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Incremental Lines in Dental Cementum as Age Estimation Biomarker

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Abstract: Age estimation is an important part of forensic identification, and teeth are among the most reliable structures used for this purpose. One of the promising histological methods is tooth cementum annulation (TCA), which involves counting of incremental growth lines in the cementum to estimate chronological age. Cementum incremental lines formed through the rhythmic deposition of mineralized tissue, appear as alternating dark and light bands and are thought to reflect annual biological cycles. The constant and non-adaptive growth of acellular cementum is considered as the ideal part of cementum to be used in cementochronology analysis. Therefore, this study aimed to explore the principles, applications, and potential of TCA. Currently, TCA or cementochronology has been widely used as an age estimation method in the forensic field, but the results of existing studies to date vary due to different preparation techniques and analysis techniques. A standardized protocol for cementochronology has now been adopted in multiple studies, often conducted in ISO 9001-certified laboratories to ensure consistency in histological preparation. While this standardization has improved methodological reliability, further research is still needed to evaluate the accuracy and applicability of cementochronology for age-at-death estimation in various forensic contexts. However, its implementation in peripheral or resource-limited settings remains challenging, as the method is invasive, requires specialized equipment, and involves laboratory-based histological processing. This review highlights that these incremental lines, particularly in acellular cementum that formed consistently over time, show a strong correlation with chronological age. Tooth cementum annulation has shown promising results and is considered a valuable complementary method for forensic age estimation.
Keywords: age estimation; tooth cementum annulation; cementochronology; forensic odontology

INTRODUCTION

Age estimation is an age determination technique that has been applied in various fields such as anthropology, archaeology, and forensic odontology. In the field of forensic odontology, age estimation on teeth is an essential tool for identifying unidentified humans, both burials and living humans. In the case of unidentified bodies, determining age is a mandatory step, especially if there is no antemortem information or data available on the body. Dental structures can resist various external influences, including temperature changes and heating processes.[1,2] Dental age estimation can be done by visual observation, radiography, histology, biochemistry, as well as genetics and epigenetics or deoxyribonucleic (DNA).[3]

Most age estimation methods rely on macroscopic analysis of the morphological characteristics of bone or tooth structures, the accuracy of which decreases when the condition of the bone or tooth structure is no longer perfect due to various intrinsic and extrinsic factors. However, even if macroscopic features are difficult to evaluate or there is fragmentation in tooth samples, tooth structure can still be useful as a tool for estimating age through histological examination. Histologically, a person's age can be gathered by counting incremental lines with the help of a microscope.[3] These lines reflect the regular growth process and periodic development of tooth tissue and can be found in the enamel, dentin, and cementum.[4]

Cementum is a hard tissue that surrounds the dentin and functions as the point of connection for the periodontal ligament fibres, which secure the tooth to the alveolar bone.[5] Cementum is composed of non-mineralized collagen fibres. Over time, the collagen bindings will undergo mineralization, resulting in the accumulation of hydroxyapatite crystals on the collagen. The crystals exhibit diverse orientations, resulting in the formation of a visual pattern characterised by alternating layers of dark and light lines when observed through a microscope. The dark and light lines are known as cementum annulation lines or tooth cementum annulation (TCA). This TCA, also known as cementochronology, is regarded as a method for estimating the age in human identification compared to other morphological or histological characteristics.[5,6] Unlike macroscopic methods, which tend to lose reliability when skeletal structures are incomplete or damaged, TCA remains effective in such conditions. It shows a strong correlation with chronological age and often yields more precise estimates, typically within two to five years of the actual age, making it particularly useful in forensic cases involving fragmented or poorly preserved remains.[7,8] A systematic review and meta-analysis by Suciyanie et al[9] reported that while root dentin translucency generally showed higher accuracy, TCA performed better in younger adults (15–44 years) and maintained a strong correlation with chronological age.

