

Different Histological Stains Used for Assessing the Morphology of Cementum - A Review

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Abstract

Cementum is a unique least mineralised tissue with only little information known on cementogenesis. Hence various stains have been identified to view the morphology of cementum. Cementum is critical for attachment of the tooth to the surrounding periodontal ligament (PDL). This study aims to review various stains used for assessing the cementum morphology. The study setting is a review where the information about the topic is obtained from various search engines like pubmed and google scholar by searching the keywords and the study was made. The cementum is a distinct and unique mineralized hard tissue which shares many similar properties with the dentin and bone. There are various methods of histotechniques for studying morphology and development of cementum. Upon overview of various histological approaches for studying and staining of cementum through light microscopy are H&E, Toluidine Blue, Alcian blue, Picrosirius red, etc. Bone sialoprotein, osteopontin, DMP1, etc., are the staining methods using immunohistochemistry. Cementum annulations are used in forensic analysis to determine the age of the body. Hence, studying about these stains might help to understand the morphology of cementum deeply and thus give accurate details.

Keywords: Stains; tissue fixing; cementum; microscope.

Introduction

Cementum is a thin, mineralised tissue covering the root dentin surface and is one of the least mineralised tissues to be understood with little known information on cementogenesis^{1,2}. Histological stains used for aiding the microscopic study of tissues. They consist of dyes that are capable of binding with these tissues and stain them based on their composition, affinity, interaction between stain and the dye used. Various stains when combined with other stains have been found to be more

effective. The most commonly used stain is H and E. Modified Gallego's stain was originally derived from Lille's stain that is used in differential staining of hard tissue of teeth^{3,4}. The best stain that is used to identify stained decalcification and ground tissue of the tooth is the cresyl violet. Other stains like Toluidine blue, PAS under conventional light and fluorescent microscopy can be used⁵. Additional imaging methods like scanning and transmission electron microscope and micro CT etc can also be used for histological staining.⁶

The stains are used to provide contrast to colourless cells and tissues for rapid ease and evaluation of the morphology. It also enhances fibrillar collagen to detect cementum defects. The advantages of these stains are that they can be used to view the ground section of the tooth, considered as an alternative to immunohistochemistry by pathologists as it gives proper insight to character and mineralised deposits. But it has certain limitations that though H and E and TB stains offer results, it is not optimal for cementum visualisation. No single stain

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describes the nature of hard tissue.^{6,7}

Gallego in 1954 did a study to identify the mineralised components using gallego stain in decalcified and ground tissue. Robert J. Levey in 1956 did a study on differential staining of cementum using modified gallego's iron fuchsin stain and found that it stained red whereas dentin and bone stained green. Sandhya et al in 2015 conducted differential staining of hard tissue of tooth, bone and pathological lesions using the stains to identify the nature of calcifications and found that enamel stains were pink and immature hard tissue deposition stained shades of red and green.⁷

Previously, our team had conducted numerous organisational studies⁸⁻¹⁴ and surveys¹⁵⁻²² for the past 5 years. Now we are focusing on applying this knowledge to write a review on new advancements in various fields. The research was done as it provided faster and better diagnosis to trace pathological lesions. Hence the study aims to review all the stains used to assess cementum

Materials and Methods

The study setting of this research is a review. The information about the topic is obtained by searching for keywords and the data was collected through search engines like pubmed and google scholar. The articles collected were related to data that included histological stains, IHC stains, promising new stains and microscopes for visualising cementum. It excluded articles related to other categories of Hard tissues.

ANIMAL AND HUMAN SAMPLES

The most commonly used models are rodents especially transgenic mice to study the critical regulators of cementum formation but it is technically challenging because the tissues are small and stages of tooth development occur rapidly compared to humans²³. Hanford miniature pigs were used to harvest tissues of mandibular first molars at 13 - 16yrs of age at University of Washington and human samples from de identified teeth and discarded dental tissues were obtained for conducting research activities. All these procedures were approved by their respective institutional committees^{6,24}

