

# Light Microscopic Study of Cementum Under Different Histological Stains.

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## Abstract:

### Background:

Cementum is a least understood hard tissue. Some authors consider it to be a variant of bone while others do not. It is gaining much attention these days much as it plays an important role in periodontal regeneration. There are not much employed methods to standardise studies involving cementum.

### Aim:

The present study aims to study and compare the morphology of cementum using different histological stains under light microscopy.

### Materials and methods:

Extracted and decalcified human Teeth were stained using three different histologic stains H and E, Alcian Blue- NFR and toluidine blue and observed under light microscope.

### Results:

Hematoxylin and eosin staining staining of cementum with eosinophilic staining of dentin. Under toluidine blue staining, cementum stained deep blue in contrast to the underlying dentin which stained pale blue. Alcian blue- NFR stain showed pink staining of Cementum and underlying dentin also the lacunae in the cellular cementum showed blue staining. There was adequate contrast between the cementum and dentin in all the three stains. There was no significant difference in the staining intensity between cellular and acellular cementum. The incremental lines were better observed in H and E and toluidine blue staining.

### Conclusion:

These findings underscore the fact that cementum is rich in extracellular matrix proteins but lacks significant proteoglycan content which is usually restricted to the lacunae and canaliculi.

**Key words:** Cementum, Hematoxylin and eosin, Alcian blue, Nuclear fast red, Toluidine blue O

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## INTRODUCTION:

The cementum is a thin, mineralized tissue covering the root dentin.<sup>1</sup> It is avascular, inorganic, dense, inert, extremely narrow layer of tissue that provides attachment of the tooth to the periodontal ligament.<sup>2</sup> It also has reparative and adaptive functions. It also helps in maintaining the integrity of root surface and supports tooth.<sup>3</sup> Cementum is of two types acellular extrinsic fiber cementum or acellular or primary cementum and cellular intrinsic fiber cementum or cellular or secondary cementum.<sup>1</sup> Cementum is of two types acellular extrinsic fiber cementum or acellular or primary cementum and cellular intrinsic fiber cementum or cellular or secondary cementum. It is present primarily as acellular cementum on the cervical root and helps in tooth attachment to adjacent PDL and cellular cementum covering the apical root helps in post eruption tooth movement and adaptation to occlusion.<sup>1,4</sup> cementum is closely related to bone. Both are mineralized tissues with organization matrix ratio but some differences have been reported. The organic matrix of cementum and bone consists of type 1 collagen (90% organic content and non collagenous matrix protein (NCPs). NCPs include osteopontin, bone sialoprotein, osteocalcin, fibronectin, and several species of proteoglycans, such as decorin (DCN), biglycan (BGN), lumican (LM) and fibromodulin (FM).<sup>4</sup> Its mineral part comprises 45-50% of its volume, mostly calcium and phosphate in the form of hydroxyapatite.<sup>5</sup> Bone is extensively vascular and it is remodelled throughout life

but cementum is avascular and it does not undergo any remodelling.<sup>4</sup> over the years few stains have been employed to study cementum.<sup>6-11</sup> However there is not a single specific stain that's could describe the nature of this least understood hard tissue in terms of composition and genesis. Following these lines, the present Study was designed to study the structure of cementum using three different histological stains namely H and E, alcian blue nuclear fast red and toluidine blue under light microscopy.

## MATERIALS & METHODS:

### Sample collection:

Extracted human teeth were collected that includes maxillary second premolars, maxillary canine, mandibular molars, maxillary molars, mandibular premolars, maxillary incisors were included in the study. The extracted teeth were formalin fixed and decalcified using 25% formic acid. The solution was periodically changed until the tooth decalcified. End point of decalcification was assessed manually until the teeth were flexible. The decalcified teeth were formalin fixed, processed, and paraffin embedded. 30 micro meter sections were made on glass slides using a semiautomated (Leica RM 2245) microtome.

### Histological staining:

The sections were deparaffinised in xylene, dehydrated in alcohol and rehydrated in distiller water.

### Hematoxylin and eosin staining:

For H&E staining deparaffinised rehydrated sections were stained in hematoxylin for 7 minutes and differentiated in

acid alcohol and immersed in ammonia for blueing. The sections were gently rinsed under running tap water for 2 minutes, dipped in eosin Y, dehydrated in series of alcohol, cleared in xylene, air dried and mounted with DPX mountant.

**Toluidene blue staining:**

Deparaffinised rehydrated sections were stained in 0.1% toluidene blue for 3 minutes, rinsed in distilled water for 5 minutes dehydrated in series of alcohol. Sections were then cleaved in xylene, dried and mounted in DPX mountant.

