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Original Research Article

Application of MV10B Stain for Paraffin Sections of Teeth

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ABSTRACT

Introduction: Haematoxylin and eosin is the most commonly used stain and can be considered the gold standard for staining technique, but the preparation of this stain can be cumbersome for untrained personnel. The present study is an endeavour in the search for an ideal stain that is both easy to prepare and simple to use.

Material and Methods: We have described a cost effective method for staining decalcified paraffin sections of tooth with a new broad spectrum stain – MV10B (Methyl Violet 10B). The tooth was fixed in 10% neutral formalin and then decalcified. Paraffin sections were cut at 6 micron thickness. A solution of MV10 B was prepared by dissolving 0.25 grams of the dye in powder form with 100 ml of distilled water. The slides were taken to water through decreasing grades of alcohol after adequate dewaxing with xylene. The prepared stain was applied for 10 minutes and the slide was mounted in DPX after washing in 95% alcohol, dehydration and clearing.

Result: The tissues of the tooth were stained in varying grades of violet. The stain gives adequate contrast to the tissue for viewing under a microscope and also for photography.

Conclusion: MV10 B can be used as a quick, simple and cost effective staining method for paraffin embedded tooth sections.

Key words: Tooth, MV10 B stain, paraffin sections

INTRODUCTION

Staining of tissues is essential to visualize clearly the different components that constitute the tissue. Different components have varying affinity for most of the stains used and render a visible contrast to the otherwise lackluster appearance of the components, depicting them clearly different from each other when studied under a microscope. Haematoxylin and eosin are the most commonly used stains. This method can be considered the gold standard in routine staining but requires a lot of steps. The preparation of the stock solutions of the two stains is not so simple. Trained personnel are very much essential to get good results. A search for the ideal stain that is both easy to prepare as well as gives excellent results in most tissues has been going on for a long time. In the present study we describe a procedure for staining paraffin embedded tooth sections with methyl violet (MV10 B) stain. MV10B belongs to the group of triphenyl methane

dyes. It is a blue anionic dye with quite a large dye structure and is water soluble and alcohol insoluble. The method that has been presented here has been used to stain myelinated fibers, nerve cells, ovary and gastrointestinal tissues and has been found to yield good results. [1, 2, 3] The same stain has been tried on decalcified paraffin embedded tooth sections.

MATERIALS AND METHODS

Teeth were carefully sectioned and to include the pulp in the section, a bur was used to open the root apex through which the fixing solution could enter.

The specimen was rinsed thoroughly in running water and then placed in 10% neutral formalin for fixation.

The fixed specimen was decalcified by suspending the specimen in 5% nitric acid. The specimen was kept in acid for 8 to 10 days and the solution was changed daily. If a sharp needle passed through the specimen then it indicated that it was completely decalcified. To remove all acid the specimen was washed in running water for 24 hours after complete decalcification. [4] After this the specimen was processed routinely and embedded in paraffin and cut at 6 microns thickness. The following method of MV10 B staining was done.

Preparation of stain:

MV10B stain 0.25 gms (powder)

Autoclaved distilled water – 100 ml,

The powder was dissolved in the distilled water and filtered with Whatmann filter paper. It was then stored in a brown bottle at room temperature.

Staining procedure:

Step 1: Sections were dewaxed in xylene

Step 2: Sections were brought to water (hydration) through consecutive gradations of alcohol (100%, 90% and 70%) for 4 minutes each. After hydration the slides were kept in distilled water for 2 to 3 minutes.

Step 3: Sections were stained with the prepared solution of MV10 B for 10minutes. (Timing for this has to be standardized as per the lab conditions, the procedure described here is at 28 degree room temperature)

Step 4: Sections were decolourised with 95% alcohol until the alcohol ran clear (about 10 to 15 seconds)

Step 5: Sections were dehydrated in 100% alcohol

Step 6: Sections were cleared in xylene and mounted with DPX

RESULTS

Application of MV10 B in staining of paraffin embedded tooth sections yielded good results as shown in the Figure 1, 2,2a, 3 and 3a.