This study was conducted using a literature review method and focusing on the fundamental principles of cementum incremental lines and their significance in age estimation. In Indonesia, the application of cementochronology is still limited, and its potential has not been fully explored, showing the need for further research and validation in local forensic practice. Scientific articles were searched online through several databases, including PubMed, *ScienceDirect*, and *Google Scholar*, covering the past ten years using the keywords “Forensic Dentistry”, “Forensic Odontology”, “Cementochronology”, “Tooth Cementum Annulation”, and “Age Estimation”. This review is based on 27 selected articles.

Incremental Lines on Teeth

Incremental lines on teeth are microscopic structures found within the enamel, dentin, and cementum**.** These lines reflect the periodic growth process of tooth tissue. Incremental lines only be seen using special microscopes or imaging techniques that allow researchers to study the microscopic details of tooth tissue. Incremental lines can be used as time markers in forensic and archaeological studies to determine a person's age at death or to understand growth patterns over the life span.[10,11] The pattern and frequency of incremental lines can provide information about an individual's general health and nutritional status during the period of tooth formation because factors such as nutritional stress or disease can influence the formation of these lines.[10,11]

Enamel contains daily incremental lines known as cross-striations and long-period lines referred to as striae of Retzius, which reflect the circadian rhythm of ameloblast activity. These line patterns allow for the estimation of the timing and duration of crown formation. However, once the crown is fully formed, enamel apposition ceases, limiting its use for age estimation to individuals who have not yet reached full maturity.[8]

Primary dentin also exhibits daily incremental lines called von Ebner lines and long-period lines known as Andresen lines, both of which are associated with the circadian cycle of odontoblast activity. Unlike enamel, dentin continues to be deposited even after tooth development is complete. However, the formation of secondary dentin and intratubular dentin does not follow an incremental pattern, thus limiting the application of incremental dentin for age estimation to younger individuals.[8]

Cementum considered the most reliable dental tissue for age estimation through incremental line analysis. In contrast to enamel and dentin, which form incremental structures based on a daily circadian rhythm, cementum deposition is regulated by a circannual biological clock, resulting in the formation of annual growth lines. These lines continue to develop throughout life, making cementum particularly valuable for estimating age in both immature and mature individuals.[8]

Incremental Lines in Acellular Cementum

The majority of acellular cementum is found in the form of acellular extrinsic fiber cementum (AEFC) which is rich in extrinsic fibers. AEFC cementum is mostly found in the cervical and middle third of the root. Unlike cellular cementum, AEFC does not have cementocytes, lacunae, and canaliculi. AEFC grows very slowly with regular formation and a higher degree of mineralization (Figure 1).

The main function of AEFC is to bond with the periodontal ligament to achieve a strong tooth support system, but no reparative or regenerative function. Tooth movement or the process of occlusal adaptation of the teeth has minimal influence on AEFC, unlike cellular cementum.[12,13] Various light microscopy techniques such as transmitted light, polarized light, or phase contrast showed regular accumulation of AEFC by layers of cementum deposited parallel to the root surface, known as growth lines or incremental lines.[10,12]

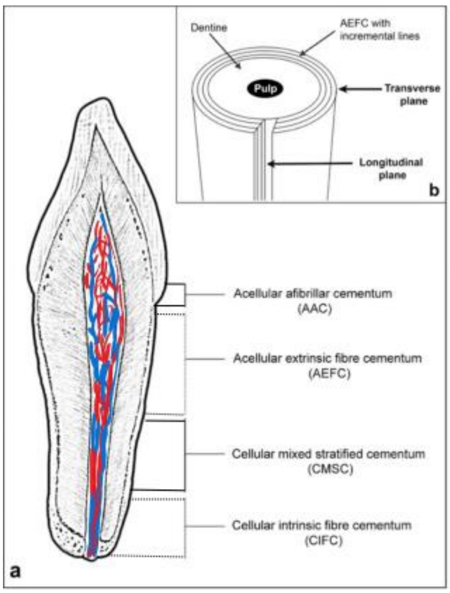

Figure 1. Types of cementum. (a) Distribution of cementum types based on their location on the tooth root. Acellular cementum (AAC and AEFC) is found from the cervical region to the middle third of the root, while cellular cementum (CIFC and CMSC) predominates in the apical region (b) Schematic illustration showing the orientation of incremental lines in AEFC relative to dentin and pulp on the inner side of the tooth root in both longitudinal and transverse sections. Source: Hinrichs et al, 2022 (modified).[12]