**Alcian blue-nuclear fast red staining:**

Deparaffinised sections were placed in 1% alcian blue in 3% acetic acid solution for 30 minutes. The sections were gently rinsed in tap water for 10 minutes. They were counter stained in 0.1% nuclear fast red for 5 minutes, rinsed in tap water for 15 dips, cleared in xylene, air dried and mounted in DPX mountant.

**RESULTS:**

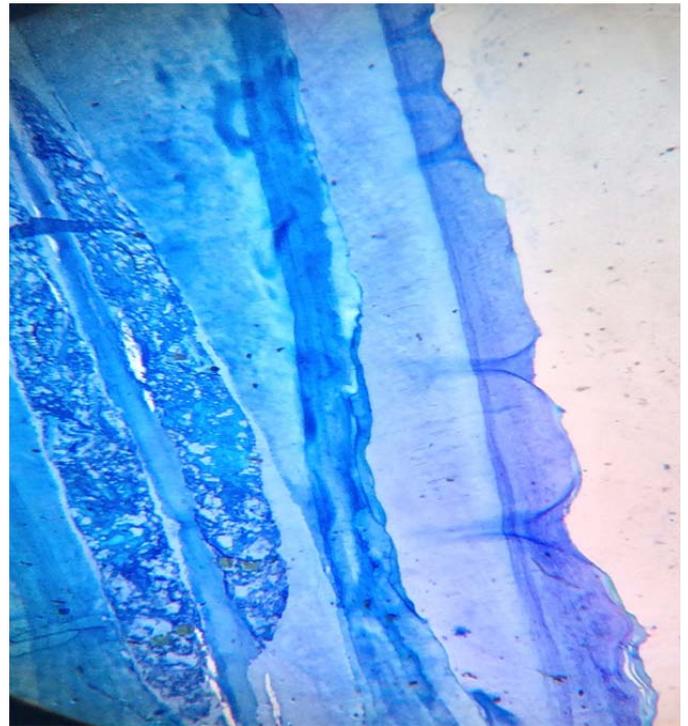
The present study was an attempt to study cementum stained by various histologic stains by light microscope. Hematoxylin and eosin staining of decalcified paraffin embedded sections showed basophilic staining of cementum with eosinophilic staining of dentin. There was adequate contrast between the cementum and dentin. The Cemento dentinal junction was evident. The incremental lines of cementum were also basophilic and prominent in H and E staining. There was no difference in the staining intensity between cellular and acellular cementum (Fig 1).



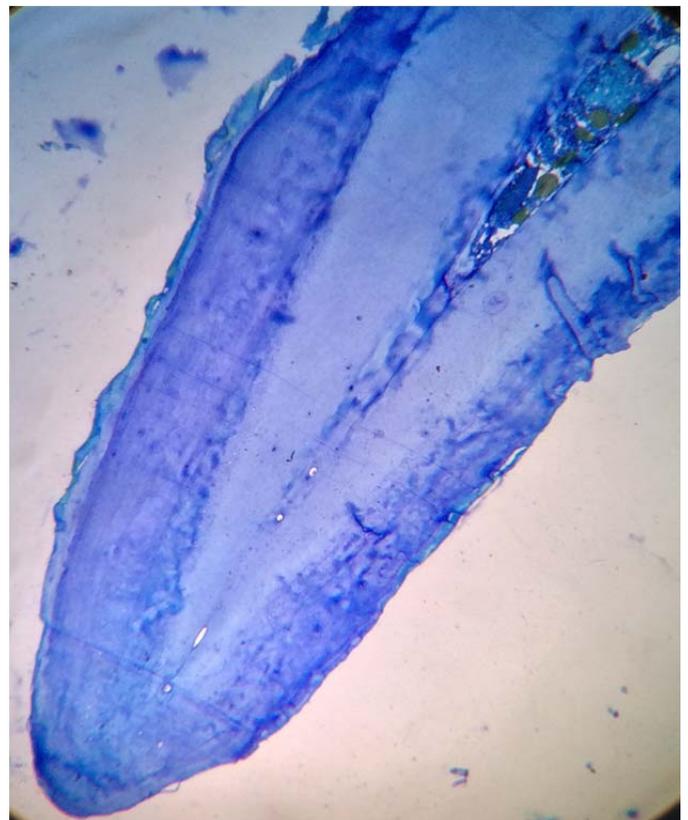
**Figure 1:** Photomicrograph showing basophilic staining of cementum and well appreciated Incremental lines (Hand E stain, 10x)

