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Cyanoacrylate, glycerine jelly and Canada balsam for tooth section mounting: A comparative *in vitro* study

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Abstract:
Ground sections of teeth are essential for studying dental microstructures, but evidence comparing mounting media remains limited. Therefore, it is of interest to evaluate the cyanoacrylate adhesive, DPX, glycerine jelly and Canada balsam using 40 extracted permanent teeth divided into four groups (n=10). Ground sections (0.25 mm) were prepared, mounted and microscopically assessed for enamel, dentinal, dentinoenamel junction and cemental features using one way ANOVA. DPX showed significantly better visualization of most microscopic structures than the other media (P<0.001), while a few features showed no significant differences. DPX was the most effective mounting medium and it is preferred for permanent mounting of dental ground sections.

Keywords: Dental ground sections, mounting media, dibutyl phthalate xylene (DPX), cyanoacrylate adhesive, optical clarity

Background:
Dental ground sections are essential for studying tooth morphology, microstructure, pathology and dental education because they preserve the natural mineralized architecture of enamel, dentin and cementum **[1].** These sections allow visualization of microscopic features such as enamel prisms, dentinal tubules, Hunter–Schreger bands and incremental lines, supporting developmental, pathological, forensic and comparative studies **[2].** The quality and durability of ground sections depend partly on the mounting medium, which should provide optical clarity, dimensional stability, suitable refractive index and resistance to discoloration and microbial growth **[3]**. Canada balsam has traditionally been preferred for its transparency and favorable refractive index, although its prolonged curing time, xylene requirement and tendency to yellow have encouraged alternative mountants **[4]**. Glycerine jelly is inexpensive and reversible but has limited long-term stability because of its hygroscopic nature and susceptibility to microbial growth **[5]**. Cyanoacrylate provides rapid polymerization, clear bonding and good moisture resistance, making it a potential alternative for permanent dental ground sections **[6].** Synthetic resin media such as DPX offer rapid drying and good optical clarity but may be prone to brittleness and cracking during prolonged handling **[7]**. Although mounting media are widely used in histopathology, comparative evidence regarding their suitability for dental ground sections remains limited **[8-10].** Therefore, it is of interest to compare and evaluate the cyanoacrylate adhesive, glycerine jelly, Canada balsam and DPX as mounting media for dental ground sections to identify the most effective option for teaching, research and forensic applications**.**

Materials and Methods:
An *in vitro* observational study was conducted; ground sections of 40 freshly extracted teeth were taken. The study was conducted in Department of Oral and Maxillofacial Pathology and Oral Microbiology, Dental College and Research Centre, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh.

Study design and study population:
This *in vitro* comparative study was conducted on forty freshly extracted permanent human teeth. The specimens were randomly allocated into four equal groups (n = 10) according to the mounting medium used for preparation of the ground sections. Group I comprised ground sections mounted using cyanoacrylate adhesive, Group II comprised ground sections mounted using dibutyl phthalate xylene (DPX), Group III comprised ground sections mounted using glycerine jelly and Group IV comprised ground sections mounted using Canada balsam.

Selection criteria:
Permanent teeth extracted exclusively for prosthetic, periodontal, or orthodontic reasons were included in the study. Only single-rooted and double-rooted permanent teeth were selected. Teeth exhibiting trauma, hypercementosis, excessive attrition, external or internal root resorption, developmental anomalies, dental caries, previous endodontic treatment or restorations and teeth with more than two roots were excluded from the study.

Armamentarium:
The equipment used during the study included a micromotor, straight handpiece, carborundum cutting disc with mandrel, binocular light microscope and slide warmer. The instruments utilized were glass slides, coverslips, tweezers, Petri dishes, Arkansas stones (coarse and fine), 250 mL beakers, glass stirrers, syringes with needles, slide racks and slide storage boxes. The chemicals employed included xylene, glycerol, 10% buffered formalin, ethyl alcohol, phenol, gelatin powder, distilled water and pumice powder. The mounting media evaluated were cyanoacrylate adhesive, glycerine jelly, dibutyl phthalate xylene (DPX) and Canada balsam. Gloves, surgical masks, head caps and clear nail polish were used as consumables during specimen preparation.

