Original study

Efficacy of cyanoacrylate adhesive as a mounting medium for soft tissue sections as an alternative to Kirkpatrick and Lendrum's DPX

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Abstract

Introduction: Mounting is the final stage in histologically preparing a slide and creates a permanent bond between the coverslip and the slide, preventing breakage, air drying artefacts, and stain fading.

Materials & methods: In this study, fifty slides were prepared, twenty five of which were mounted using DPX and twenty five using cyanoacrylate adhesive. The slides were assessed for clarity of nuclear and cytoplasmic details, adhesion between coverslip and slide, and the presence of air bubbles by two blinded observers.

Results: The results showed that adhesion between coverslip and slide was better when mounted with cyanoacrylate adhesive, but better clarity of nuclear and cytoplasmic details and minimal air bubble entrapment was seen when tissue sections were mounted using DPX.

Conclusion: Therefore, the use of cyanoacrylate adhesive cannot be an alternative to DPX for mounting soft tissue sections.

Key words: Adhesive, Alternative, Cyanoacrylate, DPX

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Introduction

Mounting is the final stage in histologically preparing a slide, which creates a permanent bond between the coverslip and the slide, preventing breakage, air drying artefacts, and stain fading. (1) Aqueous and resinous mounting media are the two broad categories of mounting media. media (2,3).

Mounting media should ideally have a refractive index as close as possible to that of the fixed tissue. (approximately 1.53). This would render it transparent, making only the stained tissue elements visible (1,3). mounting medium with an RI too far either side of 1.53 will lead to poor clarity and contrast (1,4).

Kirkpatrick and Lendrum's DPX is the most commonly used routine mountant, comprising distyrene (a polystyrene), dibutyl phthalate (a plasticizer), and xylene (a solvent).In contrast, cyanoacrylate adhesive has been used as a mounting medium for ground sections of teeth and resin-embedded semithin sections for electron microscopy. (5-8). In this study, the efficacy of cyanoacrylate adhesive as a mounting medium for soft tissue sections was assessed as an alternative to DPX.

Materials and Methods

Twenty five formalin-fixed paraffin-embedded tissue blocks were used to prepare fiftty slides, with two sections of 3µm thickness cut from

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each block using a soft tissue microtome. The slides were stained with haematoxylin and eosin and deparaffinized using xylene and alcohol. The slides were then washed and counterstained with eosin before being dehydrated using descending grades of alcohol. The twenty five control slides were mounted using DPX by coverslip method, while the twenty five test slides were mounted using cyanoacrylate adhesive. The slides were assessed for clarity of nuclear and cytoplasmic details, adhesion between coverslip and slide, and the presence or absence of air bubbles by two blinded observers. The inter-observer agreement was assessed by kappa statistics and Independent t-test was used to assess the p-value using IBM SPSS software version 20.

Results

The slides were given to two observers and graded from 0 to 3, on the basis of the following characteristics- clarity of nuclear details, clarity of cytoplasmic details, adhesion between coverslip and slide and presence of air bubbles.

The results showed that cyanoacrylate adhesive provided better adhesion and minimal air bubble entrapment between coverslip and slide than DPX. However, DPX provided better clarity of nuclear and cytoplasmic details compared to cyanoacrylate adhesive.

Cohen's kappa statistics was run to determine if there was agreement between the two observers. There was fair agreement between the two observers. An independent t-test was run to determine the statistical significance for each criteria.

Table 1: The table depicts the p value of the various criteria's assessed in the study.

Characteristic feature	p-Value
Nuclear Details	0.328
Cytoplasmic Details	0.328
Adhesion	0.03
Presence of Air bubbles	0.05

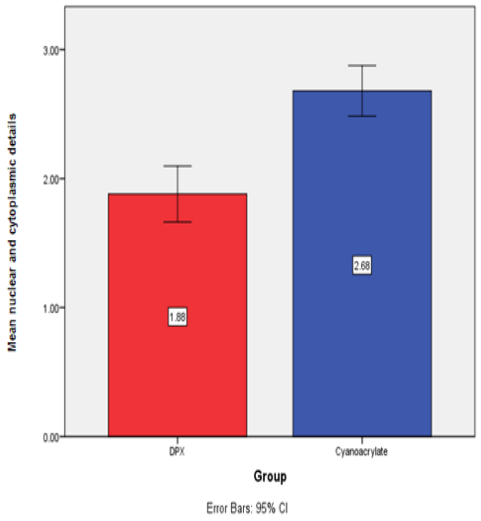

Figure 1: Shows the mean nuclear and cytoplasmic details between the groups.

