

# Mounting Media - An Untouched Aspect

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## ABSTRACT

**Introduction:** Mounting a tissue specimen is essential for preserving the specimen during storage as well as for enhancing imaging quality during microscopy. Samples are mounted in a wide variety of media, with a corresponding range of properties, for examination under microscope in the biomedical sciences. The mounting medium holds the specimens in place between the cover slip and the slide.

**Objective:** This review is an attempt to summarize on various types of mounting media and their uses in histopathology as less is discussed about mounting media in the literature.

**Material and methods:** Data was obtained and analyzed from previously published literature and electronic database searches from PubMed and Google Scholar.

**Results:** Mounting media for permanent slides can be categorized into water-based and organic solvent based mounting media. Different mounting media are used for electron microscopy, immunofluorescence slides and ground sections.

**Conclusion:** There are many commercial and home-made mounting media available.

Refractive index plays an important role in choosing a mountant. A mounting media should be chosen which suits the preservation of the required sections for future research.

**Key words:** Coverslip, Canada balsam, mounting media

Oral and Maxillofacial Pathology Journal (2020): <http://www.ompj.org/archives>.

## INTRODUCTION

Mounting is the last step in the series of histological preparation of a slide. This protects the cell film from damage, air drying and stain fading. For proper visualization of 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. A mounting medium with an RI too far either side of 1.53 will provide poor clarity and contrast<sup>1</sup>.

One of the major causes of image degradation in microscopy is due to improper matching of immersion medium and mountant. Mismatching the refractive index of mountant and objective immersion medium results in spherical aberration. Refractive Indices of few of the materials used in the histopathology laboratory are: Water- 1.333, Glycerol-1.466, Glass-1.52, Zeiss Oil-1.515 and Diamond-2.42<sup>3</sup>. "Oil objectives are designed and corrected on the assumption that the refractive indices of the immersion and embedding media are equal ( $n = 1.52$ ). For water objectives, this index is assumed to be  $n = 1.33$  for both media." The mountant and the immersion medium should be matched within 0.01-0.05 ideally to three decimal places. Changes in refractive index causes light to deviate from its expected path<sup>3</sup>.

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**How to cite this article:** Saify F, Tiwari N. Mounting Media - An Untouched Aspect. Oral Maxillofac Patho J 2020;11(1): 20-24

**Source of support:** Nil

**Conflict of interest:** None

## An Ideal Mounting Media<sup>4,5</sup> should

1. Have refractive index as close as possible to that of glass, i.e. 1.5.
2. Be colorless, transparent and not cause stain to diffuse or fade.
3. Be dry to a non-stick consistency and harden relatively quickly.
4. Not shrink back from the edge of cover-glass.
5. Be able to completely permeate and fill tissue interstices without any adverse effect on tissue components.
6. Be resistant to contamination particularly microbial growth.
7. Protect the section from physical damage and chemical activity (oxidation and changes in pH).

8. Be completely miscible with dehydrant or clearing agent and remain stable once set.

9. Set without crystallizing, cracking or shrinking (or otherwise deform the material being mounted) and not react with, leach or induce fading in stains and reaction products.<sup>4,5</sup>

### Composition of mountants

The major ingredients in mountants include a base, an antifade reagent and sometimes a plasticizer to set. Commercial products often include sodium azide as preservative<sup>3</sup>.

#### 1. Base constituents:

The base component of a mountant is either aqueous (RI ~1.34); glycerol (RI~1.47); natural oil (RI~1.53); or plastic (RI~1.51). The base-ingredient is the major determinate of the refractive index of the medium.

#### 2. Antifade constituents.

The bleaching process is thought to involve reaction of the excited-dye molecule with molecular oxygen. Most antifade reagents are reactive oxygen species scavengers. Agents such as NPG (N-propyl gallate), DABCO (1,4-diazabicyclo[2.2.2]octane), 4-POBN (4-Piry-dyl-1-oxide)-N-tert-butyl nitron and PPD (P-phenylenediamine) reduce bleaching and are often added as 'antifade' reagents to mounting media<sup>3</sup>.