Toluidine Blue staining of decalcified paraffin embedded sections. The cementum stained deep blue in contrast to the underlying dentin which stained pale blue. It also allowed adequate visualisation of cemento dentinal Junction. The incremental lines of cementum were also better appreciated

under toluidine blue staining. However there was no difference in the staining intensity between cellular and acellular cementum. (Fig 2 and 3)

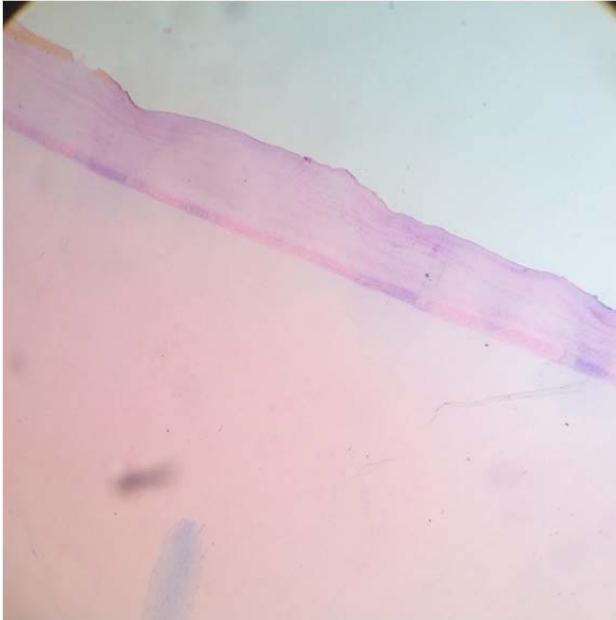


**Figure 2:** Photomicrograph showing deep blue staining of acellular cementum compared to paler staining of dentin (Toluidine blue O stain, 10x)

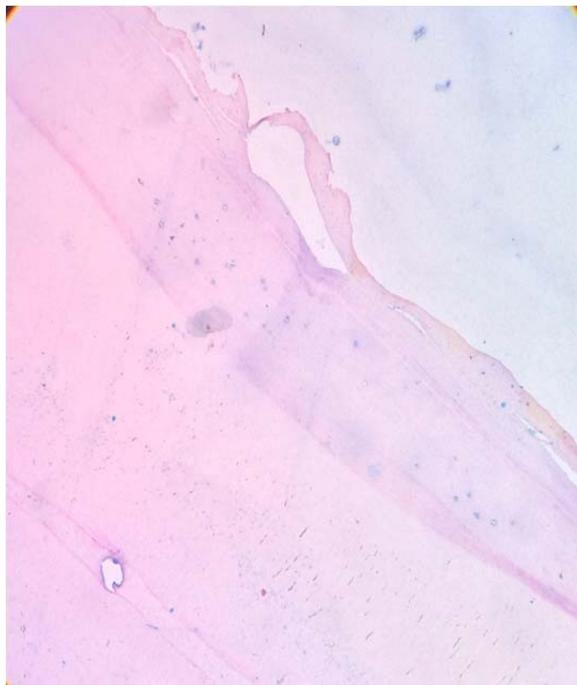


**Figure 3:** Photomicrograph showing deep blue staining of cellular cementum (Toluidine blue O stain, 10x)

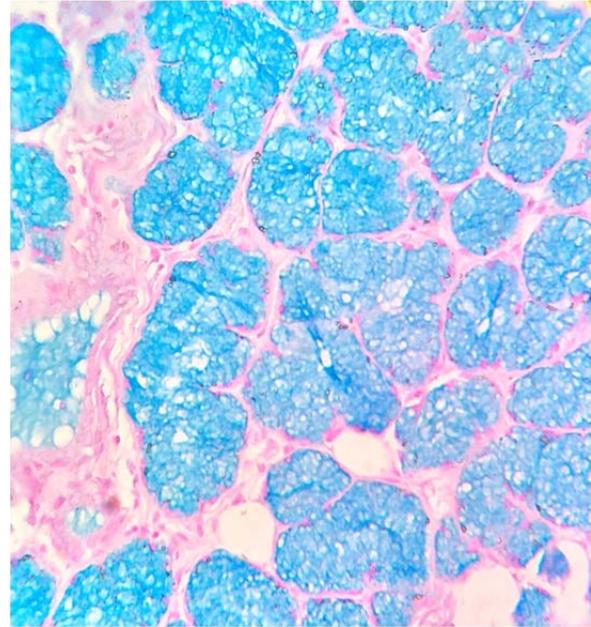
Alcian blue stain with nuclear fast red counter staining revealed pink areas of dentin and cementum. The cementum showed few areas of patchy alcian blue staining in cervical and in the apical region. The dentin layer stained pale pink compared to that of cementum. The cemento Dentinal junction and incremental lines stained pink. no difference in the staining intensity between cellular and acellular cementum was observed in Alcian blue staining but for the lacunae of the cellular cementum that showed blue staining. (Figure 4,5 and 6)