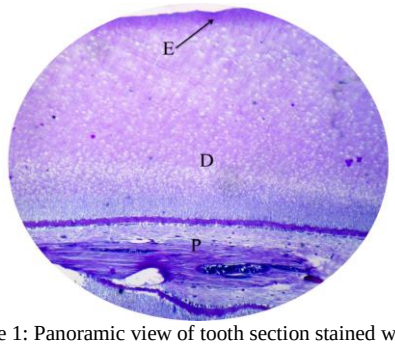

Figure 1: Panoramic view of tooth section stained with MV10B(x100): E- Enamel, D- Dentine, P- Pulp cavity

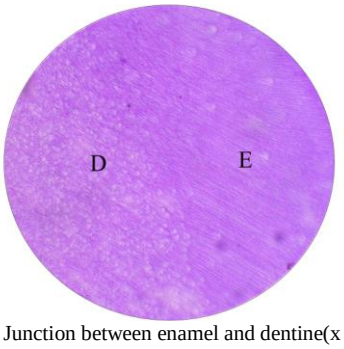

Figure 2: Junction between enamel and dentine(x 400): D-Dentine, rounded clear areas of dentine tubules are seen, E-Enamel, parallel lines of enamel rods are seen.

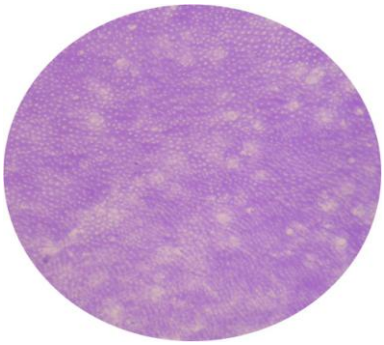

Figure 2a: Closer view of the dentine tubules.

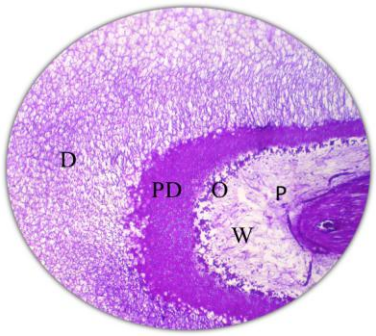

Figure 3: Junction between dentine and pulp cavity(x100): D-Dentine, PD-Predentine, O-Odontoblasts, P-Pulp cavity,W-Cell free zone of Weil.

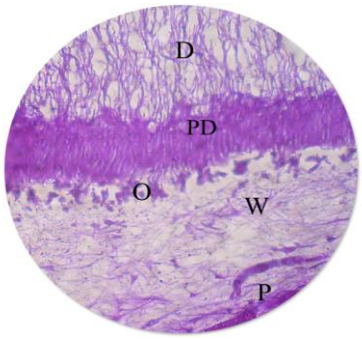

Figure 3a: Closer view of the junction between dentine and pulp cavity (x400): D-Dentine, PD-Predentine, O-Odontoblasts, P-Pulp cavity, W-Cell free zone of Weil.

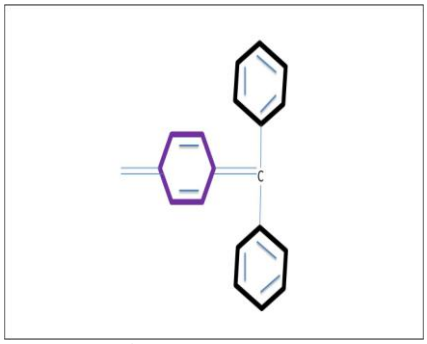

Figure 4: Structure of the triaryl methane dye structure of MV10 B. The purple ring is the quininoidchromophore component of the methyl violet dye.

DISCUSSION

The search for a substitute for haematoxylin and eosin has been an ongoing endeavour for a long time. A few dyes reported by Lillie et al were phenocyanin TC, gallein, fluorone black, alizarin cyanin BB and alizarin blue S. They mentioned that Celestin blue B with an iron mordant to be quite successful if properly handled to prevent gelling of solutions. The authors reported that the above stains still lacked the diverse capability of haematoxylin. [5, 6, 7, 8] Hong and Simpson [9] and Llewellyn [10] have also reported the use of Mordant Blue 3, but this stain has been used to stain methacrylate sections. None of the mentioned dyes have gained much popularity.

