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Short communication

Improving the visibility of tooth cementum annulations by adjustment of the cutting angle of microscopic sections

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Abstract

Age at death assessments by counting tooth cementum annulations (TCAs) in unstained undecalcified microscopic ground sections of (single rooted) teeth is, amongst others, problematic because of the unclear distinction between the bright and darker annulations. Counting is hampered by optical superimposition of the tangentially positioned layers of cementum in the section since 'regular transverse sections' run perpendicular to the axis of a cone-shaped root with its yearly deposited cone-shaped layers of cementum. This study demonstrates that to improve the visibility of the annulations, the cutting angle should be perpendicular to the exterior of a root, not perpendicular to its axis. The site where the cut hits the root perpendicular should show the best possible distinction between the TCAs. Here, superimposition of the now vertical positioned layers within the section will result in increased contrast between bright and darker layers. A procedure for such preparation is given.

Keywords: Tooth; Cementum; Annulations; Microscopy

1. Introduction

The increasing popularity of assessing the age at death, or the age of affliction of health insults during life of deceased persons, by counting tooth cementum annulations (TCAs) is in part frustrated by technical difficulties ensuring the stratigraphy of these appositional layers of acellular cementum are clearly visible through the microscope [1]. During life, cementoblasts deposit their product, cementum, onto the roots of teeth. Attributed to seasonal influences after eruption, this deposition accumulates in more or less 'regular' layers [2–5]. As a result, a combined set of a bright and a darker microscopic layer of cementum is assumed to represent a full year of deposition activity. Difference in appearance between bright and dark layers has been primarily ascribed to differences in collagen fiber orientation and degree of mineralization [5,6]. Since cone-shaped roots are thought to be covered yearly by new cone-shaped sheaths of cementum, the elapsed time in years after eruption is supposed to show as a series of distinct TCAs in a microscopic section through the root. Due to simple root form, single rooted teeth should demonstrate this stratigraphy with the least amount of distortion. One is advised to count TCAs in transverse sections taken from the middle third of the root because of the risk of cementum resorption by odontoclasts near the gingival junction from periodontitis and of cementum erosion due to neck caries and brushing, and additionally, the risk of atypical extra cementum apposition (hypercementosis) and of remodeling activity at the apical end of the root in the elderly [4,7–10].

In practice, TCA-counting has shown to be cumbersome for many reasons. Preparation of hard tissue sections is thought to be expensive and problematic to perform. Optically, it is difficult to observe the annulations through the microscopy of unstained undecalcified transverse sections. Once visible, distinctions between different sets of annulations (years) often appear to be vague. Finally, some individuals seem to accumulate multiple annulations per year [8,11–14]. At times it appears as if this trend, known as the 'doubling' phenomenon, only happens during discrete periods of life. Of all these complications, this paper focuses on improving the cutting angle of the sections, with the intention that remaining difficulties can be addressed with the best possible chance of success.

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2. Materials and methods

Routine procedures for age at death determination of individuals of unknown identity at the Department of Pathology of The Netherlands Forensic Institute and at Barge's Anthropologica of the Leiden University Medical Center include the analysis of age related changes at the sternal end of the fourth rib [15], the assessment of the status of bone tissue remodeling in the anterior femoral shaft [16] and the counting of tooth cementum annulations. As a consequence 100 single rooted teeth, one tooth per individual, were extracted and prepared for TCA analysis from 28 forensic and 71 archaeological cases. From these teeth, two types of plastic embedded microscopic sections were produced and inspected. One type consisted of sections cut perpendicular to the root axis, the other of sections cut perpendicular to the exterior of the root. The following procedure was applied:

(1) By means of a small folded paper stabilizer cut from a self-adhesive label, a tooth was positioned with its long axis positioned as centrally as possible in a rectangular plastic container (Fig. 1).
(2) Subsequently a thermosetting plastic medium was poured into the container with the embedded tooth. We used Epon (5.66 g glycide ether 100; 3.1 g dodecenylsuccinic anhydride; 2.92 g methyl norbonene-2,3-dicarboxylic anhydride; 1% of total weight 2,4,6-tris-phenol).
(3) After hardening the transparent plastic overnight and, after subsequent extraction of the cast, further trimming of the block was conducted to ensure that the long axis of the tooth root ran parallel to two adjacent longitudinal sides of the block.
(4) With a thin permanent marker two lines were drawn onto one of the transparent surfaces of the rectangular plastic

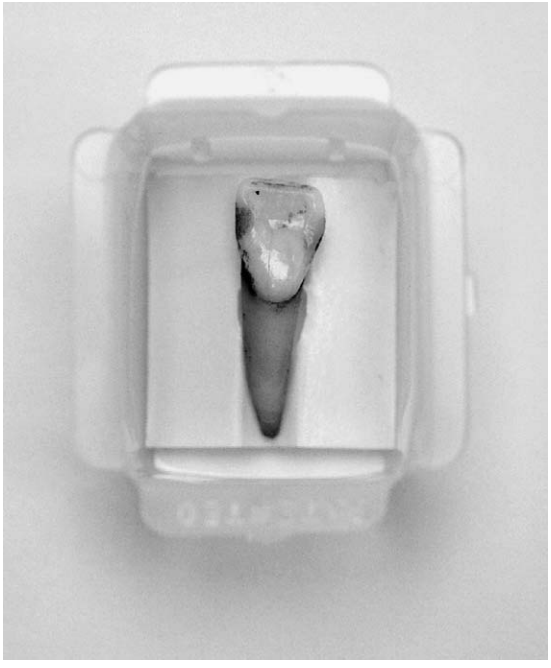

Fig. 1. By means of a small folded paper stabilizer cut from a self-adhesive label, a tooth is positioned with its long axis as centrally as possible in a rectangular plastic container.

Fig. 2. A line parallel to the exterior of the root has been drawn onto the transparent surface of the rectangular plastic block.

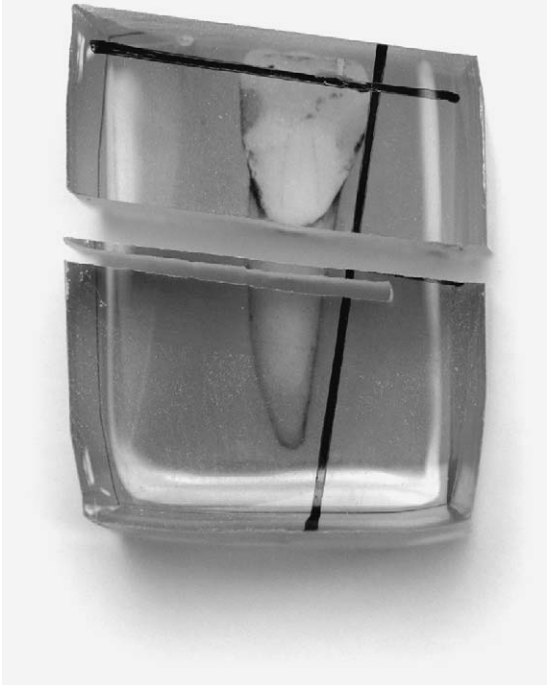

Fig. 3. Two cuts have been made perpendicular to the drawn line running parallel along the exterior of the root. The line perpendicular to that line is used as a guide for sawing. Later, for orientation during microscopy, the outer edge of the plastic slice where both cuts had hit the root perpendicular will be marked with a permanent marker.