**Figure 4:** Photomicrograph showing pink staining of acellular cementum and pale pink staining of underlying dentin (Alcian Blue- Nuclear fast Red stain, 10x)



**Figure 5:** Photomicrograph showing alcian blue staining of lacunae in cellular cementum. (Alcian Blue- Nuclear fast Red stain, 10x)



**Figure 6:** Photomicrograph showing mucous gland acini stained with Alcian blue and Nuclear fast red.

#### DISCUSSION:

Cementum is a least understood and less studied hard tissue of the tooth. It bears similarities to bone but at the same time is different from bone in being avascular, not innervated and the lack of continuous remodelling. Cementum has gained much importance in the present day due to the growing interest in periodontal regeneration of which cementum plays an integral part. On reviewing the literature, there are very few studies<sup>1</sup> that have studied the cementum on histological grounds. The present study was an attempt to study the light microscopic features of cementum using various stains and correlate it with its nature and composition.

Histological stains are dyes that bind to various tissues. The process involves multiphase interaction of the tissues with the solutions of the staining reagents. These interactions result in the staining of tissues based upon the composition, affinity and interaction between the stain and the dye.<sup>12</sup> Hence, special stains form an integral part of routine histopathology as an adjunct to Haematoxylin and eosin, and give meaningful diagnostic information of the tissue available. This was harnessed in the present study to understand the structures of cementum using various histologic stains.

Hematoxylin is a basic dye and eosin Y is an acidic dye. These two are used in combination to visualize the tissues in routine histologic examination. In the present study H&E staining of the cementum showed weakly basophilic staining of acellular and cellular cementum. However the contrast between the cemental layer and dentin was well appreciated with dentin staining pale pink compared to cementum. This could be attributed to the presence of acidic extracellular matrix proteins in this layer.<sup>13,14</sup> Though some studies<sup>1</sup> have reported variations in the basophilic nature (staining intensity) between cellular and acellular cementum in Hand E staining, it was not discernible in the present study. This might reflect either a similar

composition of ECM or a variation in the staining intensity brought about by acid decalcification.

Toluidine blue stain is also a basic dye, along with the property of metachromasia staining tissue in varying intensities of blue. Intense staining was observed with cementum and pulpal tissue while the layer of dentin stained pale blue. This strong affinity of a basic dye to the cemental layer once again underscores the presence of acidic extracellular matrix proteins in the cementum.<sup>13,14</sup>

However, there was no visible difference in the staining intensity between the cellular and acellular cemental layers. Alcian Blue –NFR stain has been previously used to stain cementum in mouse models. Alcian blue stains acidic mucopolysaccharides. The small integrin binding ligands N-linked glycoprotein family members BSP and OPN all fall under the category of acid mucopolysaccharides and proteoglycans.<sup>16,17</sup> Though previous studies<sup>1,15</sup> have reported alcian blue staining of acellular cementum and attributed it to the rich ECM content, this finding was not observed in the present study. The present study showed a pale pink to pink staining of the cementum layer with very few patchy areas of blue staining in areas of dentin and cementum. The possibility of a technical variation was ruled out as the control sections of mucous gland acini had stained positive for alcian blue. The blue staining of the previous studies could also be due to the proteoglycan content. In the present study the lack of staining might be due to the lack of proteoglycan content in the layer of acellular cementum. It might also reflect that proteoglycans are present during initial cementogenesis and are lost with older age. However the lacunae of the cellular cementum showed blue staining this may be because of the proteoglycans which are normally present around the lacunae and canaliculi.<sup>18</sup>

#### CONCLUSION:

Cementum is a unique hard tissue. Knowledge of the cementogenesis is essential so as to develop therapeutic strategies for cemental regeneration. Though this would involve more molecular characterisation both in terms of origin, structure and composition, the present study was a small step to characterise the composition based on the staining affinity. It was evident that the cemental layer is rich in Extracellular matrix proteins. However more studies are need so that it could be translated to therapeutics.

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