TISSUE FIXATION, DECALCIFICATION, PROCESSING AND EMBEDDING

It was found that pig and mouse tissue was fixed in Bouin's solution for about 18-24hrs and by storing it in 70% ethyl alcohol at 4 degree Celsius and human tissues were fixed in 10% neutral buffered formalin. Mouse samples which are of 8yrs age or more were decalcified in acid flagellates solution (AFS) for 2 to 3 weeks at 4 degree Celsius. Molar Pig tissue was decalcified

in 8% EDTA and 25% formic acid. The human molar teeth were decalcified in 20% of EDTA at 4degree Celsius for about 5weeks and these decalcified tissues were processed by automatic tissue processors embedded in paraffin in base molds at 5micron in thickness for mouse and human and 7microns for pig. Bouin's solution is used as it provides high quality morphology along with in situ hybridisation detection of mRNA but might also cause demineralisation of bone and teeth thus making it a poor choice. Hence EDTA and AFS considered a better choice than it⁶

HISTOLOGICAL STAINING

Hematoxylin and Eosin Stain

It is the most common differential stain used. It consists of 2 components haematoxylin and eosin which are differentiated in acid alcohol. Hematoxylin has properties similar to that of basic dye staining nuclei of the cell intense blue and other tissue components in varying shades of blue whereas Eosin Y is an acidic dye that counterstains cytoplasm and extracellular fibres pink and red blood cells intensely red. It showed that cementum was found as basophilic spheroidal lobule staining while dentin was found with eosinophilic staining with pale pink colour due to the presence of extracellular matrix. CEJ and incremental lines are prominent under this stain. AEFC layer of cervical root shown as a basophilic layer formed on the root dentin surface. It is widely used in pathological biopsies for providing contrast to colourless cells and tissues to study their morphology apart from histological staining. It also gives a good contrast between dentin and AEFC and acellular cementum and mantle dentin.^{27,25}

Toluidine Blue

Basic dye that is used without any counterstain. Contains deparaffinized sections that are stained and dehydrated in alcohol, after which they are cleared

in xylene. It showed deep blue cementum and pale blue dentin and allowed actual visualisation of CEJ. AEFC stained dark blue. It also stains acidic mucopolysaccharides. The small integrin binding ligands N-linked glycoproteins family members BSP and OPN fall under mucopolysaccharides [It enabled better differentiation under light microscope and produced maximum contrast between cementum and cementoid, bone and osteoid thereby exhibiting metachromasia property. Apart from this, it has been used to visualise mouse C1FC in transgenic mouse models and CDJ in human teeth. It is found markedly improved when compared to H and E. Some studies have talked about the staining of acellular cementum due to the presence of rich ECM content ^{25,26,27}

Alcian Blue With Nuclear Red Fast Staining

It is counterstain that contains deparaffinized sections in 1% alcian and 3% acetic acid sol. cleared in xylene and stain acidic mucopolysaccharides. It showed pink areas of cementum and blue patchy areas of dentin. The cervical and apical regions of dentin were pale pink and the contrast was found to be more appreciable. It provided excellent contrast for the acellular cementum layer present in mice by showing intense blue colour and PDL in bright pink colour and also provided fine details about dentinal tubules. It is superior to H and E and TB stains but rarely used ^{25,28,29}

Picrosirius Red (PR) Staining

Strong acidic dye that was used to stain collagen by reacting with basic amino groups. It contains deparaffinized sections in 0.2% of aq sol. of phosphomolybdic acid hydrate rinsed in deionised water and placed in 0.4% direct red and 1.3% of 2,4,6-trinitrophenol and 0.4% of HCL and left for dehydration. When combined with polarised light microscopy, it enhances birefringence of collagen fibre varying from maximum intensity to lesser intensity depending on the organisation and angle of collagen and PDL fibres compared to angle of polariser and makes visible the sharpey's fibres inserting through the acellular cementum along with tissue intrinsic fibres. It was also observed that different types of collagen display different interference colors under this staining in polarised light. Collagen1 forms bright yellow or red colour and its fibres are thick and tightly packed whereas collagen3 displays green

colour forming thin fibres and loosely packed. But Yellow fibres are found predominant than green fibers wh lesser amounts of types 4,5,6. ^{30,31,32,33}

IMMUNOHISTOCHEMISTRY STAINS

Bone Sialoprotein

It shows very strong localisation in thin acellular extrinsic fibre cementum layer and strong staining in bone with a little staining in soft tissues. It is also present in PBL layer and diffuse localisation to pick C1FC layer. It is important in cementogenesis ^{2,34,35}