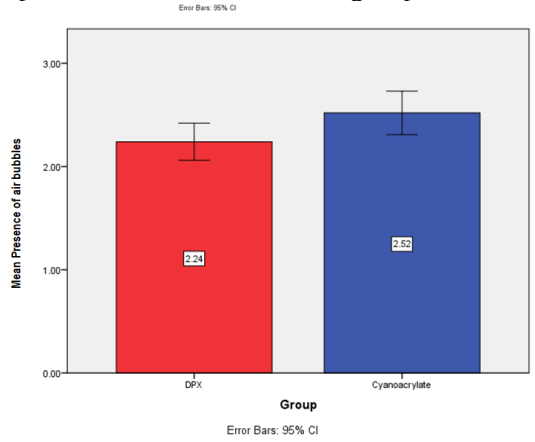

Figure 2: Shows the mean presence of air bubbles between the groups.

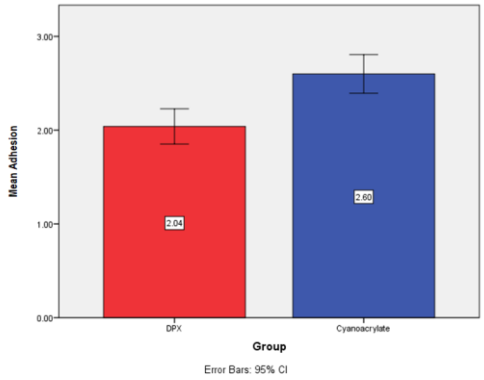

Figure 3: Shows the mean adhesion between the groups.

Results of the t-test were tabulated (Table 1and graphs 1,2 & 3)

Therefore, the use of cyanoacrylate adhesive cannot be an alternative to DPX for mounting soft tissue sections.

Discussion

The mounting media is the solution in which the tissue specimen is embedded, which is protected by a coverslip. It can be a liquid, a gum, or a resinous substance that is soluble in water, alcohol, or other solvents. The primary function of mounting material is to protect the specimen from damage caused by air drying artefacts and stain drying. The mounting media creates a physical bond between the slide, specimen, and coverslip, resulting in the formation of a transparent film.(1–4)

The mounting material should be colourless, transparent, and rapid to set. Its refractive index should be closer to that of the fixed tissue segment. It should shield the tissue from both physical and chemical harm. It should have no effect on the tissue components or staining. It should harden without crystallising, breaking, or shrinking as it cools.

The most common mounting medium, D.P.X., is a colourless, neutral medium made by dissolving distyrene in Xylene (3). When drying, it shrinks significantly and should be applied liberally to the slide. They set fast and frequently withdraw from the coverslip's edge (4). Prolonged exposure to D.P.X. (8) may cause skin and eye irritation.

Cyanoacrylate adhesive is a clear acrylic resin made of methyl alpha-cyanoacrylate that is also known as 'instant glue' because to its quick setting ability (5). It is a popular household item that may be purchased in 1ml squeezable bottles (6). The glue bond is created through an immediate

polymerization of cyanoacrylate monomers (5). As an alternate mounting medium for ground sections of teeth (6) and resin-

embedded semithin sections for electron microscopy (5), cyanoacrylate adhesive has been employed.

In our work, we evaluated the performance of Cyanoacrylate adhesive to that of D.P.X. as an alternate mounting medium for soft tissue sections. When comparing D.P.X mounted soft tissue sections to Cyanoacrylate adhesive mounted sections, it was discovered that the clarity of nuclear and cytoplasmic features was improved by 5% (Fig.1 and 2). D.P.X has a refractive index of 1.5240 (2), whereas Cyanoacrylate adhesive has a refractive value of 1.439 (10). Light bends towards the normal when it enters a dense medium (11). As a result of the higher refractive index of D.P.X., the clarity of nuclear and cytoplasmic features was improved.

When the coverslip was mounted with Cyanoacrylate glue, the adhesion between the coverslip and the slide improved by 10% (Fig.3). The adhesive bond is created through an immediate polymerization of cyanoacrylate monomers. As the reaction progresses, the monomers continue to link, resulting in a lengthy polymer chain that forms an extremely strong bond between the coverslip, tissue slice, and slide (5,6,8). When compared to D.P.X, cyanoacrylate adhesive exhibits superior adherence between the coverslip and slide.

When compared to D.P.X., air bubble entrapment was 40% higher in Cyanoacrylate adhesive mounted tissue slices. This could be because D.P.X has a higher viscosity than Cyanoacrylate adhesive. D.P.X has a viscosity of 600-700 mPa-s (12) whilst Cyanoacrylate glue has a viscosity of 900-1600 mPa-s (10). As a result, more air bubble entrapment is seen on mounting soft tissue sections with Cyanoacrylate adhesive.

Except for the existence of air bubbles, none of the criteria show statistical significance. As a result, cyanoacrylate

adhesive cannot be used in place of D.P.X for mounting soft tissue slices.

Conclusion

Mounting is an essential stage in the histological preparation of a slide, and DPX is the most commonly used routine mountant. Although cyanoacrylate adhesive has been used as a mounting medium for ground sections of teeth and resin-embedded semithin sections for electron microscopy, it cannot be used as an alternative to DPX for mounting soft tissue sections. It is crucial to choose the right mounting medium for specific tissue sections to obtain the best results.

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