Methyl violet was the first synthetic dye used to demonstrate amyloid as long ago as 1875 by Cornil, although the results were inconsistent and often difficult to observe. It has been postulated that an affinity for one of the choromophores of methyl violet may be responsible for the resulting polychromasia. [11, 12] The chemical name for MV10 B is *hexamethyl pararosaniline chloride*. Methyl violet is an organic compound made of a mixture of tetra-, penta-, and hexa- methyl pararosaniline. The group that makes an organic compound coloured is called as a chromophore. MV10B is a triaryl methane dye that has a quininoid arrangement of one of three benzene rings as shown in Figure 4 as the actual chromophore. As all the 3 benzene rings are equal, any of them can take up the quininoid arrangement. [13]

Usually the classroom slides for tooth are ground sections or haematoxylin and eosin stained paraffin sections. The structures in the pulp cavity are usually lost during preparation of the ground sections. Here we have described a broad spectrum

single dye for staining that gives a good contrast for viewing the section of tooth. The staining procedure is complete within 10 to 15 minutes and the section is ready for viewing immediately. The MV10 B stain that has been used is a single stain that gives adequate results. The structures constituting the tooth can be clearly delineated as can be viewed from the Figures 1, 2, 2a, 3 and 3a. The dye methyl violet is readily available in the market at a reasonably cheap price. The preparation of the stain and the staining technique is simple enough to be done without much training. The stain can be used as a broad spectrum stain. It can even be used by novices as a stepping stone before proceeding to more complex stains.

CONCLUSION

MV10 B stain can be used as simple, single stain cheaper alternative to haematoxylin and eosin staining for paraffin embedded tooth sections that give consistent results.

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REFERENCES

1. Ravindranath R, Victor R, Ramos A, VSumitra, D Broslis. Longevity of paraffin sections in neurohistology.Int J Health Sci Res. 2013; 3(1):39-42.
2. Ravindranath R, Victor R, Ramos A, V Sumitra, VarshaMokhasi. MV10B stain as a broad spectrum stain in histology - a substitute for haemotoxylin& eosin stain. Int J Health Sci Res. 2012; 2(8):80-84.
3. Dorathy JTF, Victor R, Sumitra V, Roopa Ravindranath, Varsha Mokhasi. A simple cost effective rapid differential staining technique for myelinated fibres and nerve cells.Int J Health Sci Res. 2012; 2(8):43-46.
4. Satish C, Shaleen C, Mithilesh C, Nidhee C; Textbook of dental and oral histology with embryology, 1st edn -Jaypee Brothers Medical Publishers (P) Limited 2004: p.285-86.
5. Lillie RD, Pizzolato P, Donaldson PT. Hematoxylin substitutes. A study of phenocyanin TC and the use of afterchromemordanting in histology. Am J ClinPathol. 1975 Jun; 63(6):876-85.
6. Lillie RD, Pizzolato P, Welsh RA, Holmquist MD, Donaldson PT, Berger C (1973) A consideration of substitutes for alum hematoxylin in routine histologic and cytologic diagnostic procedures. Am J Clin Path. 60: 817-819.
7. Lillie RD, Pizzolato P, Donaldson PT.Hematoxylin substitutes: a survey of mordant dyes tested and consideration of the relation of their structure to performance as nuclear stains. Stain Technol. 1976 Jan; 51(1):25-41.
8. Lillie RD, Pizzolato P, Donaldson PT.Hematoxylin substitutes: fluorone black and methyl fluorone black (9-phenyl- and 9-methyl-2, 3, 7-trihydroxy-6-fluorone) as metachrome iron alum mordant dyes.Stain Technol. 1975 Mar; 50(2):127-31.
9. An evaluation of solochrome cyanine R.S. as a nuclear stain similar to haematoxylin.
   Hogg RM, SimpsonR.An evaluation of solochrome cyanine R.S. as a

nuclear stain similar to haematoxylin.Med Lab Technol. 1975 Oct; 32(4):301-6.
10. Llewellyn BD.Mordant blue 3: a readily available substitute for hem atoxylin in the routine hematoxylin and eosin stain. Stain Technol. 1974 Nov; 49(6):347-9.
11. John D Bancroft, Marilyn Gamble; Theory and practice of histological techniques, 6th edn- Churchill living stone Elsevier 2008:p.269.
12. Windrum GM, Kramer H. Fluorescense microscopy of Amyloid. Archives of Pathology 1957; 63:373.
13. Cook DJ, Pallister CJ; Cellular Pathology, 1st edn-Butterworth Heinemann 1998:p.73.

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