### Classification of mounting media

Mounting media can be categorized into water based (Aqueous/hydrophilic/non-adhesive) and Organic solvent based (Resinous/hydrophobic/adhesive). Water based media are further classified as water soluble mounting media that solidify and water soluble mounting media that remains liquid. Organic solvent based are further classified as natural and Synthetic<sup>3,4,6</sup>

#### 1. Water based mounting media

They are used for mounting sections from distilled water when the stains would be decolorized or removed by alcohol and xylene as would be the case with most of the fat stains (Sudan methods). These media are of three types, the syrups, gelatin media and gum arabic media<sup>7</sup>. Aqueous mounting media require the addition of bacteriostatic agents such as phenol, crystal of thymol or sodium merthiolate to prevent the growth of fungi<sup>4</sup>.

##### ➤ Water soluble mounting media that solidify

**a. Glycerol jelly:** Water-based mounting media are useful for making permanent mounts of water organisms, algae, protozoa, etc. Glycerol jelly is one of the most difficult mounting mediums to use, but sometimes there is no other satisfactory alternative to an aqueous mounting medium. Glycerol jelly according to Kissler (not Kaiser) is commonly used to preserve pollen samples. The bottle with the solid glycerol jelly must first be warmed in a water bath to make it liquid. Do not make it too hot, otherwise it will not solidify any more. The specimen is submerged in the warm jelly and the cover glass is placed on top. Treating some specimens with organic solvent-based mounting media would cause them to shrink or change their shape in other unacceptable ways. The advantage of Glycerol jelly is that it is water-based and this avoids the need of alcohol dehydration, does not dissolve pigments such as chlorophyll from the specimen, and does not shrink. The disadvantages include the need for a potentially toxic antiseptic in the jelly, the difficulty of mounting the specimens and the need to seal the cover slip with nail polish<sup>6</sup>.

**b. Glycerine jelly:** This is usually regarded as the standard mountant for fat stains. Dissolve the gelatin in the distilled water in a conical flask in water bath and add glycerine and phenol mix well and store<sup>7</sup>.

**c. Glycerine-glycerol:** This commonly utilized aqueous mountant is a mixture of glycerol and gelatine and has a RI of 1.47. It should set quite hard but for long-term preservation sections are best ringed and sealed. Compared to other solvents, it is cheap, safe and quick to use with little preparation. Ringing the coverslip with a hydrophobic seal will extend the life of mounted sections, although cationic dyes will diffuse into the medium over time<sup>4</sup>.

**d. Aqua-Poly/Mount:** It is a water-soluble, non-fluorescing mounting medium formulated for mounting sections from aqueous solutions. Useful for immunofluorescent techniques as it enhances fluorescent stains and can be used for frozen sections, fat stains and immunohistochemical stains when aqueous mounting medium is required. It is supplied in convenient 20ml squeeze bottles and easily removed by soaking in water or buffer. Refractive Index is 1.454 - 1.4608.

**e. Fluorsave:** It is highly recommended in immunofluorescent microscopy having strong antifade properties. It hardens after about 1 hour, but shrinkage after 1 week can damage tissue<sup>5</sup>.

**f. Farrant's medium:** It is prepared by dissolving gum arabic in the distilled water with gentle heat, add glycerin and arsenic trioxide. It is also recommended for fat stains<sup>7</sup> (RI = 1.43).

**g. Polyvinyl alcohol:** Polyvinyl alcohol, often used as a mountant in immunofluorescence microscopy, has been recommended as an alternative for glycerine jelly. Adding paraphenylenediamine to the preparation is effective in retarding photo fading<sup>4</sup>.

**h. Highman's medium (RI = 1.52):** Recommended with the metachromatic dyes especially methyl violet<sup>4</sup>.

**i. Apathy's medium (RI = 1.52):** It is one of the most useful aqueous mountants for fluorescent microscopy, being virtually nonfluorescent. Dissolve the ingredients with the aid of gentle heat<sup>4</sup>.

##### ➤ Water soluble mounting media that remains liquid<sup>6</sup>

**a. Glycerol:** It is possible to make permanent mounts by embedding the specimen either in pure liquid glycerol or a specified glycerol-water mixture. The glycerol-water mixture can be adjusted to an appropriate refractive index by adding water, which lowers the refractive index. However, a high concentration of glycerol should be maintained to prevent the risk of fungal growth in the medium. Making liquid permanent slides is somewhat more advanced. Algae and other water organisms can be embedded this way. The sides of the cover slip are sealed with nail polish two or three times to prevent glycerol from leaking out. The advantage of glycerol is that fungi and algae do not shrink as much as with other mounting media. It is also not necessary to treat the specimens with alcohol or organic solvents, which may introduce artifacts and remove pigments. The disadvantage is that it is difficult to prepare slides that are truly permanent in nature. A proper sealing of the cover slip corners is absolutely necessary if one wants to store the slides over extended periods<sup>6</sup>.