The acellular cementum thickness ranges from 200 to 460 µm. Cementum gets thicker with age. This phenomenon is found not only in teeth with regular occlusion but also in impacted teeth. Under a light microscope, AEFCs appear as lamellae parallel to the root surface, where the darker lines are incremental lines. Incremental lines are formed during the slowing cementogenesis phase. On Scanning Electron Microscopy (SEM) examination, the light-dark pattern seen on a light microscope is visualized as collagen fibrils areas that are densely arranged and areas of collagen fibrils that are more sparsely arranged. The two areas alternate to form a lamellar pattern. Areas with more sparsely arranged fibrils appear to resemble indentations.[12]

Incremental Lines in Cellular Cementum

Cellular cementum is predominantly found in the apical and interradicular areas of roots. The thickness of cellular cementum ranges from tens to hundreds of micrometers, depending on the type of tooth. The maximum thickness of cellular cementum in incisors is 400-600 µm, in canines is around 500 µm, in premolars 300-1000 µm, while in molars is 700-1500 µm.[13,14] There are two types of cellular cementum, namely cellular intrinsic fibers cementum (CIFC) which is more deposited in the apex and furcation areas, and cellular mixed stratified cementum (CMSC) which is more dominant in the transition area between the middle third and the apex of the root.[12,13]

Cementum Incremental Lines for Age Estimation

Principles of Cementochronology as a Tool for Age Estimation

Cementum is the part of the tooth that is most often used as additional information to determine the estimated age of death in mammals. As with enamel and dentin, it is known that incremental lines of cementum demarcate mineralized tissue deposits.[8,13,14,15] However, in contrast to dentin and enamel, cementum is deposited continuously throughout life. On the other hand, dentin stops being deposited after the dental pulp cavity is filled and enamel is no longer deposited after the tooth is completely formed.[8,15,16] Cementum grows from a single mineralization front and is avascular so it almost does not undergo remodelling or resorption.[13,16,17] Deposits continuous cementum provides longitudinal information about the factors that influence cementum growth from the time the root edification process begins until the tooth is lost or until the end of life.[8,16,18]

Periodic variations in cementogenesis activity cause differences in matrix composition, degree of mineralization, changes in crystal orientation, and changes in the orientation of collagen fibrils in cementum. This activity is believed to cause the appearance of incremental lines of cementum.[12,14–16,18,19] In mammals terrestrial, periodic deposits of dental cementum in the form of alternating bright dark bands or lines are associated with seasonal changes.[12,15,16,20,21]

The incremental line which is a dark and thin line separating two translucent bands represents a slower or relatively inactive phase of cementogenesis in winter. Meanwhile, incremental lines can be considered as resting lines.[15,16,20] Previous studies suggest that the mineral content of incremental lines is higher than other areas in acellular and cellular cementum, so incremental lines are considered analogous with cement lines demarcating osteons in bone tissue, supporting the theory that incremental lines are lines of rest.[12,14–16,19,2122] Recent research by Takahashi et al[14] who carried out cellular cementum analysis using light microscopy, SEM, and contact microradiography, showed that the incremental lines have lower mineral content than other areas of cementum. Nevertheless, Takahashi et al. still agree with the theory which states that incremental lines are formed when cementogenesis activity decreases. Based on the similarity of findings regarding the structure of cementum fibrils with previous research by Chen et al,[23] Takahashi et al[14] proposed a modified model of the cycle of cementum formation. Cellular cementogenesis occurs through alternating phases of high activity and low activity. At the beginning of the low-activity phase, cementoblasts secrete little or no fibrils and minerals so incremental lines full of fibrils and minerals are formed. When cementoblasts are active, the process of fibril formation and cementum mineralization begins to occur. In the high activity phase, cementoblasts form lamellar structures that contain high mineral content. After that, the cementoblasts return to the low activity phase. These stages repeat until incremental lines and lamellae of cellular cementum are formed.[14] Currently, the exact mechanism for the formation of incremental lines in acellular cementum is unknown, but it is thought to be not completely different from the mechanism found in cellular cementum (Figure 2). However, it should be remembered that acellular cementum showed an appearance of thinner incremental lines and more regular intervals.[10,12,13]