Osteopontin

It is present widespread in PDL, highlighted in AEFC layers as strong as BSP. It is also present in C1FC thereby providing a good contrast from underlying dentin and bone in definite patterns indicating its presence in cemental lines. It provides cell Matrix interactions during bone remodelling and is used in investigating developmental biology of the cementum and thereby used often as great markers. ^{2,6,36,37}

Dentin matrix acidic phosphoprotein1

It is found in higher concentrations around osteocytes and then Dentinal tubules. It was found in cellular cementum's Matrix mainly around the embedded Cementocytes parallel to osteocytes³⁸. It is said to localise around a cellular cementum and study on transgenic mice revealed the potential role of DMP1 at cementum Dentin interface⁶. It is often weak when compared to BSP and OPN. Hence not recommended as a primary marker for that tissue³⁹

MICROSCOPE

Light Microscope

It is used mainly to estimate the age to the nearest accuracy for all age groups. It has lesser r value and its reliability is 74 percent but better than stereo microscope. When the relationship between chronological and estimated age was found using light microscope, it showed stronger r value ^{40,41}

Phase Contrast Microscope

Cementum annulations are more clearly visible in this microscope and hence found reliable for counting

the incremental lines and also used for age estimation. It shows stronger r value, ^{41-43,40}

Polarised Microscope

When correlation between chronological age and number of cementum annulations were found in both males and females using polarised microscopes, it showed a higher r value than all other microscopes. Better among other microscopes used, for assessing cementum annulations. But this statement was contradicted in a study conducted by Kaur et al where they found that the cementum annulations were clearly visible under the phase contrast microscope. It showed better discernibility and annulation, hence preferred over light microscope. It shows less standard error of estimate (SEE) ^{41,40-44}

Stereo Microscope

When a stereo microscope was used to find the relationship between the estimated and chronological age, it showed a weaker r value correlation and the p values produced by it are not found significant among all age groups when compared to all other microscopes, hence not considered that much. A significant coefficient of correlation of about 0.000 was found with all microscopes except stereomicroscope ⁴⁰

PROMISING STAINS

Gallego's Iron Fuchsin Stain

Human teeth use this stain. Apart from this, calcified and noncalcified tissues can also be used.

It showed that cementum was stained in contrast to dentin and PDL. Bone reversal lines was found to be positively stained ^{40,45}

Sudan Black B

Species used here are rats and human fetal tissues. For this stain, pyridine treatment is necessary before processing of tissue. Not used to study cementum explicitly but clear clear pattern of staining can be found for bone reversal lines and mineralisation front seen in dentin was also found in cementum as well ⁴⁶

Procion Dyes

The species used here are mice, rats, rabbits, cats, monkeys and even dogs. 12 structurally related dyes

were evaluated to see their ability to serve as vital dyes for mineralisation structures. Some were found to be capable to mineralise even in decalcified tissue by light microscopy ⁴⁷

Metal Staining, Rio Hortega, Polak Mitochondria And Achucarro - Hortega Tannic - Silver Methods

The species used here is hypercementosis human teeth. It makes an emphasis on cementum shape, Sharpey's fibre insertion and cementocyte imaging ⁴⁸

Fluorescent Labeling With Vital Dyes (Tetracycline, Calcein, Alizarin)

The species used here are monkeys. This is used to track cementum regeneration through vital dye dynamic histomorphometry ⁴⁹

Cresyl Violet And Periodic Acid Schiff Staining Method

The species used here is human teeth. It is used to mark cementum incremental lines ⁵⁰

The limitation of the study was that more number of articles are needed to analyse the application of alcian blue and PR staining as these are being rarely used by researchers even though they have a lot of advantages. Cementum annulations are usually used to estimate the age of the dead body. Hence, the future aim is directed towards approaches of newer stains and establishing a better understanding the morphology of cementum and its use in forensic department

Conclusion

Cementum is a unique and distinct mineralised tissue. Hence, different approaches for studying cementum is very essential to develop therapeutic strategies in regeneration of cementum.

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Ethical Clearance: As it is a review article so it is not required.

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