**b. Water:** In spite of low RI (1.333), water serves as a convenient temporary mountant for some whole specimens for examining certain microorganisms like delicate algae, particularly when checking sections during the staining procedures<sup>4</sup>.

**c.2,2 -Thiodiethanol:** It is a new water soluble mounting medium for high resolution optical microscopy and a nontoxic embedding

medium. 2,2 -thiodiethanol (TDE), which by being miscible with water at any ratio, allows fine adjustment of the average refractive index of the sample ranging from that of water (1.33) to that of immersion oil (1.52). TDE thus enables high resolution imaging deep inside fixed specimens with objective lenses of the highest available aperture angles and has the potential to render glycerol embedding redundant. The refractive index changes due to larger cellular structures such as nuclei, are largely compensated. Additionally, as an antioxidant, TDE preserves the fluorescence quantum yield of most of the fluorophores<sup>9</sup>.

Other aqueous mountants available are Hydramount and Aqualite.

### 1. Organic Solvent Based mounting media

These are natural or synthetic resins dissolved in benzene, toluene or xylene and are used when a permanent mount is required and frequently used in routine H and E staining procedures. The RI of hydrophobic (adhesive) mountants usually approximates that of tissue proteins (fixed) and they provide firm adhesion of the coverslip. These mountants are the type most frequently used<sup>4</sup>.

#### ➤ Natural Resinous Media

**a. Canada balsam** [RI:1.52-1.54]: Canada balsam, a natural mounting medium obtained from the balsam fir tree (*Abies balsamea*) was first described as a suitable mounting media by Andrew Pritchard in the 1830's<sup>4</sup>. The optical properties are nearly identical with those of glass. The dried resin is freely soluble in xylene and other organic solvents. It has the advantage that its optical properties do not deteriorate with age. Permanent slides mounted with Canada balsam have been stored for a century and are still useful. The disadvantage of Canada balsam is that the wet specimens must first be dehydrated in alcohol and then transferred to xylene (which is toxic) before embedding. Other disadvantages include yellowing with age and slow to harden. It becomes increasingly acidic over time and hence cationic dyes are poorly preserved<sup>6</sup>.

**b. Euparal:** This mounting medium was invented in 1904 by Prof. G. Gilson, Professor of Zoology at Louvain University, Belgium and contains sandarac, eucalyptol, paraldehyde, camphor, and phenyl salicylate. Euparal possesses a nice odor due to the natural oils that are included. It is regarded as a good permanent preservative, proven over the passage of time, of consistent quality, safe, quick and easy to use and good optically with low RI. One big advantage of Euparal is, that the specimens can be transferred directly from alcohol to it without the need of toxic solvents. Major disadvantage is the relatively long drying time (a few days to weeks) and some fading may occur in hematoxylin stained section.<sup>4,6</sup>

**c. Phenol and Dammar Balsam:** (RI:1.52-1.54) These are similar to Canada balsam and are rarely used as mountant because of dirt and impurities usually present and difficulty of filtering the prepared mountant<sup>7</sup>.

**d. Castor oil:** Pure Castor oil has been used in the medical field for its healing properties. It is used in manufacturing of lubricants, dyes, resins and paints. Castor oil is clear colorless viscid oil and has excellent keeping quality and good refractive index [1.47-1.48]. Castor oil showed superior quality 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<sup>10</sup>

#### ➤ Synthetic Resinous Media

**a. DPX:** It is the most commonly used routine mountant. A mixture of distyrene (a polystyrene), a plasticiser (tricresyl phosphate), and xylene called DPX, was introduced in 1939 and later modified by the substitution of a more satisfactory plasticiser, dibutylphthalate (butyl, phthalate, styrene - BPS). As DPX contains xylene, always perform procedure in a fume hood and use forceps to handle the slides. DPX requires dehydration of tissue<sup>5</sup>. They set quickly and in doing so often retract from the edge of the coverslip, but this can be prevented by adding a plasticizer, which is thought to resist the effect by forming a mesh with the polymerized plastic. It has a greater advantage over balsam that slides can be cleaned of excess mountant simply by stripping it off after cutting around the edge of coverslip<sup>4</sup>.