Preparation of glycerine jelly:

Glycerine jelly was prepared by dissolving 10g of gelatin powder in 30 mL of distilled water with gentle heating and continuous stirring until complete dissolution. Subsequently, 70 mL of glycerol and 1 mL of phenol were added and the mixture was gently heated with continuous stirring until a homogeneous solution was obtained. The prepared mounting medium was allowed to cool before use.

Preparation of ground sections:

Immediately after extraction, all teeth were cleaned of adherent soft tissue debris and stored in 10% buffered formalin until further processing. Each tooth was sectioned longitudinally using a carborundum cutting disc mounted on a straight handpiece under continuous water irrigation to prevent overheating. The obtained sections were initially ground on the coarse surface of an Arkansas stone until a thickness of approximately 1 mm was achieved. Final thinning was carried out using the fine surface of the Arkansas stone until a uniform thickness of approximately 0.25 mm was obtained. The ground sections were then immersed in absolute ethyl alcohol (99.9%) for 30 minutes for dehydration, followed by clearing in xylene for 24 hours before mounting.

Mounting procedure:

The prepared ground sections were mounted using four different mounting media for comparative evaluation. For Group I, each ground section was placed on a clean glass slide; and three to four drops of cyanoacrylate adhesive were applied. A coverslip was immediately positioned over the specimen to ensure rapid and uniform adhesion while minimizing air entrapment. For Group II, the ground sections were mounted using dibutyl phthalate xylene (DPX). Three to four drops of DPX mountant were placed over the section on a clean glass slide, following which a coverslip was carefully positioned to obtain a permanent mount.

For Group III, glycerine jelly was gently warmed to approximately 40°C until liquefied and free of air bubbles. The molten jelly was applied over the prepared ground section placed on a clean glass slide and a coverslip was positioned carefully. The mounted slide was placed on a slide warmer maintained at 40–45°C for approximately 30 minutes to facilitate adequate penetration of the mounting medium into the section. After cooling, the margins of the coverslip were sealed with clear nail polish to prevent dehydration and prolong the stability of the preparation. For Group IV, the prepared ground sections were mounted using Canada balsam. Canada balsam was applied to both the glass slide and the coverslip and gentle heating was employed to reduce viscosity and facilitate even distribution of the mounting medium. The tooth section was carefully positioned between the two balsam coated surfaces, followed by further gentle heating to ensure complete flow of the balsam and elimination of trapped air before permanent sealing. All mounted slides were allowed to cure completely before microscopic evaluation.

Results:

Forty dental ground sections were equally distributed among four experimental groups according to the mounting medium used, with 10 specimens in each group (25% per group). The distribution of specimens across the four experimental groups is presented in **Table 1**. Descriptive statistics, including mean visualization scores and standard deviations for individual microscopic features, are summarized in **Table 2**. Representative microscopic images demonstrating the visualization of dental hard tissue structures using the four mounting media are illustrated in **Figure 1**. One-way analysis of variance (ANOVA) revealed statistically significant differences among the four mounting media for most of the evaluated microscopic features. DPX consistently demonstrated the highest mean visualization scores for several enamel and dentin structures. Among the enamel features, DPX showed higher mean scores for Striae of Retzius (3.30 ± 0.82), primary curvatures (3.90 ± 0.74), enamel tufts (4.10 ± 0.74) and enamel spindles (4.00 ± 0.82), with statistically significant differences among the groups ($P < 0.001$). However, enamel lamellae showed no statistically significant difference among the four mounting media ($P = 0.435$), despite DPX exhibiting the highest mean score (3.70 ± 0.95), as detailed in **Table 2**. Regarding dentinal structures, DPX demonstrated the highest mean scores for dentinal tubules (4.20 ± 0.79), incremental lines of von Ebner (3.40 ± 0.70) and incremental lines of Salter (3.00 ± 1.05), with statistically significant intergroup differences ($P < 0.001$). Similarly, statistically significant differences were observed for secondary dentin ($P = 0.009$), tertiary dentin ($P = 0.007$) and contour lines of Owen ($P = 0.040$). Although DPX exhibited the highest mean scores for these features, the magnitude of variation differed across the groups. For Hunter–Schreger bands, DPX recorded a mean score of 3.20 ± 0.92, followed by cyanoacrylate adhesive and glycerine jelly (both 2.40 ± 0.70 and 2.40 ± 0.52, respectively), with a statistically significant overall difference ($P < 0.001$). For the dentinoenamel junction (DEJ), DPX demonstrated a higher mean visualization score (4.10 ± 0.88) compared with cyanoacrylate adhesive (2.80 ± 0.92), glycerine jelly (1.60 ± 1.26) and Canada balsam (1.80 ± 0.79). This difference was statistically significant ($P < 0.001$). Similarly, DPX demonstrated higher mean scores for Sharpey's fibers (3.70 ± 0.95), Tomes granular layer (3.70 ± 0.95) and the hyaline layer of Hopewell-Smith (3.70 ± 1.06), with statistically significant differences among the four groups ($P < 0.001$). These findings indicate better visualization of several key structural interfaces and cementodentinal features with DPX, as summarized in **Table 2** and visually demonstrated in **Figure 1.** In contrast, no statistically significant differences were observed among the four mounting media for dead tracts ($P = 0.650$), interglobular dentin ($P = 0.154$), or cementocytes ($P = 0.253$). Although DPX demonstrated numerically higher mean scores for these features, the observed differences were not statistically significant. This suggests that the mounting medium did not significantly influence the visualization scores of these particular structures in the present study. Overall, DPX showed the highest mean visualization scores for most of the evaluated microscopic features, followed generally by cyanoacrylate adhesive, whereas glycerine jelly and Canada balsam exhibited comparatively

lower scores. The comparative findings presented in **Table 2**, together with the representative microscopic appearances in **Figure 1**, indicate that DPX provided better visualization of the majority of dental hard tissue structures examined in this study.

Table 1: Distribution of dental ground sections according to experimental groups

Group	Mounting medium	Number of specimens (n)	Percentage (%)
Group I	Cyanoacrylate adhesive	10	25
Group II	Dibutyl phthalate xylene (DPX)	10	25
Group III	Glycerine jelly	10	25
Group IV	Canada balsam	10	25
Total		40	100

Table 2: Comparison of microscopic feature visualization scores among four mounting media

Microscopic feature	Cyanoacrylate Mean±SD	DPX Mean±SD	Glycerine Jelly Mean±SD	Canada Balsam Mean±SD	P value
Striae of Retzius	2.50±0.85	3.30±0.82	1.50±0.71	1.70±0.48	<0.001**
Primary curvatures	2.40±0.97	3.90±0.74	2.20±0.63	1.90±0.74	<0.001**
Enamel tufts	2.70±0.82	4.10±0.74	2.00±1.05	2.10±0.74	<0.001**
Enamel spindles	2.90±1.20	4.00±0.82	2.50±0.71	2.40±0.84	<0.001**
Enamel lamellae	3.30±1.06	3.70±0.95	3.20±0.63	3.10±0.74	0.435 (NS)
Secondary dentin	1.60±0.70	2.00±1.41	1.20±0.92	0.50±0.53	0.009*
Tertiary dentin	0.60±0.84	1.90±1.73	0.50±0.71	0.30±0.48	0.007*
Hunter–Schreger bands	2.40±0.70	3.20±0.92	2.40±0.52	1.50±0.71	<0.001**
Dead tracts	0.80±1.03	1.30±1.77	1.10±1.37	0.60±0.97	0.650 (NS)
Dentinal tubules	3.30±0.67	4.20±0.79	2.80±0.79	2.60±0.70	<0.001**
Incremental lines of von Ebner	1.60±1.43	3.40±0.70	1.50±0.71	1.20±0.63	<0.001**
Incremental lines of Salter	2.50±0.85	3.00±1.05	1.60±0.52	1.60±0.70	<0.001**
Contour lines of Owen	2.10±0.99	2.80±0.92	2.20±0.79	1.70±0.48	0.040*
Interglobular dentin	2.20±1.14	2.70±1.25	1.60±1.26	1.80±0.79	0.154 (NS)
DEJ	2.80±0.92	4.10±0.88	1.60±1.26	1.80±0.79	<0.001**
Sharpey's fibers	1.80±1.03	3.70±0.95	1.50±0.53	2.20±0.63	<0.001**
Cementocytes	1.80±1.48	2.70±1.70	1.60±1.17	2.40±0.97	0.253 (NS)
Tomes granular layer	2.60±0.97	3.70±0.95	1.60±1.35	2.20±0.79	<0.001**
Hyaline layer of Hopewell-Smith	1.60±1.26	3.70±1.06	1.50±0.71	2.10±0.99	<0.001**