Based on observations in terrestrial mammals, if one incremental band is analogous to a "winter" band and one light band is analogous to a "summer" band, a pair of dark and light can be equivalent to one year's time.[15,16,18,20] Cementum apposition is estimated to begin when the roots are fully formed, which generally coincides with the end of tooth eruption.[21,24] The age estimation method used cementum incremental lines, which is also called tooth cementum annulation. In humans, it was developed on the basis of extrapolation from observations of annual cementum deposits in mammals and the assumption that cementum apposition begins when tooth eruption ends.[21] An estimated age is obtained by adding the total number of incremental lines and translucent bands on the dental cementum preparation to the average time of tooth eruption, which indicates the individual's age when the tooth is lost or the individual's age at death (Figure 3).[8,18,21,24–26]

Figure 2. Cellular cementum in the third molar, dominated by cementocytes and showing irregular, thicker incremental lines. (Author's documentation)

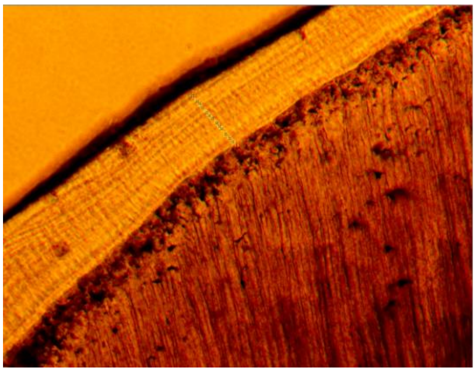

Figure 3. Cementum annulation measurement using ImageJ software. (Author's documentation)

It is known that acellular cementum and cellular cementum have different structural characteristics and different functions. Therefore, it is important to determine which cementum to calculate the incremental line for age estimation in humans. CIFC cementum has a complex function because it contains not only intrinsic fibers but also extrinsic fibers. CIFC cementum which is rich in extrinsic fibers plays a role in the tooth support system, while CIFC which is full of extrinsic fibers or without extrinsic fibers has an adaptive role and compensates for crown attrition, and has a reparative role in reshaping the root surface during tooth movement. CMSC cementum containing stratified CIFC and AEFC also plays a role in remodelling the root surface during tooth movement in the socket. AEFC cementum with extrinsic fibers that are arranged tightly and perpendicular to the root surface is the most ideal cementum as a tooth support and does not have an adaptive role. Therefore, the AEFC growth rate is constant throughout the year and varies between 0.004 to 0.008 µm/day (1.5-3 µm/year), depending on the type of tooth. The adaptive role of cellular cementum and its structural heterogeneity means that CIFC and CMSC are not used in tooth cementum annulation analysis. Therefore, the ideal cementum annulation technique is to use a tooth root preparation where 60-90% of the root length is acellular cementum.[8]

Tooth selection, the specific root area and type of cementum examined, histological preparation methods, and the process of line counting are key criteria that must be considered in cementochronology. Currently, there is no universal agreement on which tooth type yields the highest accuracy in this analysis. Nevertheless, many researchers prefer healthy single-rooted teeth, such as premolars and canines, as these typically present a more regular layer of acellular extrinsic fiber cementum.[8,18] Bertrand et al. emphasized that the ideal area for observation is the acellular cementum, which generally covers approximately 60–90% of the root length in single-rooted teeth. Teeth affected by severe caries, periodontitis, root resorption, or other pathological conditions should be avoided, as such conditions can interfere with the deposition pattern of cementum lines and complicate annual line interpretation.[8]