**b. Permout:** It is a toluene-based synthetic resin mounting medium. The product sets more quickly than DPX and has a lower viscosity<sup>5</sup>

**c. Cytoseal 60:** It is an inexpensive, toluene-based low viscosity mounting medium whose base constituent is poly (methylmethacrylate). It can form bubbles when drying<sup>5</sup>.

**d. Eukitt:** It is a very fast drying general-purpose resin-based mounting medium. Eukitt will solidify in about 20 minutes. The specimens must be free of water and placed first in alcohol and then in xylene prior to mounting. The use of xylene is a disadvantage, as it is harmful when inhaled. Eukitt can also be diluted by xylene to adjust its viscosity<sup>6</sup>.

**e. Plastic UV Mount Mounting Media:** This medium is designed for coverslipping epoxy, methacrylate, and deparaffinized sections directly from water or alcohol. Plastic UV Mount matches the refractive index of JB-4 embedded sections, since the JB-4 embedding resin is not removed prior to staining. Methacrylate based Plastic UV Mount avoids optical distortion and improves the final image. When applied to sections with a small amount of xylene, Plastic UV Mount "hardens" permanently within 2 minutes with exposure to long UV light n supplied from UV Polymerization Lamp, thus obviating leaching of stains. Slides must be coverslipped for viewing. The refractive index is 1.45<sup>8</sup>.

**f. PolyGlass:** PolyGlass is a liquid acrylic resin that spreads thinly and evenly forming a permanent scratch resistant layer, replacing a coverslip. It has a refractive index of 1.48 and can be removed if necessary by soaking in toluene or xylene. It is ideal for uneven thick sections that cause air bubbles under a coverslip with routine procedures and has a viscosity of 149 CPS<sup>8</sup>.

**g. CMCP Macroinvertebrate Media:** This medium is useful for mounting free living nematodes and live parasitic worms. CMCP is colorless, non-resinous, water miscible mounting medium for permanent transparent mounts. CMCP medium allows live or preserved specimens, to be mounted directly from water or alcohol. It has a refractive index of 1.41 and comes in high and low viscosity formulas<sup>6</sup>.

**h. Resin-embedded tissue:** Sections of tissue embedded in plastic compounds (such as epoxy resins) can be successfully mounted in liquid resin of the same type. Sections should be completely dry before applying mountant, which is best set using the same conditions prescribed for tissue blocks<sup>4</sup>.

**i. Photosensitive resins:** Light polymerizing resins have the advantage of very short setting times, requiring 10-30 seconds exposure

to ultraviolet light to harden completely. Once cured, the mountant cannot be dissolved or the coverslip removed (as might be necessary for restaining). They are also suitable for fluorescence microscopy<sup>4</sup>.

Other mounting media are Cellulose capratemountant [RI 1.47] and Polystyrenemountant [RI; 1.52] Diatex, Entellan, Malinol, Rhenohistol. They differ in their refractive index. All of these mounting media require the specimen to be first dehydrated in alcohol and then transferred to xylene. Some of these resins shrink significantly during the drying process. Nail polish can also be used directly as a mounting medium. The specimens must first be dehydrated in alcohol and can then be directly mounted (without xylene) in nail polish. The advantage is, that it is readily available and that it avoids the use of toxic organic solvents to treat the specimens<sup>5</sup>.

#### Ringing media/sealants:

Many aqueous mounting media remain permanently sticky with the possibility of the cover slips moving. A ringing medium is employed to seal the joint between cover slip and slide. Liquid glycerin, and gum chloral mount need to be ringed around the edge of the coverslip. The resin mountants usually do not need ringing as the solvent in the resin needs to evaporate in order to harden. Following are used as ringing media or sealants<sup>4</sup>.

**a. Entellan:** Entellan is sold as a xylene based mounting medium like DPX but contains poly (methyl methacrylate) rather than polystyrene (80kD) which is the styrene in DPX. Although sold as a mountant, Entellan has been recommended as a sealant as it sets quickly (20 minutes). It is reported to be best sealant for glycerol based mountants<sup>3</sup>.

**b. Dental or modelling wax:** This technique is safe and easy and easily reversed. The wax comes as pink coloured sheets. Heat the sheet in a glass beaker and it will become liquid at 40°C - 60°C. Use a small paintbrush for sealing the coverslip. When the wax is in touch with the coverslips at room temperature it became solid immediately<sup>3</sup>.

**c. Valap:** To seal living samples, VALAP (equal mixture of Vaseline, lanolin, and paraffin mixed on a heating plate 60°C) is reported to work well. As it melts at low temperature, it will not heat the slide and sample when applied. It forms a watertight seal<sup>3</sup>.