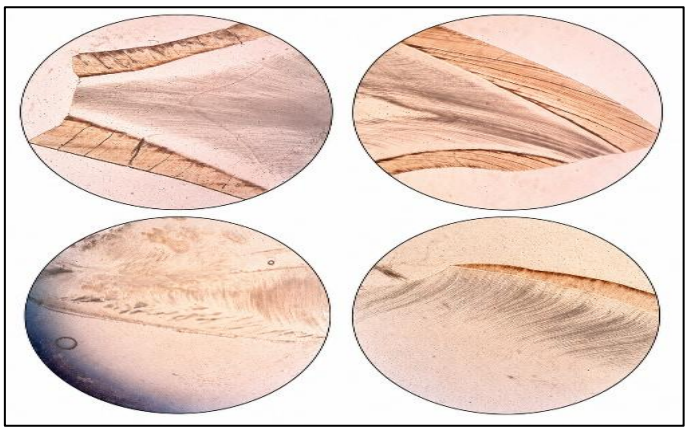


Figure 1: Representative microscopic images demonstrating the visualization of dental hard tissue structures using four different mounting media: (A) Cyanoacrylate adhesive, (B) DPX, (C) Glycerine jelly and (D) Canada balsam.

Discussion:

The present *in vitro* study evaluated the effectiveness of four mounting media, namely dibutylphthalate xylene (DPX), cyanoacrylate adhesive, glycerine jelly and Canada balsam, for visualizing microscopic structures in dental ground sections. DPX demonstrated the highest mean visualization scores for most of the evaluated structures, including enamel tufts, enamel spindles, dentinal tubules, incremental lines and the dentinoenamel junction (DEJ). These findings indicate that the choice of mounting medium influences the microscopic visualization of mineralized dental tissues. Schmid *et al.* (2021) **[11]** investigated the ageing characteristics of Canada balsam and Permount using spectroscopic and microscopic techniques. They reported progressive chemical changes and increasing coloration in Canada balsam during ageing, highlighting potential limitations in its long-term preservation. In the present study, Canada balsam showed comparatively lower visualization scores for several microscopic features than DPX and cyanoacrylate adhesive. Although the present investigation did not evaluate long-term ageing provide additional context regarding the importance of considering the stability of mounting media during permanent slide preparation. Fouquet *et al.* (2015) **[12]** evaluated high-refractive-index mounting media and demonstrated that refractive index matching could improve optical transparency, reduce spherical aberrations and enhance microscopic resolution. Their findings support the importance of optical compatibility between mounting media and specimens. Similarly, the higher visualization scores obtained with DPX in the present study may be associated with its optical properties and compatibility with mineralized dental tissues. However, Fouquet *et al.* (2015) investigated fluorescence and confocal microscopy using biological tissues rather than dental ground sections; therefore, their findings provide indirect support for the optical principles underlying the present observations. Maryam *et al.* (2022) **[13]** investigated label-free optical spectroscopy for the early detection of oral cancer, emphasizing the potential of optical techniques for identifying tissue-related changes. Although their study focused on oral cancer detection rather than mounting media or dental ground sections, it highlights the importance of optical characteristics in obtaining diagnostically useful tissue information. In the present study,

