. Mounted slides were then allowed to set. All the mounted slides were then observed, by two independent observers, for various features in the ground sections under light microscope with an attached polarizer [Table 1]. Scoring was given, as shown in Table 2, by both observers and an average was taken for each feature observed. Statistical analysis was then performed using the average scores obtained for all the features observed.

Statistical Analysis

Statistical analysis using Student's *t*-test (unpaired) of average scores was performed for each feature observed.

RESULTS

While observing the various features of enamel, dentin and cementum, the mean value of score were higher for cyanoacrylate mounted sections than DPX mounted sections. Statistically, cyanoacrylate adhesive was found to be better than DPX for observing striae of Retzius ($P < 0.0209$), enamel lamellae ($P < 0.0326$), dentinal tubules ($P < 0.0051$), interglobular dentin ($P < 0.0001$), sclerotic dentin – transmitted light (TL) ($P < 0.000012$), sclerotic dentin – polarized light (PL) ($P < 0.00025$) and Sharpey's fibers ($P < 0.0003$). (Table 3) Figure 1

shows photomicrographs of ground sections of teeth mounted with Fevikwik™ and DPX.

While comparing the technical aspects of the use of DPX and Cyanoacrylate, it was found that ease of application, setting time and absence of air bubbles were better for cyanoacrylate. Whereas DPX was more technical sensitive, longer setting time and were easy to remove with xylene.

Table 1: List of features observed.

List of features observed
Striae of Retzius
Primary curvature
Enamel tufts
Enamel lamella
Enamel spindles
Secondary dentin
Tertiary dentin
Dead tract
Dentinal tubules
Sclerotic dentin
Inter globular dentin
Incremental line of Von Ebner
Incremental lines of Salter
Cementocytes
Tome's granular layer
Sharpey's fibers

Table 2: Scoring by two observers of microscopic features.

SCORE	DISCRIPTION
0	Feature absent
1	Feature present but of poor quality
2	Quality of feature is average
3	Appearance of feature is quite good
4	Feature seen is very good
5	Excellent view of features

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.^{12,13} Dpx is the most commonly used resinous media with several advantages.⁶ There are several natural mountants such as glycerine and

also many recipes available to make own mounting medium.⁷ The natural ones may be cheaper than commercial products, but shows variable refractive indices. Choosing a right mounting media involves

Table 3: Comparison of mean scores of observation of ground section mounted with cyanoacrylate and DPX.

Features	Cyanoacrylate		DPX		P	Significance
	Mean	SD	Mean	SD		
Straie of Retzius	3.64	1.14	2.98	0.77	0.01	S
Primary curvature	4.11	0.41	3.90	0.32	0.03	S
Enamel tufts	3.16	1.10	2.75	0.56	0.35	NS
Enamel lamella	3.67	1.56	3.54	0.87	0.03	S
Enamel spindles	2.63	0.86	2.45	1.32	0.37	NS
Secondary dentin	3.76	0.78	3.43	0.64	0.58	NS
Tertiary dentin	4.23	0.58	3.98	0.63	0.45	NS
Dead tract	3.87	0.43	3.43	0.56	0.30	NS
Dentinal tubules	4.54	0.49	3.89	0.43	0.01	HS
Sclerotic dentin	4.12	0.49	3.11	0.74	0.00	HS
Interglobular dentin	3.87	0.90	2.68	0.88	0.00	HS
Incremental line of Von Ebner	2.32	0.97	2.78	0.79	0.43	NS
Incremental line of Salter	3.56	0.98	4.1	0.67	0.08	NS
Cementocytes	4.34	1.09	4.26	0.56	0.76	NS
Tome's Granular layer	4.21	0.55	3.98	0.79	0.38	NS
Sharpey's fibers	4.56	0.39	3.24	0.99	0.00	HS

Table 4: Comparison of technical aspects of application of cyanoacrylate and dpx for ground sections.

PARTICULARS	CYANOACRYLATE	DPX
Ease of application	+++++	++
Absence of air bubbles	+++++	++
Absence of discoloration	+++	+
Setting time	Less than 1 minute	16-20 hours
Ease of removal	+	+++
Ease of manipulation	+++	++

certain factors to be considered, such as, toxicity, refractive index, compatibility with specimen, pigment stability, shrinkage, durability, cost and ease of use.¹² 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.^{9,10} Cyanoacrylate adhesive [Fevikwik™] is a commonly used household item which apparently bonds anything to anything. It is purported to set fast and the bond is supposed to be quite strong. Its main

component is cyanoacrylate and is available as small 1 ml squeezable tubes.¹⁴

Although observation of most of the structures of teeth were not statistically different between the two, cyanoacrylate adhesive was found to be better than DPX for observing striae of Retzius, enamel lamellae, dentinal tubules, interglobular dentin, Sharpey's fibers and sclerotic dentin in transmitted as well as polarized light. Better visualization in Cyanoacrylate glue may be related to molecular size of the two mounting media. If the molecular size of mounting media is smaller than the pore size of enamel, they penetrate and displaces the gaseous

content or debris within the pores of enamel and the structure appear translucent when observed under transmitted light. Since cyanoacrylate adhesive has larger molecular size when compared with DPX, it is likely that there is a greater lack of penetration and therefore more prominence of these structures. The larger difference of RI between enamel and cyanoacrylate and between cyanoacrylate and glass and the lack of penetration due to larger molecular size provide greater contrast to the sections and hence hypomineralized structures are better visualized with cyanoacrylate glue.¹⁵

Regarding the technical aspects of the two mounting media, cyanoacrylate adhesive is known to set fast which is an advantage to observe ground sections earlier than DPX. Because it sets very fast, it is highly possible that the chances of air bubble entrapment are very minimal, but one has to place the coverslip properly and at a rapid pace. Another added advantage is the fact that cyanoacrylate adhesive does not cause any discoloration, unlike DPX. Cyanoacrylate glue polymerization is an irreversible process and the section cannot therefore be remounted or re-stained after the glue has set. In contrast, DPX can be easily dissolved with xylene allowing re-mounting and re-staining of these sections.

CONCLUSION

There are very few studies on the usage of mounting media for ground sections of teeth. Mounting media which are commonly used for soft tissue sections have been used for undecalcified sections of teeth and bone. Traditionally, ground sections of teeth have been mounted with DPX or Canada balsam. We have used a cyanoacrylate adhesive in our study for mounting ground sections of teeth. This preliminary study shows that it is better than DPX in observation of certain features, rapidity of setting, minimal air bubble entrapment and lack of discoloration. However, it remains to be seen whether it is better than Canada Balsam and whether it is useful in studying undecalcified sections of carious teeth (to study the zones of enamel and dentin caries), for which further studies and more number of samples are required. Moreover, use of cyanoacrylate adhesive for soft tissue sections can also be explored.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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