**d. Paraffin wax:** It is applied with a ringing iron and is satisfactory as a temporary ringing agent<sup>4</sup>.

**e. Du noyer's wax-colophonium resin mixture** is prepared by heating 10 parts of paraffin wax in evaporating dish and dissolving 40 parts of colophonium resin in it. It is a more permanent mount<sup>4</sup>.

**f. Nail polish varnish** can be applied with a brush gently. A liquid preparation sealed well with nail polish could last some months<sup>4</sup>.

**g. Asphalt based cement:** It can be applied as ringing media direct from collapsible tubes making it a permanent mount.<sup>4,7</sup>

**h. Mounting Media for Ground Sections of Teeth:** Hard tissues can be studied by either decalcification or by preparing ground sections<sup>11</sup>. Various mounting media have been tried and used for ground sections of teeth. However, there are very few studies on the use of cyanoacrylate adhesive as a mounting medium. Commonly used mounting media for ground sections of teeth are Canada balsam and Di-n-butyl phthalate in xylene (DPX). 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. Since incremental lines of Salter are hypermineralized and more glass like, they appear better when Canada balsam (RI of 1.54) is used as mounting medium<sup>12,13</sup>.

#### Mounting Medium for Immunofluorescence Microscopy:

After immunochemical staining, mounting media are used to adhere a coverslip to a tissue section or cell smear. Aqueous mounting media are generally suitable for all enzymatic label/chromogen combinations and fluorescent labels<sup>14</sup>.

#### Recommended mounting media for non-fluorescent imaging:

- ImmunoHistoMount Medium: Aqueous
- Limonene Mounting Medium: Non-aqueous

#### Recommended Mounting Media for Fluorescent Imaging

For fluorescent imaging, mounting media that preserve fluorescence in tissue and cytological smears, such as the Fluoroshield range, are used, enabling imaging of samples after prolonged storage. Aqueous Mounting Medium, Bright Mount Mounting Medium, Fluoroshield Mounting Medium are some of the examples<sup>13</sup>.

#### Cover slipping:

Bubbles may form under the cover slips if the mounting media is too thin. Incorrectly prepared resin based mountants tend to decay over time causing crystallization and cracking of mounting media. Bleaching of stain is an unwanted outcome of prolonged exposure of the sections to light. Hence, stained sections should be stored in dark storing cabinets. Presence of fingerprints can be avoided by using slide holders. If mounting bench is kept neat and tidy, unwanted elements like debris, fibers or even fungi may be prevented from contaminating the tissue sections<sup>14</sup>.

#### Mounting the sections<sup>4,7</sup>

There are mainly two ways to mount the coverslip on slides,

- A. Slide method
- B. Coverslip method

#### Slide method

1. An appropriate size of coverslip for mounting is selected and laid on the blotting paper.
2. One or two drop of mountant is placed on the slide containing section preferably in the middle to avoid trapping of air bubbles.
3. The slide is quickly inverted over the coverslip, one end is placed on the blotting paper and the other end slowly lowered until the mountant touches the coverslip.
4. The mountant spreads under the coverslip and slide and with the coverslip attached, is quickly inverted and the coverslip guided into place with dissecting needle.
5. Alternatively add the mountant on the slide as described. Place one end of the coverslip on the slide and with the aid of a dissecting needle; slowly lower the coverslip into position.

#### Coverslip method

1. Add the mountant on the coverslip in the center.
2. Bring the slide down (invert) to the coverslip and let the surface tension pull the coverslip. Use only enough mountant to fill the space on the coverslip/slide and not excess and this assessment comes with experience.
  - Too little mounting media will cause air bubble at the edges of coverslip and one will be tempted to press down on the coverslip to ensure a tight seal.
  - Too much mounting media will make it messy and move the samples around and it can make the sample impossible to image at

100x due to the very short working distance of high magnification oil immersion objective lens.

Air bubbles: If there is one odd air bubble it may be removed with gentle pressure but if there are many, instead of chasing with a dissecting needle and wasting time, put the slide back in xylene so that coverslip is separated and remount the section without air bubbles<sup>4</sup>.

### CONCLUSION:

To conclude, "No mounting media are fully satisfactory," as stated by Gutierrez<sup>4</sup>. The mounting media that physically protect the specimen and has an RI very close to that of fixed tissue and therefore, inducing a certain amount of transparency should be chosen.

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