DPX demonstrated significantly higher visualization scores for several dental structures, including dentinal tubules (4.20 ± 0.79), enamel tufts (4.10 ± 0.74) and DEJ (4.10 ± 0.88). These findings reinforce the relevance of optimizing optical conditions for the accurate examination of microscopic tissue architecture. Nevertheless, the differences in study design and imaging techniques should be considered when comparing these findings. Miranda *et al.* **[14]** compared cyanoacrylate adhesive with Canada balsam for mounting dental ground sections and reported significantly better visualization of Striae of Retzius, enamel lamellae, dentinal tubules, interglobular dentin, sclerotic dentin and Sharpey's fibers with cyanoacrylate. Similarly, the present study demonstrated higher mean visualization scores with cyanoacrylate than Canada balsam for Striae of Retzius (2.50 ± 0.85 versus 1.70 ± 0.48), enamel lamellae (3.30 ± 1.06 versus 3.10 ± 0.74), dentinal tubules (3.30 ± 0.67 versus 2.60 ± 0.70) and interglobular dentin (2.20 ± 1.14 versus 1.80 ± 0.79). The visualization of selected dental microstructures using cyanoacrylate adhesive. However, the present study further showed that DPX achieved higher mean scores than cyanoacrylate for most evaluated features. Miranda *et al.* (2025) **[14]** reviewed the clinical applications of cyanoacrylate-based adhesives in dentistry and highlighted their rapid polymerization, biocompatibility and utility in various dental procedures. Their findings support the practical advantages of cyanoacrylate adhesives, particularly their rapid setting properties. Similarly, cyanoacrylate showed satisfactory visualization performance in the present study and generally ranked second to DPX for several microscopic features. However, primarily investigated clinical applications rather than the use of cyanoacrylate as a mounting medium for dental ground sections. Therefore, their findings support its material-related advantages rather than directly establishing its superiority for microscopic visualization. An important observation in the present study was the significant superiority of DPX for several enamel and dentinal structures. It showed the highest mean scores for primary curvatures (3.90 ± 0.74), enamel spindles (4.00 ± 0.82), Hunter–Schreger bands (3.20 ± 0.92), incremental lines of von Ebner (3.40 ± 0.70), incremental lines of Salter (3.00 ± 1.05), Sharpey's fibers (3.70 ± 0.95) and the hyaline layer of Hopewell-Smith (3.70 ± 1.06). These differences were statistically significant ($P < 0.001$). However, no statistically significant differences were observed among the four mounting media for enamel lamellae, dead tracts, interglobular dentin and cementocytes. This indicates that the influence of mounting media varied according to the specific microscopic structure being evaluated. Overall, the findings of the present study are consistent with regarding the improved visualization of selected dental structures with cyanoacrylate compared with Canada balsam. The optical findings reported by Fouquet *et al.* (2015) **[12]** and the ageing observations of Schmid *et al.* (2021) **[11]** provide additional context for interpreting the performance of mounting media. Furthermore, Miranda *et al.* (2025) highlighted the practical advantages of cyanoacrylate adhesives, while Maryam *et al.* (2022) **[13]** showed the broader importance of optical techniques in tissue assessment. Collectively, the present findings indicate that DPX provided the highest visualization scores for most dental microstructures, followed by cyanoacrylate adhesive, whereas glycerine jelly and Canada balsam showed comparatively lower scores.

Conclusion:

DPX showed the best overall performance with superior clarity and minimal artifacts. Cyanoacrylate adhesive was a reliable alternative for rapid mounting, while Canada balsam performed moderately and glycerine jelly was suitable only for temporary use. Overall, the effectiveness was ranked as DPX > Cyanoacrylate adhesive > Canada balsam > Glycerine jelly, supporting DPX for permanent dental ground section mounting.

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