In addition to tooth type and cementum location, the technical stages of sample preparation from root sectioning to microscopic slide preparation, play a critical role in the accuracy of cementochronology. Differences across methods typically involve factors such as the use or omission of decalcification, whether the root is embedded in resin or left intact, the application of histological staining, longitudinal versus transverse section orientation, and variations in section thickness. Several studies, including those by Bertrand et al. and Colard et al., have implemented standardized histological protocols conducted in ISO 9001-certified laboratories. In this protocol, samples are prepared without staining and sectioned at a thickness of approximately 100 microns.[7,8,18,27]

Accuracy and precision of cementochronology for age estimation

The age estimation resulting from the increase in the number of incremental lines of cementum and the age of tooth eruption is strongly correlated with chronological age.[8] Some researchers who are sceptical of the validity of the cementum annulation method as a means of age estimation generally argue that the cementum annulation technique has no sound biological basis. This biological basis is the assumption that cementum deposits occur once a year obtained from research on animals, as well as the assumption that incremental lines begin to appear after tooth eruption.[21] Until now, there has been no research that empirically showed annual cementum deposits in humans. Nevertheless, there have been many studies evaluating the correlation between age estimation based on cementum annulation methods, as reported in a systemic review and meta-analysis by Pinto et al[25] After a systematic search, Pinto et al. found 24 studies that examined the correlation between age estimation based on the cementum annulation method and chronological age that met their inclusion and exclusion criteria. Of the 24 studies, the correlation coefficients reported ranged between 0.42 and 0.97, and only a small percentage had correlation coefficients below 0.8. Additionally, a meta-analysis by Pinto et al. showed that the correlation coefficient between the estimated age produced by the cementum annulation technique and the chronological age of healthy tooth preparations was 0.87 (95% CI 0.79-0.91). Meanwhile, a meta-analysis in research groups using dental preparations with pathologies such as periodontal disease and caries produced a correlation strength that was high, namely 0.78 (95% CI 0.58-0.89).[25] High correlation coefficient showed the strength of the linear relationship between chronological age and age estimation based on the cementum annulation method. Nevertheless, Pinto et al[25] also reported a high degree of heterogeneity among the studies they analyzed. This heterogeneity comes from the number of individuals studied, the type of teeth studied, the number of sections analyzed, as well as the preparation and analysis techniques for histological preparations.[8,25]

Colard et al[18] in their publication in 2015 stated a cementochronology procedure protocol that had been certified by ISO-9001 as an effort to standardize the stages in carrying out cementum annulation. In terms of accuracy, most studies analyzed the absolute difference between tooth age and chronological age. Accuracy values from various studies showed that the difference between tooth age based on the cementum annulation method and chronological age is two to six years.[18]

CONCLUSION

Incremental lines on teeth are microscopic structures that reflects the periodic growth processes of tooth tissue. Incremental lines can be found in enamel, dentin, and cementum. Incremental lines in cementum are formed annually and may be found both in the non-cellular and cellular cementum. The constant and non-adaptive growth of acellular cementum is considered as the ideal part of cementum to be used in cementochronology analysis.

Currently, tooth cementum annulation or cementochronology has been widely used as an age estimation method in the forensic field, but the results of existing studies to date vary due to different preparation techniques and analysis techniques. A standardized protocol for cementochronology has now been adopted in multiple studies, often conducted in ISO 9001-certified laboratories to ensure consistency in histological preparation. While this standardization has improved methodological reliability, further research is still needed to evaluate the accuracy and applicability of cementochronology for age-at-death estimation in various forensic contexts. However, its implementation in peripheral or resource-limited settings remains challenging, as the method is invasive, requires specialized equipment, and involves laboratory-based histological processing.

Conflict of Interest

The author declares no conflicts of interest in this study.

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